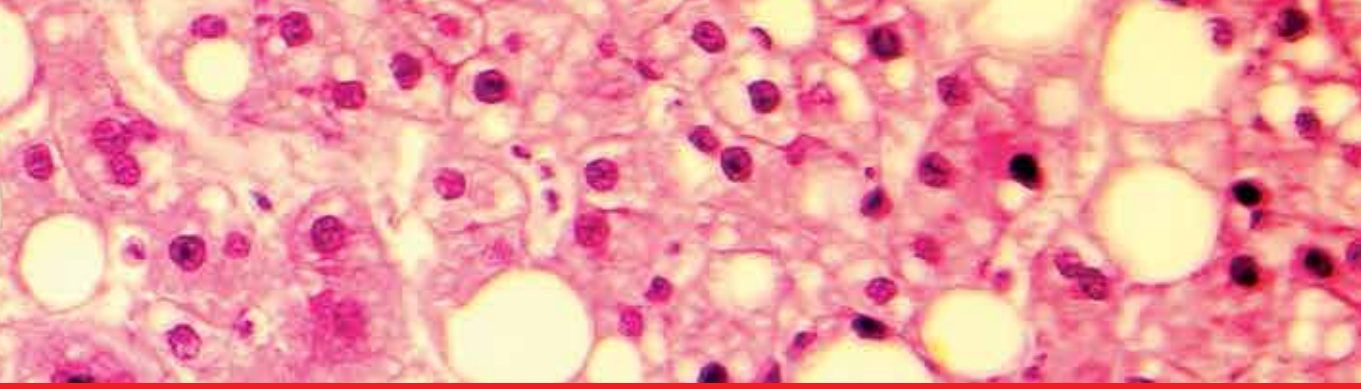
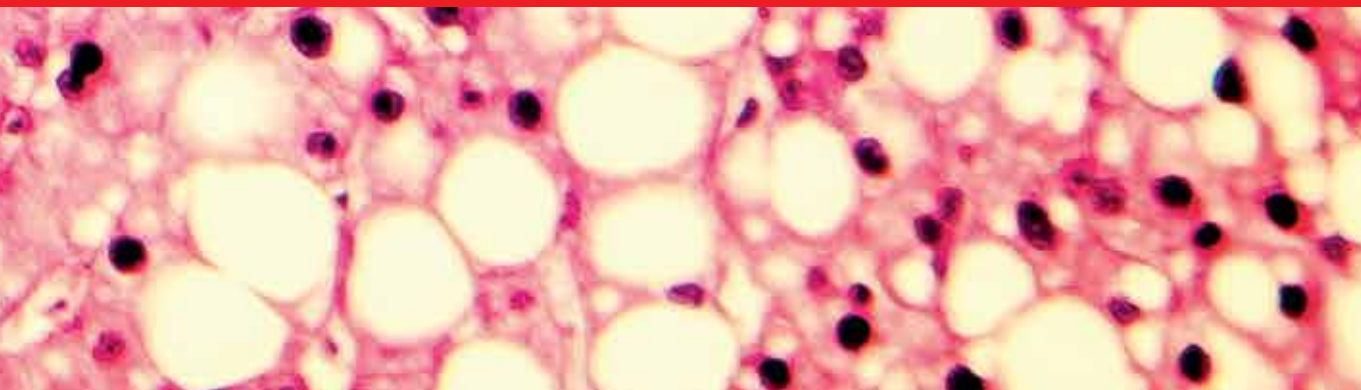


IntechOpen

Liver Transplantation

Basic Issues

Edited by Hesham Abdeldayem and Naglaa Allam



LIVER TRANSPLANTATION – BASIC ISSUES

Edited by **Hesham Abdeldayem**
and **Naglaa Allam**

Liver Transplantation - Basic Issues
http://dx.doi.org/10.5772/1473
Edited by Hesham Abdeldayem and Naglaa Allam

Contributors

John Lang Jr., Erik Stoltenberg, Alexander Vitin, Bradley Evan Hansen, Meral - Kanbak, Ayşe Heves Karagöz, Filiz Uzumcugil, Miguel Jiménez-Pérez, Ana Belén Saez Sáez-Gómez, Rocío González-Grande, Juan Miguel Rodrigo-López, Marta Malgorzata Wawrzynowicz-Syczewska, Ram Subramanian, Kathy M. Nilles, Carmen Peralta, Maria Elias-Miró, Mónica B. Jiménez-Castro, Chunbao Guo, Ling Lu, Jianhua Rao, Guoqiang Li, Xiaofeng Qian, Beicheng Sun, Xuehao Wang, Xingli Huang, Teresa Casanovas, Maria Carmen Peña-Cala, Eva Rodríguez Bruzos, Maria Jover-Cobos, Rajiv Jalan, Nathan Davies, Manuel Romero-Gomez, Matthias Heuer, Gernot Kaiser, Sven Lendemans, Andreas Paul, Claudia Fernandes, David Silveira Marinho, Fernanda Paula Cavalcante, Anne M Larson, Saleh Alqahtani, Kyota Fukazawa, Ernesto Pretto, Marcia Samada Suarez, Julio C. Hernández, Dennis Eurich, Marcus Bahra, Alejandro Mejia, Parvez S Mantry, Hector Nazario, Peter Nissen, Hans J Frederiksen, Niels H Secher, Ayman Zaki Azzam

Notice

First published in Croatia, 2012 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Liver Transplantation - Basic Issues
Edited by Hesham Abdeldayem and Naglaa Allam

p. cm.

ISBN 978-953-51-0016-4

eBook (PDF) ISBN 978-953-51-6800-3

Meet the editor

Editor, Dr Hesham Abdeldayem is a professor of surgery at the National Liver Institute, Menoufeyia University, in Egypt. He is a well known expert in the fields of hepatobiliary and pancreatic surgery and liver transplantation. He was trained at Starzl Transplantation Institute, University of Pittsburgh Medical Center, in USA. Dr Abdeldayem is a WHO-certified trainer in publishing medical journals. He is a reviewer in many medical journals, and published many papers and several books, both in English and Arabic, in the fields of liver transplantation and hepatobiliary and pancreatic surgery.

Editor, Dr Naglaa Allam is an associate professor of Hepatology at the National Liver Institute, Menoufeyia University, in Egypt. She was trained at the liver transplant unit at the Hospital of university of Pennsylvania in USA. Dr Allam has a number of international publications in the field of hepatology and liver transplantation. She is a reviewer in many journals and has also played an active role in editing books on liver transplantation.

Contents

Preface XIII

Part 1 History 1

Chapter 1 **History and Evolution of Liver Transplantation 3**
Ayman Azzam

Part 2 Ischemia/Reperfusion Injury 19

Chapter 2 **Ischemia-Reperfusion Injury Associated with Liver Transplantation in 2011: Past and Future 21**
M. Elias-Miró, M.B. Jiménez-Castro and C. Peralta

Chapter 3 **The Influences of Nitric Oxide on Liver Ischemia-Reperfusion Injury 57**
Erik Stoltenberg, Alexander A. Vitin, Bradley E. Hansen and John D. Lang, Jr.

Part 3 Immunology and Liver Allograft Rejection 73

Chapter 4 **Immunology of Liver Transplantation 75**
Ling Lu, Jianhua Rao, Guoqiang Li, Xiaofeng Qian, Beicheng Sun and Xuehao Wang

Chapter 5 **Clinical Immunosuppression 95**
Chunbao Guo

Part 4 Prognostic Factors in Liver Transplantation 123

Chapter 6 **Prognostic Factors for Survival in Patients with Liver Cirrhosis 125**
Marcia Samada and Julio C. Hernández

Chapter 7 **The Rise of Glutaminase in End-Stage Liver Diseases 145**
Maria Jover-Cobos, Nathan Davies, Rajiv Jalan and Manuel Romero-Gómez

Part 5 Quality of Life Before and After Liver Transplantation 157

Chapter 8 **Disease Targeted Measures of Health Related Quality of Life (HRQOL) in Patients with Advanced Liver Disease Before and After Liver Transplantation 159**
Teresa Casanovas Taltavull, Maria Carmen Peña-Cala and Eva Rodríguez Bruzos

Part 6 Viral Hepatitis and Liver Transplantation 191

Chapter 9 **Hepatitis C and Liver Transplantation 193**
Miguel Jiménez Pérez, Ana Belen Sáez Gómez, Rocío González Grande and Juan Miguel Rodrigo López

Chapter 10 **HCV-Recurrence After Liver Transplantation 205**
Dennis Eurich, Marcus Bahra and Peter Neuhaus

Chapter 11 **Management of Recurrent HCV and HBV Infections After Liver Transplantation 225**
Marta Wawrzynowicz-Syczewska

Part 7 Other Indications for Liver Transplantation 249

Chapter 12 **Role of Liver Transplantation in Acute Liver Failure 251**
Saleh A. Alqahtani and Anne M. Larson

Chapter 13 **Liver Transplantation for Hepatocellular Carcinoma (HCC) 277**
Alejandro Mejia, Hector Nazario and Parvez Mantry

Chapter 14 **Liver Transplantation Due to Abdominal Trauma 303**
Matthias Heuer, Sven Lendemans, Gernot M. Kaiser and Andreas Paul

Part 8 Anesthesia and Periopertive Period 319

Chapter 15 **Intensive Care Management of Patients Prior to Liver Transplantation 321**
Kathy M. Nilles and Ram M. Subramanian

Chapter 16 **Anesthesia in Liver Transplantation 333**
Meral Kanbak, Ayse Heves Karagoz and Filiz Üzümcügil

Chapter 17 **Cardiovascular Monitoring and Substitution of the Blood Volume During Liver Transplantation 359**
Peter Nissen, Hans J. Frederiksen and Niels H. Secher

Chapter 18 **The Post-Reperfusion Syndrome (PRS): Diagnosis, Incidence and Management 385**
Kyota Fukazawa and Ernesto A. Pretto, Jr.

Chapter 19 **Malignant Hyperthermia in Liver Transplantation 397**
Cláudia Regina Fernandes,
David Silveira Marinho and Fernanda Paula Cavalcante

Preface

Liver transplantation is one of the few truly life-saving and life-altering procedures in medicine, but at the same time it is a highly risky procedure. Thus, it is not to be considered as a cure but more as a swap, where the benefits and risks must be balanced against the risks and benefits of no-transplant. Although the basic principles of liver transplantation have not changed, the field of liver transplantation is still young, evolving and dynamic.

This book covers a wide spectrum of topics including history of liver transplantation, ischemia-reperfusion injury, immunology of liver transplantation, viral hepatitis and liver transplantation, other indications for liver transplantation, prognostic factors and perioperative period.

The authors of the chapters are experts in their respective fields. They are pioneer proponents in different aspects of liver transplantation and come from many centers across the world. The interdisciplinary approach and the authority of the contributors resulted in a valuable reference to anyone interested in developing a global view in liver transplantation including medical students, residents, fellows, nurses, and practicing physicians and surgeons as well as researchers in the field of liver transplantation.

This book is dedicated to our Patients without whose goodwill and trust no progress in medicine would be possible. As the editor, I wish to thank all the authors for their co-operation and desire to share their precious experience with the medical community. On their behalf, I wish to express hope that our publication will facilitate access to the latest scientific achievements in the field of liver transplantation all across the world.

To all my colleagues at the National Liver Institute in Egypt who supported me, and embraced me with their warm feelings: I love you all. To all my professors who so generously guided me by their example, wisdom and insights: thank you. Finally, to Ms. Romana Vukelic, the publishing process manager, with whom editing this book was a real pleasure. Thank you, Romana.

Hesham Abdeldayem, MD.
Professor of Surgery
National Liver Institute
Menoufeyia University
Egypt

Part 1

History

1

History and Evolution of Liver Transplantation

Ayman Azzam
Alexandria University
Egypt

1. Introduction

Historically, in ancient civilization, man had already imagined changes in the morphology, structure and function of the human body. Egyptian and Greco-Roman mythology provided examples of the metamorphoses sung by Homer and Ovid, symbolic incarnations of the "comedie humaine" with its strength, weakness, vices and virtues. The liver has been the noble organ, the organ of life from time immemorial-liver in English, Leber in German, derived from the verb to live. An Indian legend from the 12th century B.C recounts the power of Shiva, who xenotransplanted an elephant head onto a child induce the Indian god Gaesha.[1] In ancient China, Yue-Jen (407-310 B.B.) induced anesthesia lasting 3 days by "the absorption of extremely strong wine, opened up the chest of two soldiers and after examining them, exchanged their hearts and transplanted them". The first reference to the concept of organ transplantation and replacement for therapeutic purposes appears to be Hua-To (136 to 208 A.D.) who replaced diseased organs with healthy ones in patients under anesthesia induced with a mixture of Indian hemp.

Although attempts at transplantation date back to ancient times, the impetus for modern transplantation was World War II and Battle of Britain. Royal Air Force pilots often were severely burned when their planes crashed. The mortality rate associated with burns corresponds to the size of the area of the skin that has been injured and the survival rate can be improved if the burned skin is replaced. For this reason, British doctors, attempted skin transplantation from other human donors as a mode of therapy. However, these attempts were uniformly unsuccessful. The transplanted skin became necrotic and fell off over several days.[2] This problem led investigators in 1940s to study skin transplantation in animal models. It remained for Sir Peter Medawar in 1944 to establish that the failure of a skin graft to "take" was the result of a process later termed immunological rejection.[3] Later studies by Gowens in 1948 revealed that lymphocytes play a major role in transplant rejection.[4] In 1951, it was shown that cortisone therapy significantly prolonged survival of skin allograft.[5] In 1959, Schwartz and Dameshek reported drug-induced immune-tolerance using 6-mercaptopurine.[6] Later in 1961, Calne and Murray showed that azathioprine therapy suppressed the rejection reaction and prolonged allograft survival.[7]

Once clinicians were confident that adequate immunosuppression was available, solid organ transplantation for end stage organ disease entered its early investigative phase. This was not possible without the application of the principles of vascular anastomosis pioneered by Alexis Carrel in 1902, for which he was awarded the Nobel Prize for Medicine in 1912.[8]

Further refinements in surgical techniques and suture materials have enabled Murray and his colleagues to perform the first successful kidney transplant in 1955.[9] This was a living donor transplant performed between identical twins. However, later attempts to perform renal transplantation when the donor and recipient were not genetically identical failed because no effective immunosuppressive therapy was available. From the early 1960s, a combination of azathioprine and corticosteroids was used with success to prevent graft rejection after kidney transplantation. In 1963, Woodraff described the immunosuppressive effect of antilymphocytic serum which destroyed the recipient active lymphocytes.[10] The success of kidney transplantation paved the way to think and perform liver transplantation for end-stage liver disease.

In 1955, Welch reported on his efforts to transplant an auxiliary liver into the right paravertebral gutter of non-immunosuppressed mongrel dogs.[11] In 1958, Francis Moore described the standard technique of canine liver orthotopic liver transplantation.[12] In 1963, Starzl attempted the first human orthotopic liver transplantation in a 3-years-old boy who suffered from biliary atresia, however, the patient died before the operation was completed.[13] Following this first unsuccessful attempt, the procedure evolved slowly and although his series remained largely unsuccessful, many of the technical principles that still guide liver transplantation were established. In 1967, Starzl and colleagues at the University of Colorado reported the first successful clinical liver transplantation.[14]

Between 1966 and 1973, Starzl and colleagues performed three chimpanzee-to-human xenotransplantation of liver as well.[15] There have been 12 cases of clinical xenotransplantation including four cases of champazee-to-human, seven cases of baboon-to-human and one case of pig-to-human.[16]

In 1978, Roy Calne opened liver transplantation unit in Cambridge, UK, and performed the first liver transplantation in Europe and the second largest transplantation series in the world.[17] Until 1977, Starzl and Calne contributed the majority of performed liver transplantation worldwide.[18]

The first hetero-topic liver transplants in man were reported by Apsolon in 1965; however, the first long-term survivor with this technique was reported by Fortner in 1973.[19]

In 1984, Shaw et al introduced the venovenous bypass system at Pittsburg University, leading to better hemodynamic stability during the standard liver transplantation.[20]

At the same time, Broelsch et al.[21] in the USA and Bismuth et al.[22] in France performed independently the first reduced-size liver transplantation. Thereafter, Pichlmayr et al.[23] reported the first split liver transplantation 1988. Meanwhile, Tzakis et al. introduced the piggyback technique with preservation of the recipient's vena cava.[24] With the increasing number of the patients on the waiting list, transplantation of partial liver grafts from living donors evoluted to increase the donor pool. For this purpose, Broelsch et al. established the technique of segmental living donor liver transplantation (LDLT), and Strong et al. performed the first successful LDLT in 1989, implanting a left lateral segment into a pediatric patient.[25] In 1990, Broelsch et al. reported the first series of LDLT in the USA.[21] In 1991, the first domino liver transplantation using liver from donors affected by familial amyloidotic polyneuropathy type I was introduced by Holmgren at al.[26] In 1992, Belghiti and coworkers introduced a modified piggyback technique with a cavo-caval side-to-side

anastomosis.[27] One year later, Hashikura and colleagues transplanted a left hepatic lobe into an adult recipient in 1993,[28] and Yamaoka et al. implanted a right lobe into a pediatric recipient.[29] In 1996, Lo et al. performed the first successful liver transplantation using an extended right lobe from a living donor for an adult recipient.[30] In 1998, Tzakis et al. introduced liver transplantation with cavo-portal hemitransposition in the presence of diffuse portal vein thrombosis.[31] In 2002, Cherqui et al. reported first donor hepatectomy by a full laparoscopic procedure in which a left lateral lobectomy was successfully performed for liver transplantation in a child.[32]

2. Evolution of immunosuppression

Rejection of the transplant remained a major problem until cyclosporine-A was discovered by Jean Borel.[33] The 1-year survival rate following liver transplantation was 30% to 50% prior to the discovery of cyclosporine-A,[34,35] however, after the introduction of cyclosporine-A, the 1-year and 3-year survival rates were 74% and 67% in the first 1000 recipients treated with cyclosporine-A at the University of Pittsburgh in the early 1980s.[36] After these good results, growth of liver transplantation was facilitated by the conclusion of the National Institute of Health Consensus Development Conference in 1983 that liver transplantation is not an experimental procedure but an effective therapy that deserves broader application.[34] Shortly thereafter, the first monoclonal antibody OKT3 was discovered by Cosimi in 1981 and proved effective in treating acute transplant rejection and was sometimes used along with cyclosporine-A based regimen as immunoprophylaxis especially in North American Centers or to treat steroid resistant graft rejection.[37] Since then, many new immunosuppressive agents were introduced. In 1990, Mycophenolate mofetil (MMF, CellCept) was introduced by University of Wisconsin and proved, in combination with cyclosporine-A, to further reduce the incidence of graft rejection episodes better than azathioprine with less toxic effects.[38] In the same year, Rapamycin (Sirolimus) was introduced.[39] It is like cyclosporine-A but it has a different mechanism of action. It inhibits lymphocyte proliferation through prevention of ligation of IL-2 to the IL-2 receptors.[40] In 1994, Ochiai in Japan introduced tacrolimus (FK506, Prograf) and proved to reduce the incidence of transplant rejection more than cyclosporine A. It is like cyclosporine-A but hundred times more potent and is indicated in severe acute rejection resistant to standard immunosuppressive protocols and in chronic rejection.[41]

Greater understanding of the underlying liver disease, improved surgical and anaesthetic techniques, reliable immunosuppression and dependable postoperative care over the last few years have contributed towards improved results of liver transplantation. This success has resulted in a disproportionate increase in demand of liver transplantation and the appearance of a major problem of shortage of available donor organs, leading to a prolonged waiting times and high mortality on the waiting list.[42]

3. The progress in liver transplantation with donor shortage

The donor shortage together with the development of surgical skills of liver resections based on the knowledge of segmental anatomy of the liver described by Couinaud,[43] opened the door for innovative methods of transplantation including auxiliary liver transplantation, reduced-liver transplantation (RLT), split liver transplantation (SLT) and living donor liver

transplantation (LDLT).[44,45] Also, The donor shortage had led to the evolution of hepatocyte and stem cells transplantation which will be the future in the liver transplantation.

3.1 Auxiliary liver transplantation

Auxiliary liver transplantation (ALTx) consists of either implanting a healthy liver graft placed heterotopically or orthotopically while leaving all or part of the native liver. This concept was originated from an experimental work of Welch in 1955.[46, 47]

The first auxiliary liver transplantation in human was performed by Absolon in 1964,[48] and it was till 1972 when an auxiliary transplantation truly prolonged a human life.[49] During the following two decades, ALTx was done solely in a heterotopic manner - heterotopic auxiliary liver transplantation (HALTx), where a graft (usually partial) is placed below the un-resected native liver. The initial clinical results of HALTx were rather disappointing with a high rate of technical failure, probably due to inadequate portal perfusion of the graft and insufficient drainage of hepatic blood flow in an area of low pressure which had led to temporary abandonment of HALTx in the early 70s.[50-52]

Many efforts have been made ever since to improve post-transplant survival. Most notably, based upon the experiences in animal studies,[52-58] the contributions of Terpstra's group have improved the surgical techniques of HALTx with markedly increased post-HALTx survival rate.[58-63] Since 1980s', the concept of ALTx has further been extended by the introduction of a new approach –auxiliary partial orthotopic liver transplantation (APOLTx), where the left or the right lobe of the native liver is resected and replaced by an auxiliary graft.[64-71] The physiological position of the hepatic graft by this approach results in an optimal outflow pressure. Accumulating clinical results have shown a reduced incidence of post-transplant portal thrombosis.[65, 72, 73]

For certain types of non-cirrhotic metabolic disorders, such as type 1 Crigler-Najjar syndrome, urea cycle enzyme deficiencies, disorders of fatty acid metabolism, familial hypercholesterolemia, hemophilia and ornithine transcarbamylase deficiency, an auxiliary liver may correct the partial enzymatic deficiency responsible for the disease without the need to remove the otherwise normal native liver.[65, 66] A significant minority of patients with acute liver failure who fulfill the transplant criteria would have had complete morphological and functional recovery of their liver if they had not undergone orthotopic liver transplantation.[74] These considerations have led to the concept of auxiliary liver transplantation, which doesn't exclude the potential for spontaneous regeneration of the native liver and eventual withdrawal of immunosuppression drugs.[75-78]

In selected patient aged <40 years without hemodynamic instability, the use of ABO compatible, non-steatotic grafts harvested from young donors with normal liver function, can restore normal liver function and prevent the occurrence of irreversible brain damage. After standard immunosuppression, the recovery of the native liver is assessed by biopsies, hepatobiliary scintigraphy and computed tomography. When there is evidence of sufficient regeneration of the native liver, immunosuppression can be discontinued progressively. Complete regeneration of the native liver can be observed in >50% of patients, who can be withdrawn from immunosuppression. Therefore, the advantages of the auxiliary liver transplantation seem to balance with the potential inconvenience of this technique in

selected patients.[79-81] ALTx also preserves the patient's native liver, which remains accessible for future gene transfer therapy.[82]

3.2 Reduced-sized liver transplantation (RLT)

It was first reported in 1984 by Bismuth, and involves ex-vivo resection of an adult cadaveric liver in order to create an appropriate sized liver graft for an infant or small child. It was introduced as a surgical solution for decreasing the pediatric liver transplant waiting list mortality using organs from donors much larger than the recipient, but does not increase the total number of livers available for transplantation. This is because the reduced-sized portion is not used and discarded.[22]

Initially, RLT was criticized because it disadvantaged adult patients awaiting liver transplantation and was to be associated with inferior results. The allegations regarding inferior graft and patient survival were proven wrong,[83-85] and several proponents of this technique actually reported a lower incidence of vascular complications since the caliber of the hepatic artery was larger than that seen in a pediatric donor.[86] Since this technique resulted in discarding the remaining portion of liver, it clearly had a negative impact on adult population awaiting liver transplantation, and for that reason, is rarely used today.

3.3 Split liver transplantation (SLT)

In 1988, Pichlmayer in Germany and Bismuth in France simultaneously performed split liver transplantation (SLT), an ex-vivo splitting of a cadaveric liver allowing transplantation to a pediatric recipient and one adult.[23, 87] Unlike RLT, SLT resulted in an increased number of organs in donor pool with each cadaveric liver giving rise to two functioning allografts. The initial results of SLT, reported by Broelsch,[21] had a high rate of graft failure with a survival rate of only 67% in children and 20% in adults receiving a split liver transplants. In addition, 35% of patients required retransplantation and more than a quarter had biliary complications.[22] More recently, in-situ SLT has provided patient and graft survival similar to that seen in whole cadaveric transplantation.[88-90] The practical feasibility of split-liver transplantation as well as the increased safety of conventional liver surgery suddenly opened up the idea of removing part of the liver from a living donor.

3.4 Living donor liver transplantation (LDLT)

This has been made possible by recent advances in hepatic surgery; first, improved understanding of the anatomy and the techniques of hepatic resections,[91] second, growing evidence that the operative risk of partial hepatectomy in a non-cirrhotic liver is extremely low,[92, 93] third, widespread success with RLT,[25, 83-85, 94] and fourth, the successful application of SLT.[95]

3.4.1 LDLT in pediatrics

LDLT was first introduced in pediatric population. In 1988, Raia in Brazil reported the first LDLT, establishing the technical feasibility of this procedure, yet both pediatric recipients died of complications.[96] Strong and colleagues subsequently reported the first successful pediatric LDLT using a left lobe graft from the child's mother.[25] Broelsch reported the first

successful series of LDLT with an overall graft survival of 75% and patient survival of 85%.[97] Furthermore, he was the first to report a prospective ethical analysis of this radical surgical innovation prior to performing their first LDLT.[98]

LDLT in children involves the removal of an adult donor left lateral segment (segment 2 and 3). Monosegment transplantation (segment 3) was introduced in Japan to solve the problem of "Large for size" grafts in small children.[99] The donor operation has been associated with a low and acceptable risk for complications. The donors being related to the recipients (parents), the risk for the donor is balanced by the great benefit to be received by the transplant recipient, as well as the donor's psychological benefit.

LDLT was initially restricted to children with chronic disease, in relatively stable condition, in order to avoid a major psychological pressure on the potential donor.[98] With larger experience, it was extended to emergency cases such as fulminant hepatic failure. Auxiliary transplantation, initially developed in this indication,[78] and in metabolic disorders,[100] could also be performed with a living donor liver.[101-104]

The continued shortage of cadaver livers in the face of growing list of recipients plus the advantages of LDLT have led to the introduction of LDLT in adults.

3.4.2 Adult-to-adult LDLT

The expansion of LDLT to the adult population began in the countries where the availability of deceased donors was scarce, and in some cases, totally unavailable.[105-107] The law for deceased organ retrieval was instituted in Japan in 1998, however, the lack of societal acceptance of organ retrieval from brain dead donors resulted in live donation being the main source of grafts for patients awaiting transplantation in Japan and other countries in Asia.[108]

On November 2, 1993, the Shinshu group performed the first successful adult-to-adult LDLT.[28] By June 2002, there were 433 adult LDLT cases recorded in European Liver Transplantation Regestery,[109] with 3 years graft and patient survival rate of 65% and 68% respectively. According to the United Network for Organ Sharing (UNOS), 731 adult LDLT cases have been performed in the United States by October 2001. The 3 years graft survival was 47% between 1998 and 1999 (n=156) but it improved significantly to 61% between July 1999 and June 2001 (n=285).[110] According to the Japanese Liver Transplantation Society, 1063 adult LDLT procedures were performed in Japan by the end of 2002. The 5 years survival rates were 83% in children and 69% in adults.[111] The lesser outcome in adults compared to that in children indicates that problems remain in adult LDLT.

In LDLT, donor safety must be assured. This may be achieved by optimizing graft size to ensure safety of both donor and recipient, technical expertise in liver procurement from the donor as well as ethical problems of using non-related live donors. As regarding the optimum size of the graft, it was found that, a graft volume of >40% of the recipient standard liver volume is necessary,[112] while for the living donor the remnant liver mass must be more than 30% of the whole liver.[113] The term "standard liver volume" has become a key concept in LDLT and it has been estimated using the following formula:[114]

$$\text{Standard liver volume (SV) in ml} = 706.2 \times (\text{body surface area } [m^2]) + 2.4.$$

In order to obtain the optimum graft size in adult-to-adult living donor transplantation, many graft types has been introduced. The strategy of selection of left or right liver graft is influenced by the patient's preoperative condition as patient with advanced liver disease require a larger liver mass.[115] The model for end-stage liver disease (MELD) score could become a satisfactory criterion for differentiating between high and low-risk patients and therefore determine the type of graft to use.[116] In the initial adult LDLT procedures only a left liver graft was used. In 1998, the Shinshu group reported satisfactory results using a left liver graft in 13 patients.[107] To cover wide range of recipient body weight, the right lobe graft was introduced in 1998 in Kyoto university.[117] In the same year, the University of Colorado group also introduced the right liver graft in adult LDLT,[118] the group performed 80 adult LDLT. In the first 10 cases, the right lobe graft was procured without the middle hepatic vein (MHV), 3 grafts were lost. As a result, the group included the MHV in the right lobe graft in the subsequent 70 cases. No graft loss was experienced.[119] The reason may be due to the prevention of congestion of the anterior segment of the right lobe which is drained by the MHV. However, the right lobe graft including the MHV was first introduced by the Hong Kong group in 1996.[106] In this situation, the volume of the remnant liver should be at least greater than 30% and the anatomy of vein 4 must be precisely evaluated before this procedure is accepted. However, the outcome of initial 8 donors and recipients were not without complications, one recipient died and the recipients as well as the donors experienced high morbidity.[106] The next 92 patients subsequently received extended right liver grafts (right lobe graft including the MHV) with the following innovations: elimination of venovenous bypass from the routine protocol, preservation of segment 4 venous drainage for donors, venoplasty of MHV and right hepatic vein (RHV) into a single orifice for better venous return and easy vein reconstruction in recipients and preservation of the blood supply to the right hepatic ducts. Over time the mortality rate of recipient decrease from 16% in the initial 50 cases to 0% in more recent patients.[120]

Lee, aggressively reconstructed the MHV tributaries in right liver grafts without the MHV trunk and named this type of graft a modified right lobe graft.[121] Ghobrial, also recommended reconstruction of the MHV tributary veins when the RHV in the graft was <1.5 cm in diameter.[90] All MHV tributaries with a size >5 mm should be preserved during donor hepatectomy and reconstructed with autogenous interposition vein grafts.[122]

Right hepatectomy imposes an increased surgical risk on the donor due to the reduced residual liver volume. A recent report indicated that in 25% of potential donors, the right liver had an estimated volume of >70% of the whole.[123] Since safe donation was possible only when estimated residual liver volume was >30%, right hepatectomy is not possible for some potential donors. The University of Tokyo group was the first to design the right lateral sector graft consisting of segment 6 and 7 in those donors with right livers over 70% of liver volume and the estimated volume of the right lateral segments is greater than that of the left liver and at the same time >40% of the recipient's standard liver volume.[124] Between January 2000 and April 2001, 6 of 32 adult-to-adult LDLT with a right lateral sector graft were performed. The postoperative course was uneventful in all donors and all recipients survived the operation.[125]

Lee et al, were the first to devise dual grafts from 2 living donors.[126] Most commonly, both donors donate the left liver or left lateral segment. The first left liver graft is orthotopically implanted in the original left position, the second left liver graft is rotated 180

degrees and positioned heterotopically in the right upper quadrant fossa. Because the bile duct is now located behind the portal vein and hepatic artery, bile duct reconstruction is necessary before reconstruction of vessels. An interposition vein graft might be necessary for the reconstruction of the hepatic or portal vein. By the end of 2003, this technique was used in 93 patients with satisfactory results. However the procedure has limited appeal due to the high requirements of economic and medical resources including 3 operating rooms and 3 surgical teams working simultaneously.[127]

4. Hepatocyte and stem cells transplantation

Additional approaches, as therapeutic alternative in attempt to reduce the significant mortality in the waiting list for liver transplantation is hepatocyte transplantation. A number of experiments have shown the feasibility of total liver parenchymal cell replacement by transplanted hepatocytes.[128-132] Hepatocyte transplantation might be able to bridge a period needed for regeneration of the acute liver failure patient's own liver or stretch the waiting time for a suitable liver donation. Although the first animal experiments with this technique began in 1967 [133], it was first applied in humans only in 1992.[134] Isolated Hepatocyte transplantation has long been recognized as a potential treatment for life-threatening liver disease. The basis for proceeding with clinical trials has been provided by extensive laboratory work in animal models.[135-140] The most important advantage of this treatment compared to liver transplantation, is its simplicity, since no surgery is required for cell implantation. The cell transplantation has been used for, temporary metabolic support of patients in end-stage liver failure awaiting whole-organ transplantation, as method to support liver function and facilitate the regeneration of the native liver in cases of fulminant hepatic failure, and in a manner similar to gene therapy as a form of "cellular therapy" for patients with genetic defects in vital liver functions. The patients can be treated by the infusion of 10^7-10^{10} allogenic hepatocytes, obtained from adult cadaveric livers, into the splenic artery or portal vein.[141] The main obstacle to wider usage of hepatocyte transplantation is the rapid elimination of the transplanted hepatocytes by recipient macrophages.[142]

Alternatives to the transplantation of allogenic human hepatocytes include the transplantation of hepatocytes derived from fetal, adult, or embryonic stem cells, engineered immortalized cells, or hepatocytes derived from other animal species.[143] Stem cells are one of the best approaches to obtaining cell stores. This approach can be used for clinical treatment by selecting small cell population that could effectively repopulate the host liver.[144]

5. References

[1] Calne R. The history and development of organ transplantation: biology and rejection. Bailliere's clinical gastroenterology. 1994 Sep;8(3):389-97.
[2] Hansen TH, Carreno BM, Sachs DH. The major histocompatibility complex. In: Paul WE, ed. *Fundamental immunology*. New York: Raven Press 1993:577-628.
[3] Medawar P. The behavior and fate of skin autografts and skin homografts in rabbits. J Anat. 1944;78:176.
[4] Gowen JW. Inheritance of immunity in animals. Ann Rev Microbial. 1948;2:215.
[5] Billingham RE, Krohn PL, Medawar PB. Effect of cortisone on survival of skin homografts in rabbits. Br Med J. 1951 May 26;4716:1157-63.

[6] Schwartz R, Stack J, Dameshek W. Effect of 6-mercaptopurine on antibody production. Proc Soc Exp Biol Med. 1958 Oct;99(1):164-7.
[7] Calne RY, Murray JE. Inhibition of the rejection of renal homografts in dogs by Burroughs Wellcome 57-322. Surg Forum. 1961;12:118-20.
[8] Carrel A. La technique opertoire des anastomoses vasculaires et la transplantation des visceres. Lyon Med. 1902;98:859.
[9] Murray JE, Merrill JP, et al. Renal homotransplantation in identical twins. Surg Forum. 1955;6:432.
[10] Woodruff MF, Anderson NA. Effect of Lymphocyte Depletion by Thoracic Duct Fistula and Administration of Antilymphocytic Serum on the Survival of Skin Homografts in Rats. Nature. 1963 Nov 16;200:702.
[11] Welch CS. A note on transplantation of the whole liver in dogs. Transplant Bull. 1955;2:54.
[12] Moore FD, Wheele HB, Demissianos HV, Smith LL, Balankura O, Abel K, et al. Experimental whole-organ transplantation of the liver and of the spleen. Ann Surg. 1960 Sep;152:374-87.
[13] Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the Liver in Humans. Surgery, gynecology & obstetrics. 1963 Dec;117:659-76.
[14] Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, et al. Orthotopic homotransplantation of the human liver. Ann Surg. 1968 Sep;168(3):392-415.
[15] Starzl TE, Marchioro TL, Porter KA, Iwasaki Y, Cerilli GJ. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation. Surgery, gynecology & obstetrics. 1967 Feb;124(2):301-8.
[16] Taniguchi S, Cooper DKC (1997). Clinical xenotransplantation: a brief review of the world experience. In: Cooper DKC, Kemp E, Platt JL, White DJD (eds) Xenotransplantation: the transplantation of organs between species. Springer, Berlin Heidelberg New York, p779.
[17] Calne RY, White DJ, Rolles K, Smith DP, Herbertson BM. Prolonged survival of pig orthotopic heart grafts treated with cyclosporin A. Lancet. 1978 Jun 3;1(8075):1183-5.
[18] Eghtesad B, Kadry Z, Fung J. Technical considerations in liver transplantation: what a hepatologist needs to know (and every surgeon should practice). Liver Transpl. 2005 Aug;11(8):861-71.
[19] Fortner JG, Kinne DW, Shiu MH, Howland WS, Kim DK, Castro EB, et al. Clinical liver heterotopic (auxiliary) transplantation. Surgery. 1973 Nov;74(5):739-51.
[20] Shaw BW, Jr., Martin DJ, Marquez JM, Kang YG, Bugbee AC, Jr., Iwatsuki S, et al. Venous bypass in clinical liver transplantation. Ann Surg. 1984 Oct;200(4):524-34.
[21] Broelsch CE, Emond JC, Whitington PF, Thistlethwaite JR, Baker AL, Lichtor JL. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg. 1990 Sep;212(3):368-75; discussion 75-7.
[22] Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery. 1984 Mar;95(3):367-70.
[23] Pichlmayr R, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. [Transplantation of a donor liver to 2 recipients (splitting transplantation)--a new method in the further development of segmental liver transplantation]. Langenbecks Arch Chir. 1988;373(2):127-30.

[24] Tzakis A, Todo S, Starzl TE. Orthotopic liver transplantation with preservation of the inferior vena cava. Ann Surg. 1989 Nov;210(5):649-52.
[25] Strong RW, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. N Engl J Med. 1990 May 24;322(21):1505-7.
[26] Holmgren G, Steen L, Ekstedt J, Groth CG, Ericzon BG, Eriksson S, et al. Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet. 1991 Sep;40(3):242-6.
[27] Belghiti J, Panis Y, Sauvanet A, Gayet B, Fekete F. A new technique of side to side caval anastomosis during orthotopic hepatic transplantation without inferior vena caval occlusion. Surgery, gynecology & obstetrics. 1992 Sep;175(3):270-2.
[28] Hashikura Y, Makuuchi M, Kawasaki S, Matsunami H, Ikegami T, Nakazawa Y, et al. Successful living-related partial liver transplantation to an adult patient. Lancet. 1994 May 14;343(8907):1233-4.
[29] Yamaoka Y, Washida M, Honda K, Tanaka K, Mori K, Shimahara Y, et al. Liver transplantation using a right lobe graft from a living related donor. Transplantation. 1994 Apr 15;57(7):1127-30.
[30] Lo CM, Fan ST, Liu CL, Lo RJ, Lau GK, Wei WI, et al. Extending the limit on the size of adult recipient in living donor liver transplantation using extended right lobe graft. Transplantation. 1997 May 27;63(10):1524-8.
[31] Tzakis AG, Kirkegaard P, Pinna AD, Jovine E, Misiakos EP, Maziotti A, et al. Liver transplantation with cavoportal hemitransposition in the presence of diffuse portal vein thrombosis. Transplantation. 1998 Mar 15;65(5):619-24.
[32] Cherqui D, Soubrane O, Husson E, Barshasz E, Vignaux O, Ghimouz M, et al. Laparoscopic living donor hepatectomy for liver transplantation in children. Lancet. 2002 Feb 2;359(9304):392-6.
[33] Borel JF, Baumann G, Chapman I, Donatsch P, Fahr A, Mueller EA, et al. In vivo pharmacological effects of ciclosporin and some analogues. Adv Pharmacol. 1996;35:115-246.
[34] National Institutes of Health Consensus Development Conference Statement: liver transplantation--June 20-23, 1983. Hepatology (Baltimore, Md. 1984 Jan-Feb;4(1 Suppl):107S-10S.
[35] Scharschmidt BF. Human liver transplantation: analysis of data on 540 patients from four centers. Hepatology (Baltimore, Md. 1984 Jan-Feb;4(1 Suppl):95S-101S.
[36] Iwatsuki S, Starzl TE, Todo S, Gordon RD, Esquivel CO, Tzakis AG, et al. Experience in 1,000 liver transplants under cyclosporine-steroid therapy: a survival report. Transplant Proc. 1988 Feb;20(1 Suppl 1):498-504.
[37] Cosimi AB, Colvin RB, Burton RC, Rubin RH, Goldstein G, Kung PC, et al. Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. N Engl J Med. 1981 Aug 6;305(6):308-14.
[38] Barry JM. Immunosuppressive drugs in renal transplantation. A review of the regimens. Drugs. 1992 Oct;44(4):554-66.
[39] Strepkowski SM. Sirolimus, a potent new immunosuppressive drug for organ transplantation. Ann Transplant. 1996;1 (3):19-25.
[40] Klagehpour K, Pyronnet S, Gingras AC, al e. Translational homeostasis: eukaryotic translation initiation factor 4E control of 4E-binding protien 1 and p70 s6 kinase activities. Mol Cell Biol. 1999;19:4302-20.

[41] Takada Y, Ueda M, Ishikawa Y, Fujimoto Y, Miyauchi H, Ogura Y, et al. End-to-side portocaval shunting for a small-for-size graft in living donor liver transplantation. Liver Transpl. 2004 Jun;10(6):807-10.
[42] Modan B, Shpilberg O, Baruch Y, Sikuler E, Anis E, Ashur Y, et al. The need for liver transplantation: a nationwide estimate based on consensus review. Lancet. 1995 Sep 9;346(8976):660-2.
[43] Couinaud C. Le foie. Etudes anatomiques et chirurgicales. Paris: Masson. 1957.
[44] Smith B. Segmental liver transplantation from a living donor. J Pediatr Surg. 1969 Feb;4(1):126-32.
[45] Dagradi A, Marzoli GP, Radin S, Sussi PL, Dagradi V, Zannini M, et al. [Possibilities of sectional liver transplantation in man]. Langenbecks Arch Chir. 1968;322:533-7.
[46] Marchioro TL, Porter KA, Dickinson TC, Faris TD, Starzi TE. Physiologic Requirements for Auxiliary Liver Homotransplantation. Surgery, gynecology & obstetrics. 1965 Jul;121:17-31.
[47] Nagashima I, Bergmann L, Schweizer R. How can we share the portal blood inflow in auxiliary partial heterotopic liver transplantation without portal hypertension? Surgery. 1994 Jul;116(1):101-6.
[48] Zhao D, Zimmermann A, Kuznetsova LV, Wheatley AM. Regression of bile duct damage and bile duct proliferation in the non-rearterialized transplanted rat liver is associated with spontaneous graft rearterialization. Hepatology (Baltimore, Md. 1995 May;21(5):1353-60.
[49] Svensson G, Naredi P, Hafstrom L, Tufveson G. Quantitative measurements of collateral arterial blood flow in nonarterialized rat liver grafts. Transpl Int. 1994;7(2):136-9.
[50] Howden B, Jablonski P, Grossman H, Marshall VC. The importance of the hepatic artery in rat liver transplantation. Transplantation. 1989 Mar;47(3):428-31.
[51] Sumimoto R, Shinomiya T, Yamaguchi A. Influence of hepatic arterial blood flow in rats with liver transplants. Examination of donor liver-derived serum class I MHC antigen in rats with liver transplants with or without hepatic arterial reconstruction. Transplantation. 1991 May;51(5):1138-9.
[52] Post S, Menger MD, Rentsch M, Gonzalez AP, Herfarth C, Messmer K. The impact of arterialization on hepatic microcirculation and leukocyte accumulation after liver transplantation in the rat. Transplantation. 1992 Nov;54(5):789-94.
[53] Taub R. Liver regeneration 4: transcriptional control of liver regeneration. Faseb J. 1996 Mar;10(4):413-27.
[54] Gentile JM, Grace JT, Jr. A cell growth stimulating factor in partially hepatectomized rat serum. Surgical forum. 1968;19:62-3.
[55] Morley CG, Kingdon HS. The regulation of cell growth. I. Identification and partial characterization of a DNA synthesis stimulating factor from the serum of partially hepatectomized rats. Biochimica et biophysica acta. 1973 May 10;308(2):260-75.
[56] Michalopoulos GK, DeFrances MC. Liver regeneration. Science (New York, NY. 1997 Apr 4;276(5309):60-6.
[57] LaBrecque D. Liver regeneration: a picture emerges from the puzzle. The American journal of gastroenterology. 1994 Aug;89(8 Suppl):S86-96.
[58] Moolten FL, Oakman NJ, Bucher NL. Accelerated response of hepatic DNA synthesis to partial hepatectomy in rats pretreated with growth hormone or surgical stress. Cancer research. 1970 Sep;30(9):2353-7.

[59] Zhao D, Zimmermann A, Wheatley AM. Morphometry of the liver after liver transplantation in the rat: significance of an intact arterial supply. Hepatology (Baltimore, Md. 1993 Feb;17(2):310-7.
[60] Burdelski M, Rogiers X. Liver transplantation in metabolic disorders. Acta gastro-enterologica Belgica. 1999 Jul-Sep;62(3):300-5.
[61] Kren BT, Trembley JH, Fan G, Steer CJ. Molecular regulation of liver regeneration. Annals of the New York Academy of Sciences. 1997 Dec 31;831:361-81.
[62] Morsiani E, Aleotti A, Ricci D. Haemodynamic and ultrastructural observations on the rat liver after two-thirds partial hepatectomy. Journal of anatomy. 1998 May;192 (Pt 4):507-15.
[63] Zhang XK, Gauthier T, Burczynski FJ, Wang GQ, Gong YW, Minuk GY. Changes in liver membrane potentials after partial hepatectomy in rats. Hepatology (Baltimore, Md. 1996 Mar;23(3):549-51.
[64] Neuschwander-Tetri BA, Nicholson C, Wells LD, Tracy TF, Jr. Cholestatic liver injury down-regulates hepatic glutathione synthesis. The Journal of surgical research. 1996 Jul 1;63(2):447-51.
[65] Chandler JG, Lee S, Krubel R, Rosen H, Nakaji NT, Orloff MJ. The inter-liver competition and portal blood in regeneration of auxiliary liver transplants. Surgical forum. 1971;22:341-3.
[66] Shaw BW, Jr. Auxiliary liver transplantation for acute liver failure. Liver Transpl Surg. 1995 May;1(3):194-200.
[67] Wondergem R, Potter VR. A new protocol for studying the early events during liver regeneration. Life sciences. 1978 Oct 16;23(15):1565-75.
[68] Schenk DB, Hubert JJ, Leffert HL. Use of a monoclonal antibody to quantify (Na+,K+)-ATPase activity and sites in normal and regenerating rat liver. The Journal of biological chemistry. 1984 Dec 10;259(23):14941-51.
[69] Moule SK, McGivan JD. Regulation of the plasma membrane potential in hepatocytes--mechanism and physiological significance. Biochimica et biophysica acta. 1990 Oct 8;1031(3):383-97.
[70] Kelm M, Feelisch M, Deussen A, Strauer BE, Schrader J. Release of endothelium derived nitric oxide in relation to pressure and flow. Cardiovascular research. 1991 Oct;25(10):831-6.
[71] Feelisch M, Ostrowski J, Noack E. On the mechanism of NO release from sydnonimines. Journal of cardiovascular pharmacology. 1989;14 Suppl 11:S13-22.
[72] Schoen JM, Wang HH, Minuk GY, Lautt WW. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. Nitric Oxide. 2001;5(5):453-64.
[73] Lambotte L, Saliez A, Triest S, Tagliaferri EM, Barker AP, Baranski AG. Control of rate and extent of the proliferative response after partial hepatectomy. The American journal of physiology. 1997 Oct;273(4 Pt 1):G905-12.
[74] Wolf HK, Michalopoulos GK. Hepatocyte regeneration in acute fulminant and nonfulminant hepatitis: a study of proliferating cell nuclear antigen expression. Hepatology (Baltimore, Md. 1992 Apr;15(4):707-13.
[75] Moritz MJ, Jarrell BE, Munoz SJ, Maddrey WC. Regeneration of the native liver after heterotopic liver transplantation for fulminant hepatic failure. Transplantation. 1993 Apr;55(4):952-4.
[76] Moritz MJ, Jarrell BE, Armenti V, Radomski J, Carabasi RA, Zeitoun G, et al. Heterotopic liver transplantation for fulminant hepatic failure--a bridge to recovery. Transplantation. 1990 Sep;50(3):524-6.

[77] Gubernatis G, Pichlmayr R, Kemnitz J, Gratz K. Auxiliary partial orthotopic liver transplantation (APOLT) for fulminant hepatic failure: first successful case report. World J Surg. 1991 Sep-Oct;15(5):660-5; discussion 5-6.
[78] Boudjema K, Jaeck D, Simeoni U, Bientz J, Chenard MP, Brunot P. Temporary auxiliary liver transplantation for subacute liver failure in a child. Lancet. 1993 Sep 25;342(8874):778-9.
[79] Durand F, Belghiti J, Handra-Luca A, Francoz C, Sauvanet A, Marcellin P, et al. Auxiliary liver transplantation for fulminant hepatitis B: results from a series of six patients with special emphasis on regeneration and recurrence of hepatitis B. Liver Transpl. 2002 Aug;8(8):701-7.
[80] Belghiti J, Sommacale D, Dondero F, Zinzindohoue F, Sauvanet A, Durand F. Auxiliary liver transplantation for acute liver failure. HPB (Oxford). 2004;6(2):83-7.
[81] Chenard-Neu MP, Boudjema K, Bernuau J, Degott C, Belghiti J, Cherqui D, et al. Auxiliary liver transplantation: regeneration of the native liver and outcome in 30 patients with fulminant hepatic failure--a multicenter European study. Hepatology (Baltimore, Md. 1996 May;23(5):1119-27.
[82] Nakazawa H, Genka C, Fujishima M. Pathological aspects of active oxygens/free radicals. The Japanese journal of physiology. 1996 Feb;46(1):15-32.
[83] Emond JC, Whitington PF, Thistlethwaite JR, Alonso EM, Broelsch CE. Reduced-size orthotopic liver transplantation: use in the management of children with chronic liver disease. Hepatology (Baltimore, Md. 1989 Nov;10(5):867-72.
[84] Ryckman FC, Flake AW, Fisher RA, Tchervenkov JI, Pedersen SH, Balistreri WF. Segmental orthotopic hepatic transplantation as a means to improve patient survival and diminish waiting-list mortality. J Pediatr Surg. 1991 Apr;26(4):422-7; discussion 7-8.
[85] Otte JB, de Ville de Goyet J, Sokal E, Alberti D, Moulin D, de Hemptinne B, et al. Size reduction of the donor liver is a safe way to alleviate the shortage of size-matched organs in pediatric liver transplantation. Ann Surg. 1990 Feb;211(2):146-57.
[86] Houssin D, Soubrane O, Boillot O, Dousset B, Ozier Y, Devictor D, et al. Orthotopic liver transplantation with a reduced-size graft: an ideal compromise in pediatrics? Surgery. 1992 May;111(5):532-42.
[87] Bismuth H, Morino M, Castaing D, Gillon MC, Descorps Declere A, Saliba F, et al. Emergency orthotopic liver transplantation in two patients using one donor liver. Br J Surg. 1989 Jul;76(7):722-4.
[88] Rogiers X, Malago M, Gawad K, Jauch KW, Olausson M, Knoefel WT, et al. In situ splitting of cadaveric livers. The ultimate expansion of a limited donor pool. Ann Surg. 1996 Sep;224(3):331-9; discussion 9-41.
[89] Busuttil RW, Goss JA. Split liver transplantation. Ann Surg. 1999 Mar;229(3):313-21.
[90] Goss JA, Yersiz H, Shackleton CR, Seu P, Smith CV, Markowitz JS, et al. In situ splitting of the cadaveric liver for transplantation. Transplantation. 1997 Sep 27;64(6):871-7.
[91] Bismuth H, Castaing D, Garden OJ. Segmental surgery of the liver. Surg Annu. 1988;20:291-310.
[92] Iwatsuki S, Todo S, Starzl TE. Excisional therapy for benign hepatic lesions. Surgery, gynecology & obstetrics. 1990 Sep;171(3):240-6.
[93] Bismuth H, Houssin D, Michel F. [Operative risk in hepatectomies. Experience with 154 hepatectomies]. Chirurgie. 1983;109(4):342-8.
[94] Kalayouglu M, D' Alessandro AM, Sollinger HW, et al. Experience with reduced-size liver transplantation. Surgery, gynecology & obstetrics. 1990;171:139-47.

[95] Emond JC, Whitington PF, Thistlethwaite JR, Cherqui D, Alonso EA, Woodle IS, et al. Transplantation of two patients with one liver. Analysis of a preliminary experience with 'split-liver' grafting. Ann Surg. 1990 Jul;212(1):14-22.
[96] Raia S, Nery JR, Mies S. Liver transplantation from live donors. Lancet. 1989 Aug 26;2(8661):497.
[97] Broelsch CE, Whitington PF, Emond JC, Heffron TG, Thistlethwaite JR, Stevens L, et al. Liver transplantation in children from living related donors. Surgical techniques and results. Ann Surg. 1991 Oct;214(4):428-37; discussion 37-9.
[98] Singer PA, Siegler M, Whitington PF, Lantos JD, Emond JC, Thistlethwaite JR, et al. Ethics of liver transplantation with living donors. N Engl J Med. 1989 Aug 31;321(9):620-2.
[99] Kasahara M, Kiuchi T, Haga H, Uemoto S, Uryuhara K, Fujimoto Y, et al. Monosegmental living-donor liver transplantation for infantile hepatic hemangioendothelioma. J Pediatr Surg. 2003 Jul;38(7):1108-11.
[100] Whitington PF, Emond JC, Heffron T, Thistlethwaite JR. Orthotopic auxiliary liver transplantation for Crigler-Najjar syndrome type 1. Lancet. 1993 Sep 25;342(8874):779-80.
[101] Emond JC, Heffron TG, Kortz EO, Gonzalez-Vallina R, Contis JC, Black DD, et al. Improved results of living-related liver transplantation with routine application in a pediatric program. Transplantation. 1993 Apr;55(4):835-40.
[102] Inomata Y, Kiuchi T, Kim I, Uemoto S, Egawa H, Asonuma K, et al. Auxiliary partial orthotopic living donor liver transplantation as an aid for small-for-size grafts in larger recipients. Transplantation. 1999 May 27;67(10):1314-9.
[103] Yazaki M, Ikeda S, Takei Y, Yanagisawa N, Matsunami H, Hashikura Y, et al. Complete neurological recovery of an adult patient with type II citrullinemia after living related partial liver transplantation. Transplantation. 1996 Dec 15;62(11):1679-84.
[104] Kasahara M, Kiuchi T, Uryuhara K, Ogura Y, Takakura K, Egawa H, et al. Treatment of ornithine transcarbamylase deficiency in girls by auxiliary liver transplantation: conceptual changes in a living-donor program. J Pediatr Surg. 1998 Dec;33(12):1753-6.
[105] Lo CM, Lo RJ, Lai CL, Fan ST, Wong J. Liver transplantation from cadaveric and living donors. Transplant Proc. 1996 Jun;28(3):1659-60.
[106] Lo CM, Fan ST, Liu CL, Wei WI, Lo RJ, Lai CL, et al. Adult-to-adult living donor liver transplantation using extended right lobe grafts. Ann Surg. 1997 Sep;226(3):261-9; discussion 9-70.
[107] Kawasaki S, Makuuchi M, Matsunami H, Hashikura Y, Ikegami T, Nakazawa Y, et al. Living related liver transplantation in adults. Ann Surg. 1998 Feb;227(2):269-74.
[108] Tanaka K, Kobayashi Y, Kiuchi T. Current status of living donor liver transplantation in adults. Curr Opin Organ Transpl. 2000;5:74-80.
[109] Adam R, McMaster P, O'Grady JG, Castaing D, Klempnauer JL, Jamieson N, et al. Evolution of liver transplantation in Europe: report of the European Liver Transplant Registry. Liver Transpl. 2003 Dec;9(12):1231-43.
[110] Data from US transplant organization. Available at: http:// www.ustransplant.org/index.php. Accessed on March 27. 2004.
[111] The Japanese Liver Transplantation Society. Liver Transplantation in Japan. Registery by the Japanese Liver Transplantation Society [Japanese]. Jpn J Transpalnt. 2004;38:401-8.

[112] Lo CM, Fan ST, Liu CL, Chan JK, Lam BK, Lau GK, et al. Minimum graft size for successful living donor liver transplantation. Transplantation. 1999 Oct 27;68(8):1112-6.
[113] Fan ST, Lo CM, Liu CL, Yong BH, Chan JK, Ng IO. Safety of donors in live donor liver transplantation using right lobe grafts. Arch Surg. 2000 Mar;135(3):336-40.
[114] Urata K, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, et al. Calculation of child and adult standard liver volume for liver transplantation. Hepatology. 1995 May;21(5):1317-21.
[115] Sugawara Y, Makuuchi M, Kaneko J, Kokudo N. MELD score for selection of patients to receive a left liver graft. Transplantation. 2003 Feb 27;75(4):573-4.
[116] Wiesner RH, McDiarmid SV, Kamath PS, Edwards EB, Malinchoc M, Kremers WK, et al. MELD and PELD: application of survival models to liver allocation. Liver Transpl. 2001 Jul;7(7):567-80.
[117] Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. Transplantation. 2000 Jan 27;69(2):258-64.
[118] Wachs ME, Bak TE, Karrer FM, Everson GT, Shrestha R, Trouillot TE, et al. Adult living donor liver transplantation using a right hepatic lobe. Transplantation. 1998 Nov 27;66(10):1313-6.
[119] Bak T, Wachs M, Trotter J, Everson G, Trouillot T, Kugelmas M, et al. Adult-to-adult living donor liver transplantation using right-lobe grafts: results and lessons learned from a single-center experience. Liver Transpl. 2001 Aug;7(8):680-6.
[120] Lo CM, Fan ST, Liu CL, Lai CL, Wong J. Prophylaxis and treatment of recurrent hepatitis B after liver transplantation. Transplantation. 2003 Feb 15;75(3 Suppl):S41-4.
[121] Lee S, Park K, Hwang S, Lee Y, Choi D, Kim K, et al. Congestion of right liver graft in living donor liver transplantation. Transplantation. 2001 Mar 27;71(6):812-4.
[122] Gyu Lee S, Min Park K, Hwang S, Hun Kim K, Nak Choi D, Hyung Joo S, et al. Modified right liver graft from a living donor to prevent congestion. Transplantation. 2002 Jul 15;74(1):54-9.
[123] Leelaudomlipi S, Sugawara Y, Kaneko J, Matsui Y, Ohkubo T, Makuuchi M. Volumetric analysis of liver segments in 155 living donors. Liver Transpl. 2002 Jul;8(7):612-4.
[124] Sugawara Y, Makuuchi M, Takayama T, Mizuta K, Kawarasaki H, Imamura H, et al. Liver transplantation using a right lateral sector graft from a living donor to her granddaughter. Hepatogastroenterology. 2001 Jan-Feb;48(37):261-3.
[125] Sugawara Y, Makuuchi M, Takayama T, Imamura H, Kaneko J. Right lateral sector graft in adult living-related liver transplantation. Transplantation. 2002 Jan 15;73(1):111-4.
[126] Lee S, Hwang S, Park K, Lee Y, Choi D, Ahn C, et al. An adult-to-adult living donor liver transplant using dual left lobe grafts. Surgery. 2001 May;129(5):647-50.
[127] Lee SG, Hwang S, Park KM, Kim KH, Ahn CS, Lee YJ, et al. Seventeen adult-to-adult living donor liver transplantations using dual grafts. Transplant Proc. 2001 Nov-Dec;33(7-8):3461-3.
[128] Horslen SP, Fox IJ. Hepatocyte transplantation. Transplantation. 2004 May 27;77(10):1481-6.
[129] Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis. 1999;19(1):39-48.

[130] Shafritz DA, Dabeva MD. Liver stem cells and model systems for liver repopulation. Journal of hepatology. 2002 Apr;36(4):552-64.
[131] Gupta S. Hepatocyte transplantation. J Gastroenterol Hepatol. 2002 Dec;17 Suppl 3:S287-93.
[132] Fox IJ, Roy-Chowdhury J. Hepatocyte transplantation. Journal of hepatology. 2004 Jun;40(6):878-86.
[133] Afzelius BA, Schoental R. The ultrastructure of the enlarged hepatocytes induced in rats with a single oral dose of retrorsine, a pyrrolizidine (Senecio) alkaloid. J Ultrastruct Res. 1967 Oct 31;20(5):328-45.
[134] Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc. 1992 Dec;24(6):3052-3.
[135] Arkadopoulos N, Chen SC, Khalili TM, Detry O, Hewitt WR, Lilja H, et al. Transplantation of hepatocytes for prevention of intracranial hypertension in pigs with ischemic liver failure. Cell Transplant. 1998 Jul-Aug;7(4):357-63.
[136] Nagata H, Ito M, Shirota C, Edge A, McCowan TC, Fox IJ. Route of hepatocyte delivery affects hepatocyte engraftment in the spleen. Transplantation. 2003 Aug 27;76(4):732-4.
[137] Mito M, Kusano M, Onishi T, Saito T, Ebata H. Hepatocellular transplantation -- morphological study on hepatocytes transplanted into rat spleen. Gastroenterol Jpn. 1978;13(6):480-90.
[138] Ito M, Nagata H, Yamamoto T, Yoshihara D, Fox IJ, Miyakawa S. Intrasplenic hepatocyte transplantation prolonged the survival in Nagase analbuminemic rats with liver failure induced by common bile duct ligation. Cell Transplant. 2007;16(5):547-53.
[139] Kobayashi N, Ito M, Nakamura J, Cai J, Hammel JM, Fox IJ. Treatment of carbon tetrachloride and phenobarbital-induced chronic liver failure with intrasplenic hepatocyte transplantation. Cell Transplant. 2000 Sep-Oct;9(5):671-3.
[140] Makowka L, Rotstein LE, Falk RE, Falk JA, Langer B, Nossal NA, et al. Reversal of toxic and anoxic induced hepatic failure by syngeneic, allogeneic, and xenogeneic hepatocyte transplantation. Surgery. 1980 Aug;88(2):244-53.
[141] Platt JL. New directions for organ transplantation. Nature. 1998 Apr 30;392(6679 Suppl):11-7.
[142] Nagata H, Ito M, Cai J, Edge AS, Platt JL, Fox IJ. Treatment of cirrhosis and liver failure in rats by hepatocyte xenotransplantation. Gastroenterology. 2003 Feb;124(2):422-31.
[143] Ramirez P, Chavez R, Majado M, Munitiz V, Munoz A, Hernandez Q, et al. Life-supporting human complement regulator decay accelerating factor transgenic pig liver xenograft maintains the metabolic function and coagulation in the nonhuman primate for up to 8 days. Transplantation. 2000 Oct 15;70(7):989-98.
[144] Zhang Y, Riesterer C, Ayrall AM, Sablitzky F, Littlewood TD, Reth M. Inducible site-directed recombination in mouse embryonic stem cells. Nucleic Acids Res. 1996 Feb 15;24(4):543-8.

Part 2

Ischemia/Reperfusion Injury

2

Ischemia-Reperfusion Injury Associated with Liver Transplantation in 2011: Past and Future

M. Elias-Miró[1], M.B. Jiménez-Castro[1] and C. Peralta[1,2]
[1]*Institut d'Investigacions Biomèdiques Agust Pi i Sunyer, Centro Esther Koplowitz;*
[2]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas*
Spain

1. Introduction

Liver transplantation has evolved as the therapy of choice for patients with end-stage liver disease. However, the waiting list for liver transplantation is growing at a fast pace, whereas the number of available organs is not growing at a proportional rate. The potential use of steatotic livers for transplant, one of the most common types of organs from marginal donors, has become a major focus of investigations. However the clinical problem is still unresolved since steatotic livers are more susceptible to ischemia-reperfusion (I/R) injury and, when used, have poorer outcome than non-steatotic livers. Indeed, the use of steatotic livers for transplantation is associated with increased risk of primary non-function or dysfunction after surgery. Therefore, minimizing the adverse effects of I/R injury could improve outcomes in steatotic liver surgery, increase the number both of suitable transplantation grafts and of patients who successfully recover from liver transplantation.

The present review focuses on the complexity of hepatic I/R injury, summarizing conflicting results obtained from the literature about the mechanisms responsible for it. We also review the therapeutic strategies designed in past years to reduce I/R injury, attempting to explain why most of them have not been applied clinically. Finally, we will consider new potential protective strategies that have shown promising results for I/R injury with the potential to increase the number of liver suitable for liver transplantation.

2. Hepatic ischemia-reperfusion injury associated with liver transplantation. An unresolved problem in clinical practice

Liver transplantation (LT) dates back to 1963, when Thomas Starzl carried out the first transplant on a child suffering from biliary atresia. LT has evolved as the therapy of choice for patients with end-stage liver disease. However, I/R injury, inherent in every LT, is the main cause of both initial poor function and primary non-function of liver allograft. The latter is responsible for 81% of re-transplantations during the first week after surgery (Clavien et al., 1992; Jaeschke, 1996). I/R injury is a phenomenon whereby cellular damage in a hypoxic organ is accentuated following the restoration of oxygen delivery (Jaeschke, 1998; Teoh et al., 2003; Jaeschke, 2003). In the liver, this form of injury was recognized as a

clinically important pathological disorder by Toledo-Pereyra et al. in 1975 during studies of experimental LT. However, it was not until the mid-1980s that the term reperfusion injury was generally used in the literature on LT (Teoh et al., 2003).

A variety of clinical factors including starvation, graft age, and steatosis contribute to enhance liver susceptibility to I/R injury, further increasing the patient risks related to reperfusion injury (Shah & Kamath, 2003). In clinical LT, starvation of the donor, due to prolonged intensive care unit hospitalization or lack of an adequate nutritional support, increases the incidence of hepatocellular injury and primary nonfunction (Massip-Salcedo et al., 2007).

The waiting list for LT is growing at a fast pace, whereas the number of available organs is not growing at a proportional rate. The shortage of organs has led centers to expand their criteria for the acceptance of marginal grafts, which show poor tolerance to I/R (Busuttil & Tanaka, 2003). Some of these include the use of organs from aged donors, non-heart-beating donors (NHBD), and grafts such as small-for-size or steatotic livers. However, I/R injury is the underpinning of graft dysfunction that is seen in the marginal organ (Busuttil & Tanaka, 2003). The fundamental problem with NHBD organs is the prolonged warm ischemia before cold preservation (Reddy et al., 2004). Controlled NHBDs provide organs that are far less prone to ischemic damage and tend to offer superior posttransplant function (Busuttil & Tanaka, 2003). The use of uncontrolled NHBDs is associated with a very high risk of primary nonfunction (Reddy et al., 2004).

One of the benefits of reduced-size grafts from living donors is a graft of good quality with a short ischemic time, this latter being possible because live donor procurements can be electively timed with recipient procedure (Farmer et al., 2001). On the other hand, the major concern over application of living-related liver transplantation for adults is graft-size disparity. The small graft needs regeneration to restore the liver/body ratio. It is well known that I/R significantly reduces liver regeneration after hepatectomy (Franco et al., 2004).

Donor age of more than 70 years was found to be associated with lower patient and graft survival (Busuttil & Tanaka, 2003, Casillas et al., 2006). Additionally these donors also have an increased incidence of steatosis, which may potentiate cold preservation injury (Busuttil & Tanaka, 2003). Steatotic livers are one of the most common types of organs from marginal donors. The present review will focus on this type of liver grafts. Among other factors, unhealthy lifestyles associated with the consumption of alcohol and inappropriate diets have increased the proportion of patients with steatotic livers.

Hepatic steatosis is a major risk factor for liver surgery and transplantation, and fatty livers are unsuitable for many reasons. Operative mortality associated with steatosis exceeds 14%, compared with 2% for healthy livers, and the risks of primary non-function and dysfunction after surgery are similarly higher (Casillas et al., 2006; Selzner et al., 2000). Thus, hepatic steatosis is the major cause of graft rejection after LT and exacerbates the organ shortage problem (Fernández et al., 2004). Therefore, minimizing the adverse effects of I/R injury could increase the number of both grafts suitable for transplantation and patients who successfully recover from LT. The first step towards achieving this objective is a full understanding of the mechanisms involved in I/R injury.

3. Complexity of hepatic ischemia-reperfusion injury

A large number of factors and mediators play a part in liver I/R injury (Banga et al., 2005; Casillas et al., 2006; Fan et al., 1999; Jaeschke, 2003; Lentsch et al., 2000). The relationships between the signalling pathways involved are highly complex and it is not yet possible to describe, with absolute certainty, the events that occur between the beginning of reperfusion and the final outcome of either poor function or a non-functional liver graft.

Figure 1 shows some of the mechanisms involved in the pathophysiology of I/R injury. Due to the complexity of hepatic I/R injury, the present review summarizes the established basic concepts of the mechanisms and cell types involved in this process. The lack of oxygen to hepatocytes during ischemia causes mitochondrial deenergization, ATP depletion, alterations of H+, Na+, Ca2+ homeostasis that activate hydrolytic enzymes and impair cell volume regulation and sinusoidal endothelial cells (SEC) as well as Kupffer cells (KC) swelling (Massip-Salcedo et al., 2007). This fact together with the imbalance between nitric oxide (NO) and endothelin (ET) production, contributes to narrowing of the sinusoidal lumen and thus to microcirculatory dysfunction. Capillary narrowing also contributes to hepatic neutrophil accumulation (Peralta et al., 1996; Peralta et al., 2000a). Concomitantly, the activation of KC releases reactive oxygen species (ROS) and proinflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) (Bilzer & Gerber, 2000; Lentsch et al., 2000). ROS can also derive from xanthine deshydrogenase/xanthine oxidase (XDH/XOD). Cytokines release throughout the induction of adhesion molecules (intercellular cell adhesion molecule [ICAM] and vascular cell adhesion molecule [VCAM]) and chemokines promote neutrophil activation and accumulation, thereby contributing to the progression of parenchymal injury by releasing ROS and proteases (Jaeschke, 1998, 2003; Lentsch et al., 2000). Besides, IL-1 and TNF-α recruit and activate CD4+ T-lymphocytes, which produce granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (INF-γ) and tumor necrosis factor beta (TNF-β). These cytokines amplify KC activation and TNF-α and IL-1 secretion and promote neutrophil recruitment and adherence into the liver sinusoids (Casillas et al., 2006; Selzner, 2003). Platelet activating factor (PAF) can prime neutrophils for superoxide generation, whereas leukotriene B4 (LTB4) contributes to the amplification of the neutrophil response (Jaeschke, 1998, 2003) (see Fig. 1).

The present review will present data from the literature about the possible sources of ROS, NO effects, mechanisms, and the role of some pro-inflammatory mediators such as TNF-α, and transcription factors, for example, nuclear factor kappa B (NFκB). These data will provide a better explanation on why hepatic I/R injury remains an unresolved problem in the clinical practice.

3.1 Mechanisms responsible for ROS production

The source of ROS in hepatic I/R has long been controversial. As regards the mechanisms responsible for ROS production, experiments with XDH/XOD inhibitors such as allopurinol suggest that this system is the main ROS generator in hepatocytes and it has also been implicated in LT-related lung damage (Casillas et al., 2006; Fernández et al., 2002). However, results obtained in experimental models of the isolated perfused liver have underestimated the importance of the XDH/XOD system, and suggest that mitochondria could be the main source of ROS (Jaeschke & Mitchell, 1989). On the other hand, some data challenge the

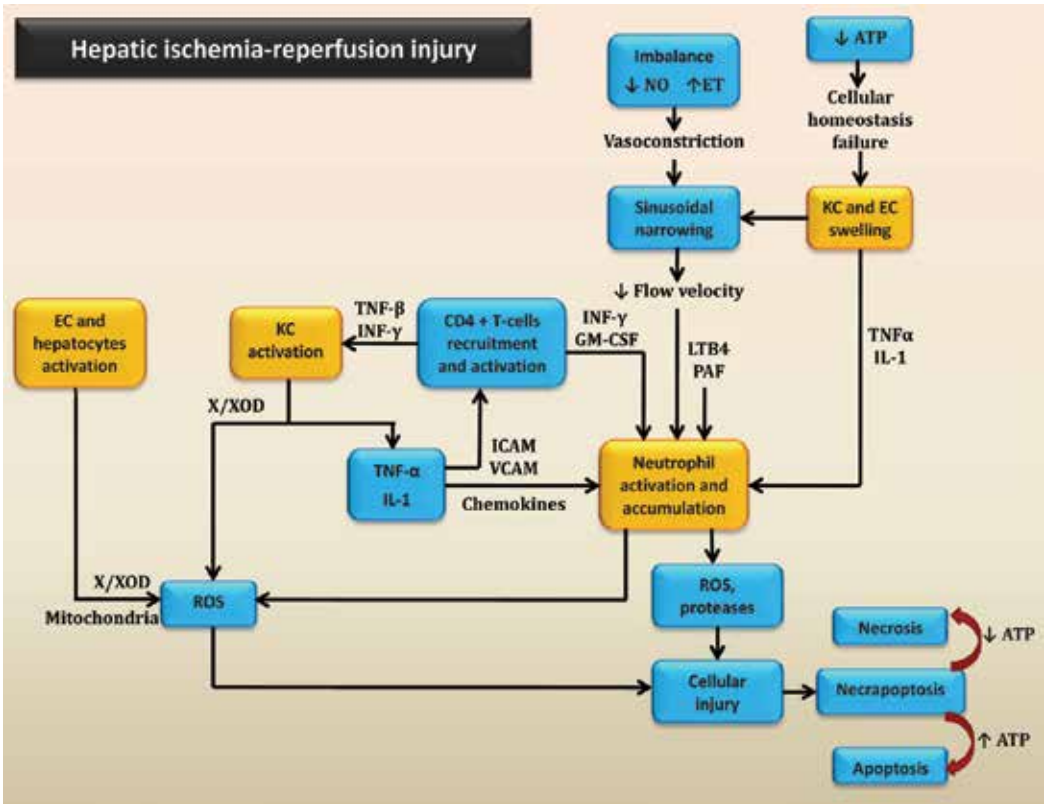


Fig. 1. Summary of the mechanisms involved in hepatic ischemia-reperfusion injury. (Bilzer & Gerber, 2000; Casillas et al., 2006; Jaeschke, 1998, 2003; Lentsch et al., 2000; Massip-Salcedo et al., 2007 ; Peralta et al., 1996, 2000a; Selzner, 2003)

pathophysiological relevance of intracellular oxidant stress during reperfusion (Grattagliano et al., 1999; Metzger et al., 1988). Grattagliano et al., 1999, demonstrated that mitochondria do not seem to actively participate in the reperfusion-induced oxidative stress. In addition, studies by Jaeschke et al. and Metzger et al. showed that the increased vascular oxidant stress after 30 and 60 min of ischemia was attenuated by inactivation of KC but not by high dose of allopurinol (Metzger et al., 1988). Interestingly, ROS release by KC occurs via the XDH/XOD system (Wiezorek et al., 1994). The conversion from XDH to XOD following cold storage is very slow in endothelial cells and hepatocytes, but much faster and higher in KC (Wiezorek et al., 1994). However, the KC function in I/R injury is still an area of active investigation. The elimination of KC did not modify the deleterious effects of I/R and the activation of neutrophils is not essential for reoxygenation injury (Imamura et al., 1995; Teoh et al., 2003). Clearly, then, there is a range of potentially conflicting results with regard to the mechanisms responsible for ROS generation in liver I/R injury. For instance, in our opinion, in order to clarify the importance of XDH/XOD versus mitochondria it should be taking into account that there are differences in the experimental models evaluated, including the times of ischemia. In this line, XDH/XOD play a crucial role in hepatic I/R injury only in conditions in which significant conversion of XDH to XOD occurs (80-90% of XOD) such as 16 h of cold ischemia. However, this ROS generation system does not appear to be crucial at

shorter ischemic periods such as 6 h of cold ischemia (Fernández et al., 2002). Thus, even after prolonged periods of ischemia, where a significant conversion of XDH to the XOD occurs, this enzyme may only play a minor role compared with mitochondria (Jaeschke & Mitchell, 1989). In contrast with the experimental studies, the clinical reports suggest that 45-65% XOD was sufficient to induce hepatic damage (Pesonen et al., 1998). In addition, the drugs used for inhibiting XDH/XOD should be considered, since, for example allopurinol, seems to have more than one mechanisms of action. It is not only a potent inhibitor of XOD, but it may also improve ischemia-induced mitochondrial dysfunction (Casillas et al., 2006; Jeon et al., 2001). In fact, evidence for reduced mitochondrial dysfunction after high doses of allopurinol was shown in a warm hepatic I/R model (Jeon et al., 2001). Similarly, in assessing the relative contribution of intracellular versus vascular oxidant stress to hepatic I/R injury, it should also be noted that oxidative stress in hepatocytes and the stimulatory state of KC after I/R depend on the duration of ischemia, and may also differ between ischemia at 4ºC and that at 37ºC, which probably leads to different developmental mechanisms of liver damage (Casillas et al., 2006). The differences in KC function in liver I/R injury cannot be attributed to the type experiment, since most authors used an *ex vivo* model of perfused rat liver. Nor could they be explained by differences in the times of cold ischemia, since the results obtained following the same ischemic period (24 h) were completely opposed (Imamura et al., 1995). The type of drug used for KC inactivation is the most probable explanation, since most of the studies implicating KC as main source of ROS used gadolinium chloride ($GdCl_3$) (Schauer et al., 2001; Zhong et al., 1996) whereas those that did not implicate KC used liposome-encapsulated dichloromethylene diphosphate (Imamura et al., 1995). Indeed, differences in the properties and action mechanisms of these two KC inhibitors have been reported.

3.2 Mediators and transcription factors in I/R injury

3.2.1 Nitric oxide

It is difficult to distinguish between beneficial and harmful mediators in I/R injury. Some authors have found that NO exerts a beneficial effect on I/R injury in different organs, tissues and cells, whereas other studies report no effect or even a deleterious action of NO (Peralta et al., 2001a). In our opinion, in addition to the differences in animal species, experimental models of hepatic I/R tested, and the dose and timing of administration of the different pharmacological modulators of NO, these differential effects of NO could be explained, at least partially, by the different source of NO. In this context, some studies suggest that although endothelial NO synthase (eNOS)-derived NO production is protective in I/R, inducible NO synthase (iNOS)-derived NO production may contribute to I/R injury. This may be a function of the NO generation kinetics of the two isoforms in I/R. The basal, low-level NO generation by the constitutively expressed eNOS isoform may abrogate the microcirculatory stresses of engraftment and reperfusion. In contrast, iNOS-derived NO cannot be generated until several hours after stimulation because of requirements for transcriptional induction of this isoform. Excess NO production may no longer be of microcirculatory benefit at this later time (Shah & Kamath, 2003). Furthermore, the excessive levels of iNOS-derived NO production may be detrimental through the generation of NOS-derived superoxide production or the generation of peroxynitrite. Additionally, whether NO is cytoprotective or cytotoxic in hepatic I/R injury may be determined at apoptosis (Casillas et al., 2006). For example, NO may promote apoptosis by inducing cytochrome c

(Cyt c) release and caspase activation (Chung et al., 2001). However, NO may also upregulate the anti-apoptotic protein Bcl-2 (Genaro et al., 1995). In addition, to understand the different results in relation with the action mechanisms of NO, it is important to clarify whether the NO source is endogenous or exogenous. In this regard, although the beneficial role of endogenous NO could be related to an attenuation of leukocyte accumulation, the exogenous supplementation of NO did not modify this parameter but was associated with an inhibition of endothelin release (Peralta et al., 2001a).

3.2.2 TNF and NFκB

Differential effects of NO mentioned above have also been reported for other mediators involved in hepatic I/R injury. According to the cell type and experimental or pathologic conditions, TNF-α is protective or injurious to the liver in the context of I/R injury. TNF-α may stimulate cell death or it may induce hepatoprotective effects mediated by antioxidant, antiapoptotic, and other anti-stress mediators coupled with a pro-proliferative biologic response (Casillas et al., 2006). For example, although the deleterious effect of the TNF-α in local and systemic damage associated with hepatic I/R is well established (Peralta et al., 1999), this mediator is also a key factor in hepatic regeneration (Teoh et al., 2003), an important process in reduced-size LT. Conversely, a study by our group found no correlation between TNF-α levels and liver regeneration in reduced-size LT (Franco et al., 2004), while Tian *et al*., 2006, linked disruption of TNF-α release to lower hepatic injury and increased liver regeneration. These divergent results about the role of TNF-α in liver regeneration could be explained by different TNF-α inhibitors or animal species utilized in these experiments as well as differences in the experimental models of LT used, including the times of cold ischemia. These differential effects observed for TNF-α can also be extrapolated to transcription factors.

It is well known that NFκB can regulate various downstream pathways and thus has the potential to be both pro- and antiapoptotic (Fan et al., 1999). Currently it is not clear whether the beneficial effects of NFκB activation in protection against apoptosis or its detrimental proinflammatory role predominate in liver I/R (Fan et al., 1999). Hepatic neutrophil recruitment and hepatocellular injury are significantly reduced when NFκB activation is suppressed in mice following partial hepatic I/R (Casillas et al., 2006). However, nuclear factor kappa B (NFκB) activation is essential for hepatic regeneration after rat LT, and reduces apoptosis and hepatic I/R injury (Bradham et al., 1999). To understand the role of NFκB in the context of hepatic I/R, is important to consider the differences in animal species used, for instance, mechanisms of protection from apoptosis might be different in rats and mice (Chaisson et al., 2002). In addition, the experimental design used to evaluate the role of this transcription factor may also be important. Thus, some studies using adenoviral vector containing a repressor to prevent NFκB activation may not accurately reflect the role of NFκB signalling in regenerating liver because adenoviral vectors themselves cause increased TNF-α levels, DNA synthesis, and apoptosis in the liver before partial hepatectomy (Iimuro et al., 1998). Moreover, to explain these apparently controversial effects of NFkB, the pattern of NFkB activation under cold ischemia conditions should be taken into account. Takahashi *et al.,* 2002, have demonstrated in rat LT that NFkB activation during reperfusion occurs in two phases. The early peak of NFkB DNA binding was found 1-3 h after reperfusion and represents the nuclear translocation of NFkB p50/p65 heterodimers, whereas the second

peak, mainly composed of p50 homodimers, was observed at 12 h post-reperfusion. In this study, the donor liver treatment with adenovirus encoding the IkB super-repressor gene cannot affect the early peak of NFkB activation, but partially inhibited the second peak of NFkB DNA binding. The results indicated that, in contrast to early NFkB activation, inhibition of the late phase of NFkB activation was not associated with variations in levels of inflammatory mediators, but rather enhanced hepatocellular apoptosis (Takahashi et al., 2002), which reinforces the dual function of NFkB in transplanted liver. Nevertheless, this hypothesis does not fully explain the differences in the results. Indeed, Bradham *et al*., 1999, observed a marked increase in apoptosis when NFkB blockade was carried out at 3 h of reperfusion, which seems to be a reperfusion time associated with the early peak of activation of NFkB. Of course, there are differences between Takahashi´s and Bradham´s studies. For example, whereas Bradham infused the adenoviral vector by endovenous injection 24 h before liver explantation, in Takahashi's study the graft was perfused with UW solution containing the adenovirus immediately before cold storage.

3.2.3 Neutrophil accumulation

Activation of neutrophils has been implicated in the hepatic microvascular dysfunction and parenchymal damage associated with I/R (Cutrin et al., 2002). Still, a controversial topic is the question of how neutrophils actually accumulate in the liver. The classical theory argues that the increased expression of adhesion molecules such as ICAM-1 and P-selectin plays a key role in neutrophil accumulation and the subsequent liver damage associated with I/R (Banga et al., 2005, Cutrin et al., 2002). In contrast, it has also been reported that neutrophil accumulation observed in the liver following I/R is not dependent on the up-regulation of either ICAM-1 or P-selectin (Peralta et al., 2001b).

To explain the results that neutrophil accumulation is not dependent on adhesion molecules, we subscribe to the theory proposed by Jaeschke, 2003. This theory argues that although P-selectin and ICAM-1 appear to be relevant for neutrophil adherence in postsinusoidal venules, the neutrophils relevant for the injury accumulate in sinusoids, which were identified as the dominant sites for neutrophil extravasation. In these capillaries, neutrophil sequestration does not depend on B2 integrins or on ICAM-1 or selectins (Essani et al., 1998; Vollmar et al., 1995; Jaeschke et al., 1996). Thus, mechanical factors such as active vasoconstriction, vascular lining cell swelling and injury, and reduced membrane flexibility after activation of the neutrophil, appear to be involved in trapping of these leukocytes in sinusoids (Jaeschke et al., 1996). The extensive vascular injury during reperfusion eliminates, in part, the sinusoidal endothelial cell barrier and the neutrophil has direct access to hepatocytes (Jaeschke, 2003; McKeown et al., 1988). Nevertheless, even with damaged but still present EC, transmigration may still be required (Jaeschke, 1998). As a consequence, I/R injury is only moderately or not at all attenuated by anti-ICAM therapies (Farhood et al., 1995; Vollmar et al., 1995). In regard with the role of P-selectin, sinusoidal EC neither contain Weibel Palade bodies nor do they transcriptionally upregulate relevant levels of P-selectin (Essani et al., 1998). However, during I/R, a number of interventions directed against selectins reduced hepatic neutrophil accumulation and cell injury (Amersi et al., 2001). Because these findings cannot be explained by the prevention of P-selectin-dependent rolling in sinusoids, it has been suggested that most liver I/R models include some degree of intestinal ischemia, which leads to neutrophil accumulation in remote organs including the liver (Casillas et al., 2006; Kubes et al., 2002). Thus the lower number of neutrophils in

the liver when selectins are blocked may be a secondary effect due to the protection of antiselectin therapy against intestinal reperfusion injury (Kubes et al., 2002).

3.3 Cell death in liver transplantation

The severity of hepatocyte damage depends on the length of time the ischemia lasts. In human LT, a long ischemic period is a predicting factor for post-transplantation graft dysfunction, and some transplantation groups hesitate to transplant liver grafts preserved for more than 10 h (Fernández et al., 2002). Some studies in experimental models of LT indicate that 24 h of cold ischemia induces low survival at 24 h after LT. However, at shorter ischemic periods, LT may also result in primary organ dysfunction. The main victims of ischemic injury are the hepatocytes and SECs. These two cell types show different responses to different types of ischemia: hepatocytes are more sensitive to warm ischemia and SECs to cold ischemia (Bilzer & Gerber, 2000; McKeown et al., 1988). Although most hepatocytes remain viable after 48 h of cold preservation and reperfusion, SECs suffer severe damage following reperfusion (40% non-viable) (Caldwell et al., 1989). The result of this sinusoidal damage is the subsequent microcirculatory abnormalities upon reperfusion, resulting in hepatocyte injury and dysfunction (McKeown et al., 1988). This contributes to the development of primary nonfunction or impaired primary function after LT. However, some studies have called the importance of sinusoidal injury into question. Huet et al., 2004, have demonstrated that damage to the extracellular matrix from prolonged preservation and reperfusion appears to be the critical factor in graft failure (Banga et al., 2005). In addition, it is possible that perturbations in hepatocyte levels of adenine nucleotides during cold storage can trigger proteolytic events that contribute to damage in the liver graft and subsequently compromise hepatic functions after LT (Kukan & Haddad, 2001). Moreover, cold ischemia profoundly disturb several key hepatocellular functions, such as volume and pH homeostasis, as well as solute transport and drug metabolism, protein synthesis and mitochondrial function. This contributes to preservation injury of the liver graft. Therefore, these observations indicate that aside from reducing EC damage, LT therapy may benefit from strategies aimed at improving the maintenance of appropriate hepatocyte functions (Kukan & Haddad, 2001; Vajdova et al., 2002).

Apoptosis has been regarded as the fate of cells experiencing I/R injury (Sasaki et al., 1996). In this line, different studies have demonstrated apoptotic death in hepatocytes and/or SECs after both cold and warm ischemia of the rat liver (Gao et al., 1998; Kohli et al., 1999). All of the aforementioned studies (Gao et al., 1998; Kohli et al., 1999; Sasaki et al., 1996) used TdT-mediated dUTP-biotin nick and labelling (TUNEL staining) for DNA ladders to demonstrate apoptosis. However, the ability of TUNEL staining to distinguish between apoptosis and necrosis has been called into question. The activation of caspases has also been used to demonstrate apoptosis in rat SECs following cold I/R (Natori et al., 1999). Indeed, use of pan-caspase inhibitors protected rat liver SECs and hepatocytes against I/R injury after prolonged periods of both cold and warm ischemia. On the other hand, other groups oppose the view that the majority of cells undergo apoptosis in response to either warm or cold I/R injury, believing that necrosis is the principle form of cell death (Massip-Salcedo et al., 2007). They believe that in a number of studies the proportion of cells undergoing apoptosis is not of significant magnitude and that the degree of caspase activation does not correlate with the number of SECs and hepatocytes supposedly undergoing apoptosis. Thus, a controversy has emerged over the past years as to whether

necrotic or apoptotic cell death accounts for the severe parenchymal injury observed during hepatic reperfusion. Although it has long been assumed that necrosis and apoptosis are different processes this may not actually be the case. First we will briefly review some basic background information on death cell signalling pathways in hepatocytes in order to understand the shared pathway that leads to both necrosis and apoptosis.

Apoptosis occurs through two main pathways. The first, referred to as the intrinsic (mitochondrial) pathway, is typically activated by a variety of stressors such as DNA damage, p53 activation, growth factor deprivation, and metabolic disturbances (Malhi et al., 2006). The second is the extrinsic pathway that is triggered through death receptors (Malhi et al., 2006). It is well known that one of the most important regulators of intrinsic pathway is the Bcl-2 family of proteins. The Bcl-2 family includes proapoptotic members such as Bax, Bak, Bad, Bid and antiapoptotic members such Bcl-2, Bcl-Xl and Bcl-W (Ghobrial et al., 2005). Following death signal, proapoptotic proteins undergo posttranslational modifications resulting in their activation and translocation to the mitochondria. Then, the outer mitochondrial membrane becomes permeable, leading to the release of Cyt c, which promotes caspase 9 activation, which then activates caspase 3 and the final stages of apoptosis (Ghobrial et al., 2005). In the extrinsic pathway, a variety of mediators, including tumor TNF-α, Fas ligand, and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) first bind to their respective death receptors, which cause receptor oligomerization and the association of various adapter proteins, including Fas-associated death domain, TNF-α receptor-associated death domain, and TNF-α receptor-associated factor. Fas-associated death domain and TNF-α receptor-associated death domain promote binding of procaspase 8 and its proteolytic activation to catalytic caspase 8. If sufficient amounts of caspase 8 are generated at the receptor, caspase 8 can directly activate procaspase 3. In hepatocytes the caspase 8 interacts with the intrinsic pathway and cleaves Bid, a BH3 only proapoptotic Bcl2 family member, to a truncated form, tBid. tBid translocates to mitochondria, causing mitochondrial permeabilization and release of mitochondrial effectors of apoptosis, such Cyt c (Yin, 2000) (see Fig. 2).

The mechanisms that induce the release of mitochondrial intermembrane proteins such as Cyt c remain controversial (Jaeschke & Lemasters, 2003). In hepatocytes TNF-α and Fas dependent signalling induce the onset of the mitochondrial permeability transition (MPT), which leads to large-amplitude mitochondrial swelling, rupture of the outer membrane, and release of Cyt c and other proteins from the intermembrane mitochondrial space (Jaeschke & Lemasters, 2003). In some models, tBid interaction with either Bax or Bak, forms channels in the mitochondrial outer membrane that release Cyt c and other proteins from the intermembrane space. If MPT onset occurs in relatively few mitochondria, the organelles become sequestered into autophagosomes for lysosomal digestion, a process that eliminates the damaged and potentially toxic mitochondria (Casillas et al., 2006; Jaeschke & Lemasters, 2003). When the MPT involves more mitochondria, mitochondrial swelling leads to outer membrane rupture and Cyt c realease. Provided that ATP is available from glycolysis and still-intact mitochondria, Cyt c activate downstream caspases and other executioner enzymes of apoptosis. When MPT onset is abrupt and involves most mitochondria, ATP becomes profoundly depleted, which blocks caspase activation. Instead, ATP depletion culminates with plasma membrane rupture and the onset of necrotic cell death (Jaeschke & Lemasters, 2003). Hence, the new term "necrapoptosis" has been coined to describe a process that begins with a common death signal and which culminates in either cell lysis

(necrotic cell death) or programmed cellular resorption (apoptosis), depending on factors such as the decline of cellular ATP levels (see Fig. 2).

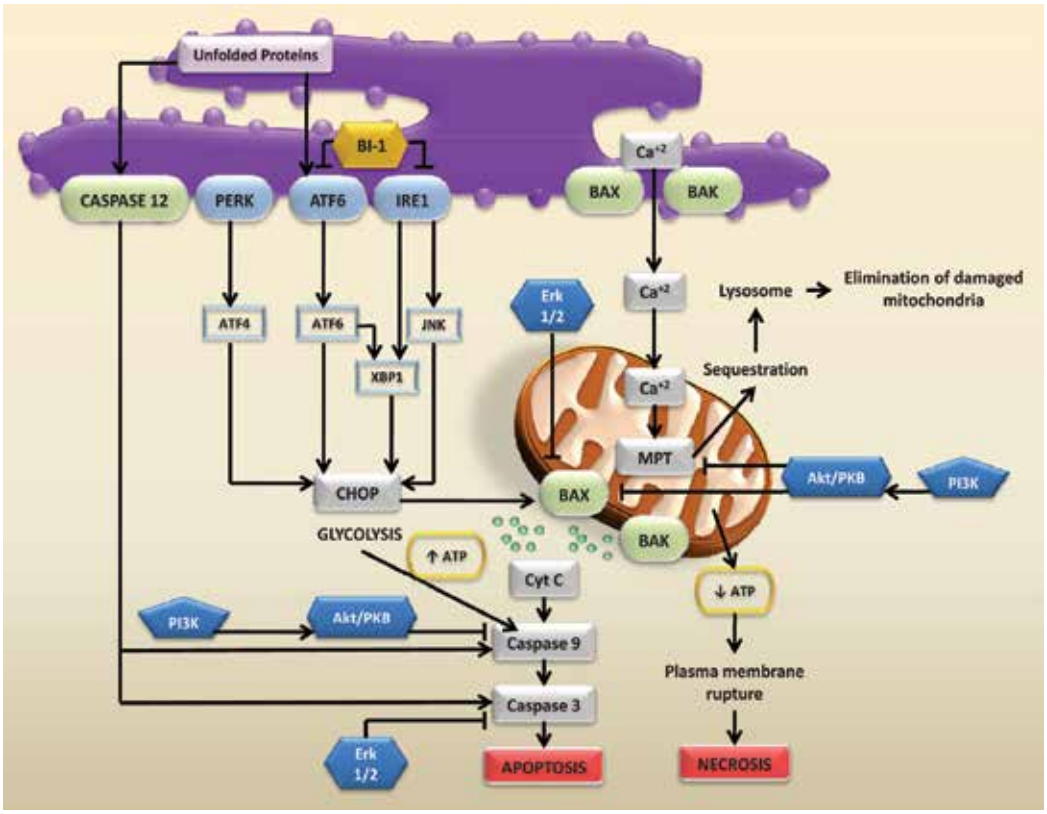


Fig. 2. Scheme of possible cell death pathway in hepatic I/R. (Alfany et al., 2009; Ben Mosbah et al., 2010; Casillas et al., 2006; Fernández et al., 2004; Ghobrial et al., 2005; Jaeschke &Lemasters, 2003; Malhi et al., 2006; Massip-Salcedo et al., 2007; Selzner et al., 2000; Yin, 2000)

4. Steatosis in hepatic ischemia-reperfusion

Several hypotheses have been suggested to explain the decreased tolerance of steatotic liver to I/R injury compared with non-steatotic livers. The impairment of the microcirculation is considered a major event of reperfusion injury in steatotic livers (Ijaz et al., 2003). A reduction in hepatic microcirculation has been observed in human fatty donor livers and in experimental models of hepatic steatosis (Ijaz et al., 2003; Seifalian et al., 1999). An imbalance between vasoconstrictors (e.g., ET1) and vasodilators (e.g., NO) negatively affect the hepatic microcirculation (Massip-Salcedo et al., 2007; Peralta et al., 2000a). In addition, fatty accumulation in the cytoplasm of hepatocytes is associated with an increase in cell volume that reduces the size of the hepatic sinusoid space by 50% compared with a normal liver and may result in partial or complete obstruction of the hepatic sinusoid space (Ijaz et al., 2003; Seifalian et al., 1999). Using Doppler flowmetry, Seifalian et al., 1999 demonstrated reduced sinusoidal perfusion in fatty human liver donors compared with healthy livers.

Analogous studies in rabbits with diet-induced steatosis confirmed that this reduction in perfusion correlated with the severity of fat accumulation in hepatocytes. The reductions in sinusoidal perfusion appear to arise initially from the effects of enlarged hepatic parenchymal cells, swollen with accumulated lipid, which widen the parenchymal cell plates and narrow and distort the lumens of sinusoids. Other investigators have shown that as a result of the structural alterations around them, the sinusoids become inefficient conduits of blood with resulting impairment of tissue perfusion, evidenced by the significant reductions in the numbers of perfused sinusoids per microscopic field (Teoh et al., 2010).

Hepatocyte damage appears remarkably higher in steatotic livers than in non-steatotic livers (Casillas et al., 2006; Selzner et al., 2000). Several evidences indicate that an increased sensitivity of fatty hepatocytes to the injurious effects of ROS could explain the poor tolerance of steatotic livers to I/R (Koneru et al., 2005; Soltys et al., 2001). It has been postulated that steatotic livers are more susceptible than nonsteatotic livers to lipid peroxidation because of either their lower antioxidant defenses or their greater production of ROS or both (Fernández et al., 2004). Mitochondrial ROS generation dramatically increases during reperfusion and mitochondrial structures are exposed to the attack of the ROS generated both outside and inside these organelles leading eventually to the dysfunction of important mitochondrial processes including those responsible for the ATP synthesis. In ROS generation systems, the inhibition of XOD with allopurinol effectively protected against the greater liver and lung damage in transplantation of steatotic livers (Fernández et al., 2004). Higher levels of IL-1β and lower IL-10 levels were observed in steatotic livers compared with non-steatotic livers after I/R. This imbalance between pro- and anti-inflammatory ILs was responsible for the vulnerability of steatotic livers to I/R (Serafin et al., 2004). Previous studies form our group indicated less glutathione (GSH) and SOD levels in steatotic livers than in non-steatotic livers as consequence of hepatic I/R (Fernández et al., 2004; Serafin et al., 2002).

It is well-known that steatotic livers synthesise less ATP than non-steatotic livers during post-ischemic reperfusion (Caraceni et al., 2005). Fatty degeneration induces a series of ultra-structural and biochemical alterations in both human and animal mitochondria. The lower ATP and adenine nucleotide content observed in steatotic livers preserved in UW solution could be caused by mitochondrial damage (Ben Mosbah et al., 2006; Caraceni et al., 2005; Massip-Salcedo et al., 2007). Caraceni et al., 2004 reported that alterations in oxidative phosphorylation during preservation is greatly enhanced by fatty infiltration resulting from damage to respiratory chain complex I and F0F1–ATP synthase. Others studies have discovered that in steatotic livers under conditions of either warm ischemia or transplantation, the content of mitochondrial uncoupling protein-2 (UCP-2) is four to five times higher than in non-steatotic livers (Chavin et al., 2004; Wan et al., 2008). This finding was associated with reduced ability to synthesize ATP upon reperfusion (Chavin et al., 2004). If cold storage time exceeds 10-12 h, complications in biliary structures occur in more than 25% of liver transplant recipients (Kukan & Haddad, 2001). Several factors, including poor recovery after ATP depletion appear to contribute to bile duct cell damage after liver transplantation. Furthermore, isolated rat bile duct epithelial cells are noticeably sensitive to oxidative stress, possibly because their cellular stores of reduced glutathione are seven times lower than those of hepatocytes (Noack et al., 1993). Taking these observations into account, bile production failure in steatotic livers could be explained, at least partially, by the lower

ATP and increased oxidative stress presented by this type of liver compared with non-steatotic liver.

Toll-like receptor 4 (TLR4) has been implicated as a mediator of steatotic liver damage after I/R (Ellett et al., 2009). The loss of TLR4 in steatotic livers from TLR4-knockout HFD animals reduces pro-inflammatory cytokines and liver injury and improves survival (Ellett et al., 2009). Although TLR4 signaling is relevant in hepatic I/R injury, there is some controversy over which of the pathways [(myeloid differentiation factor 88 (My-D88)-dependent) or Toll/IL-1 receptor domain-containing adaptor inducing interferon-β (TRIF/IRF-3 signalling pathway)] is activated in hepatic I/R (Kang et al., 2011). Neutrophils have been involved in the increased vulnerability of steatotic livers to I/R injury, especially in alcoholic steatotic livers. However, neutrophils do not account for the differentially greater injury in the non-alcoholic steatotic liver during the early or late hours of reperfusion. Similarly, the role of TNF-α in the vulnerability of steatotic livers to I/R injury may be dependent on the type of steatosis (Serafin et al., 2002). These observations could be of clinical interest because pharmacological strategies that could be effective in alcoholic fatty livers by reducing the neutrophil infiltration and or TNF-α action may not be sufficient to reduce the hepatic I/R injury in non-alcoholic fatty livers.

Cell death can occur by either necrosis or apoptosis and intracellular ATP level appear to play a role as a putative apoptosis/necrosis switch: when ATP depletion is severe, necrosis ensues before the activation of the energy-requiring apoptotic pathway (Casillas et al., 2006; Massip-Salcedo et al., 2007) (See Fig. 2). In steatotic liver graft undergoing 6 h of cold ischemia, necrosis was the predominant cell death whereas no apoptosis signs were found (Alfany et al., 2009; Fernández et al., 2004). Since apoptosis is an energy-requiring process, the impaired maintenance of ATP levels observed after reperfusion in steatotic livers submitted to long periods of cold ischemia may be linked with a failure to induce apoptosis. Thus, it is not surprising that data reported previously indicate that necrosis rather than apoptosis is the predominant process by which steatotic livers undergo cell death (Alfany et al., 2009; Fernández et al., 2004; Selzner et al., 2000).

Previous studies from our group have indicated that steatotic livers differed from non-steatotic livers in their response to UPR and ER stress. Steatotic livers showed a reduced ability to respond to ER stress as the activation of two UPR arms, IRE1 and PERK, was weaker in the presence of steatosis. (Ben Mosbah et al., 2010). Different hypotheses, including decreased ATP production and dysfunction of regulators of apoptosis, such as Bcl-2, Bcl-xL and Bax have been proposed to explain the failure of apoptosis in steatotic livers. The results on ER stress in steatotic livers undergoing I/R may throw some light on this question. Reduced proapoptotic factors related to ER stress such as caspase 12, C/EDP-homologos protein (CHOP) and Jun N-terminal kinase (JNK) were observed in steatotic livers under conditions of I/R compared with non-steatotic livers. This may be related to the reduced activation of the two UPR arms, inositol-requiring enzyme-1 (IRE1) and PERK, which are responsible for caspase 9 and 12 activation, JNK activation and CHOP induction (Ben Mosbah et al., 2010) (see Fig. 2). We believe that the damaged ER and mitochondria are intimately linked and that mitochondrial cell death and ER-induced cell death cannot be separated in hepatic I/R. Thus, caspase activation and Cyt *c* release from mitochondria consequently to hepatic I/R (Ben Mosbah et al., 2010) can be attributed to ischemic disturbance or damage to the ER. Given these results in steatotic livers under warm

ischemia conditions, it is therefore tempting to speculate that increased ER stress may be involved in the vulnerability of steatotic liver grafts to I/R injury associated with transplantation and in the sensitivity of other marginal grafts to I/R injury, such as liver grafts from aging donors. Indeed, aging donors have an increased incidence of steatosis, which may favor cold preservation injury (Busuttil & Tanaka, 2003; Massip-Salcedo et al., 2007). Alterations in the activation of inflammatory transcription factors and expression of cytoprotective proteins, increased intracellular oxidants and decreased mitochondrial function and protein misfolding accumulation, and aggregation also characterize many age-related diseases (Massip-Salcedo et al., 2007; Pallet et al., 2009).

5. Strategies designed in past years to prevent hepatic I/R injury

Despite improvements in pharmacological treatments, preservation solutions and gene therapy aimed at reducing hepatic I/R injury, the results to date have not been conclusive. Figure 3 shows some of the therapeutic strategies developed to prevent I/R injury in LT. Possible reasons for the failure of these strategies in clinical applications are now discussed.

5.1 Pharmacological treatment

Numerous experimental studies have focused on inhibiting the harmful effects of I/R-associated inflammatory response. In this respect, drugs such as chloroquine and chlorpromazine have been administered in order to prevent mitochondrial dysfunction and loss of liver cell phospholipids during hepatic ischemia. Antioxidant therapy using either tocopherol, GSH ester, or allopurinol has been applied in an attempt to inhibit ROS effects in reperfusion, and anti-TNF antiserum pre-treatment has also been employed to block the damaging effects of this cytokine. Therapies with dopamine or ATP-$MgCl_2$ have been administered to reduce hepatic I/R injury-related microcirculatory disorders. Drugs such as adenosine, NO donors, L-arginine, and anti-ICAM-1 and anti-P-selectin antibodies have been used to inhibit neutrophil accumulation. However, none of these treatments has managed to prevent hepatic I/R injury. The possible side effects of the some drugs may frequently limit their use in human LT (Casillas et al., 2006). For example, idiosyncratic liver injury in humans is documented for chlorpromazine, pernicious systemic effects have been described for nitric oxide (NO) donors, allopurinol therapy can cause haematological changes and gadolinium can induce coagulation disorders (Casillas et al., 2006).

Hepatic failures have been observed after administration of these two thiazolidinediones (TZDs) and some case reports of acute hepatotoxicity attributed to rosiglitazone have been published, including one death (Reynaert et al., 2005). The toxicity of TZDs is thought to be mainly metabolic idiosyncratic, although in some cases possible immunological mechanism has been implicated (Reynaert et al., 2005). High dose resveratrol was found to be a pro-oxidant with aggravation of liver injury; and experiments are in progress to devise a pharmaceutical form appropriate for clinical use (Hassan et al., 2008). The development of therapeutic strategies that utilize the protective effect of Heme oxigenase-1 (HO-1) induction is hampered by the fact that most pharmacological inducers of this enzyme perturb organ function by themselves and that gene therapy for up-regulation of HO-1 has potential negative side effects, which currently preclude its clinical application under these conditions (Schmidt, 2010) (see Fig. 3).

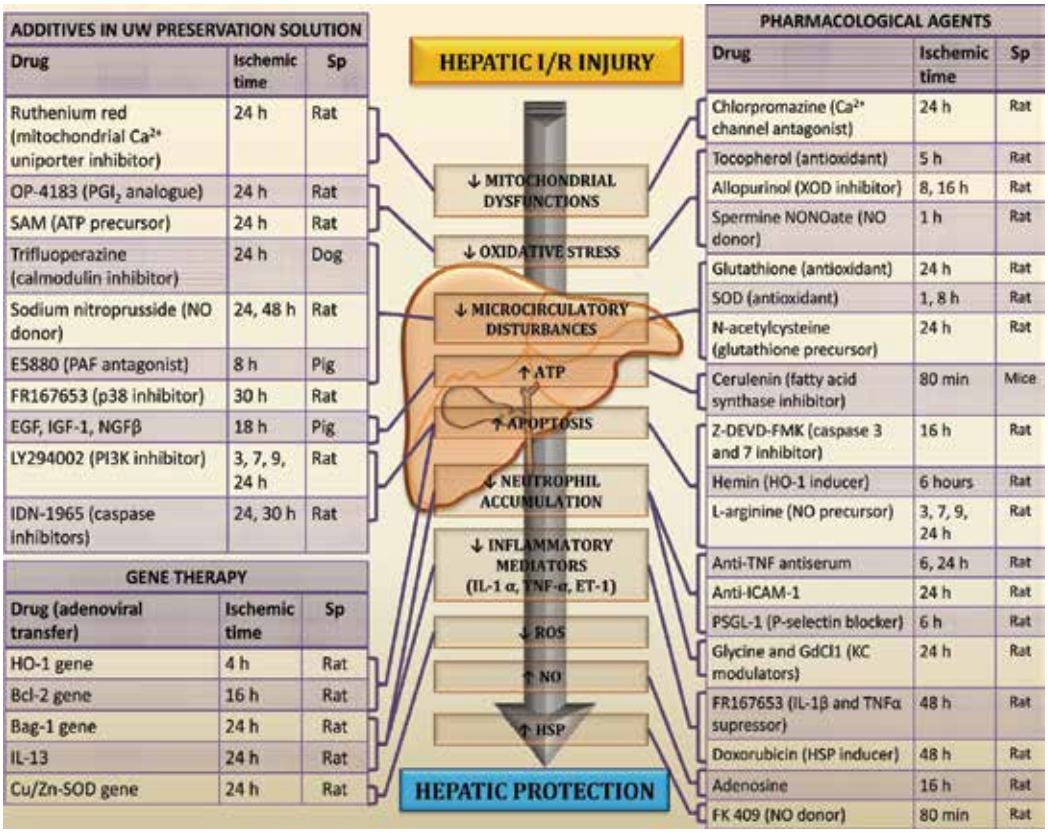


Fig. 3. Strategies used to prevent hepatic I/R injury. (Sp, species). (Carini et al., 2004; Carrasco et al., 2005; Casillas et al., 2006; Chavin et al., 2004; Cheng et al., 2003; Esfandiari et al., 2007; Fan et al., 1999; Hassan et al., 2008; Massip-Salcedo et al., 2006; Nakano et al., 2007; Natori et al., 1999; Peralta et al., 2001a, 2001b; Polyak et al., 2000; Reynaert et al., 2005; Schmidt, 2010; Selzner et al., 2000, 2003; Teoh et al., 2003; Vajdova et al., 2002; Yoshinari et al., 2001)

The difficulty of blocking the inflammation related to this process must be taken into account because, among other factors, many mediators and cell types are involved in this kind of inflammatory response. Pharmacological treatment-derived difficulties must also be considered. In this regard, superoxide dismutase (SOD) and glutathione show inadequate delivery to intracellular sites of ROS action (Polyak et al., 2000). The administration of anti-TNF antibodies does not effectively protect against hepatic I/R injury, and this finding has been related to the failure of complete TNF-α neutralization locally (Peralta et al., 2001b). Additionally, special attention should be given to drugs that suppress TNF-α, because its potential dual effects (Teoh et al., 2003). Small changes in the dose of NO donors produce totally opposite effects (Peralta et al., 2001a). Although this also occurs in non-steatotic livers, modulating I/R injury in steatotic livers poses a greater problem. Until now, data about the effectiveness of the administration of antioxidants on the deleterious effects of ROS in steatotic livers was controversial. Some studies in obese Zucker rats, a well-characterized model of nutritionally induced obesity, indicated that the administration of

tocopherol, which possesses antioxidant properties, improved tolerance to warm ischemia. However, other experimental studies in steatotic livers, induced by a choline–methionine-deficient diet, show that the administration of GSH precursors, such as N-acetylcysteine, could help to restore hepatocellular integrity in the steatotic liver but without scavenging free radical. In addition, both dietary high fat and alcohol exposure produced SOD/catalase-insensitive ROS that may be involved in the mechanism of failure of steatotic livers after orthotopic LT (Casillas et al., 2006; Massip-Salcedo et al., 2007; Serafin et al., 2002; Soltys et al., 2001).

Differences in the action mechanisms between steatotic and non-steatotic livers mean that therapies which are effective in non-steatotic livers may prove useless in the presence of steatosis, and the effective drug dose may differ between the two liver types. Findings such as these must be taken into consideration when applying pharmacological strategies in the same way to steatotic and non-steatotic livers, because the effects may be very different. Apoptosis was the predominant form of hepatocyte death in the ischemic non-steatotic liver, whereas the steatotic livers developed massive necrosis after an ischemic insult. Thus, caspase inhibition, a highly protective strategy in non-steatotic livers, had no effect on hepatocyte injury in steatotic livers (Selzner et al., 2000). For instance, whereas in an LT experimental model a NO donor reduced oxidative stress in non-steatotic livers, the same dose increased vulnerability of steatotic grafts to I/R injury (Carrasco et al., 2005). The injurious effects of exogenous NO donors on hepatic injury and oxidative stress in steatotic grafts could be explained by peroxinitrite generation caused by ROS overproduction (Carrasco et al., 2005). HO-1 activators such as cobalt (III) protoporphyrin IX, might protect both liver types against warm I/R injury. However, a lower dose of HO-1 activator was required to protect steatotic livers effectively, as steatotic livers undergoing I/R showed higher HO-1 levels than nonsteatotic livers (Massip-Salcedo et al., 2006). Furthermore, there may be drugs that would only be effective in steatotic livers. In the context of LT, steatotic donors have been reported to show a higher content of mitochondrial uncoupling protein-2 (UCP-2) and a reduced ability to synthesize ATP upon reperfusion, thus leading to increased mortality following I/R (Cheng et al., 2003). Studies by Chavin *et al* have discovered that in ob/ob mice (approximately 70%-80% of liver lipid content) expression of UCP-2 is four to five times higher than in normal liver tissues (Chavin et al., 1999; Wan et al., 2008). Hence, compounds such as cerulenin that reduce UCP-2 expression in steatotic livers, offer protection as a result of increased availability of ATP prior to I /R (Chavin et al., 2004). However, this strategy may be ineffective in non-steatotic livers because the latter do not show an overexpression of UCP-2 (Chavin et al., 1999). Similar results have been obtained with carnitine administration (Tolba et al., 2003; Yonezawa et al., 2005).

All the aforementioned results point up the fact that the different mechanisms of cell death in steatotic vs. non-steatotic livers as well the differences in the mechanisms involved in hepatic I/R injury in terms of the type of steatosis could explain the difficulties in effectively preventing steatotic livers from I/R injury. Further investigations are required to optimize some treatments because long-term therapy appears to be necessary to exert the desired effects. For example, the pre-treatment times for rosiglitazone was between 6 to 12 weeks (Nakano et al., 2007); and, S-adenosylmethionine (SAM) between 14 and 17 weeks (Esfandiari et al., 2007). Similarly, long-term IL-6 treatment (10 days) reduced hepatic steatosis and markedly prevents I/R-induced liver injury in ob/ob mice and mice fed high-

fat diets (Hong et al., 2004). However, there are obvious difficulties concerning the feasibility of long-term drug administration in some I/R processes, in particular, liver transplantation from cadaveric donors, because this is an emergency procedure in which there is very little time to pre-treat the donor with drugs.

5.2 Preservation solutions

Since its introduction by Belzer et al. in the late eighties, the University of Wisconsin (UW) solution has become the standard solution for the preservation of most organs in transplantation. The inclusion of some components in the UW solution has been both advocated and criticised. For instance, adenosine has been added to UW solution as a substrate for the regeneration of adenine nucleotides. However, simplified variants of UW solution in which adenosine was omitted were shown to have similar or even higher protective potential during cold liver storage. The colloid hydroxyethyl starch (HES) included in UW preservation solution prevents interstitial edema but produces extended and accelerated aggregation of erythrocytes that may result in stasis of blood and incomplete washout of donor organs before transplantation. Another limitation of the UW solution is that some of its constituent compounds (allopurinol, lactobionate) do not offer very good protection because they are not present at a suitable concentration and encounter problems in reaching their site of action. Indeed, studies in humans have suggested that the allopurinol in the UW preservation solution was unable to prevent the subsequent XDH/XOD-derived superoxide radical production during reperfusion (Casillas et al., 2006; Pesonen et al., 1998).

A variety of ingredients such as stable protacyclin (PGI2) analogue OP-4183, p38 mitogen-activated protein kinase (MAPK) inhibitor FR167653, NO donor sodium nitroprusside, platelet-activating factor (PAF) antagonist E5880, calmodulin inhibitors, Ca^{2+} channel blockers such as nisoldipine, trophic factors, caspase or calpain inhibitors, S-adenosylmethionine (SAM), insuline, or fructose-1,6-biphosphate (FBP) were introduced into UW preservation solution, with promising results (Casillas et al., 2006). However, none of these modifications to UW solution composition have found their way into routine clinical practice. For instance, studies aimed at enrichment of UW solution with caspase inhibitors showed that this prevents sinusoidal endothelial cells apoptosis (Vajdova et al., 2002), but it has also been demonstrated that such inhibitors have little effect on necrosis, and this could mean no protection in the steatotic liver where the predominant form of cell death is necrosis (Selzner, 2003). Along this line, addition of precursors for ATP resynthesis such as SAM only resulted in a poor initial ATP recovery during liver reperfusion (Vajdova et al., 2002) (see Fig. 3). Insulin and FBP were recommended and added to UW preservation solution with the aim of stimulating glycolysis and modulating KC activity, respectively. However, further studies showed that these modifications in UW solution may exacerbate graft ischemic injury and decrease the graft survival rate in rat LT.

The failure of UW solution enrichments could be related either to factors intrinsic to the drugs themselves (i.e. toxic side-effects, lack of specificity, etc.) or disagreement in their mechanisms of modulation. For instance, LY294002 was added to UW in order to maintain calcium homeostasis through the inhibition of phosphatidylinositol-3-OH kinase (PI3K) activity (see Fig. 3). Despite LY294002 reduces apoptosis in the grafts, the beneficial effects of the survival pathway activated by PI3K were also suppressed (Carini et al., 2004).

Additives to UW solution might further improve survival rate and graft viability if their concentration could be increased, but this is not always possible. For example, the solubility of FR167653 in UW solution was found to be limited. In addition, these additives are rinsed from the liver graft before implantation, so they should have prolonged action (Yoshinari et al., 2001). For instance, addition of precursors for ATP re-synthesis, such as S-adenosylmethionine, only resulted in a poor ATP recovery during reperfusion, since they can be rescued only partially after liver flush before implantation (Vajdova et al., 2002). Another limitation is that suitable concentrations of additives, such as caspase inhibitor IDN-1965, can be achieved only with prolonged storage of the organ in the presence of the inhibitor (Natori et al., 1999). However, this exacerbates the cold ischemic injury.

Numerous studies have reported equivalent patient and graft survival for deceased donor liver allografts preserved with UW and HTK solutions (Steawart et al., 2009). The reduced viscosity of HTK as compared to UW has been hypothesized to be protective against the development of biliary complications. However, the impact of HTK versus UW preservation on biliary complications remains unclear, as some centers report equivalent, increased or reduced rates of biliary complication with HTK preservation of deceased donor liver allografts (Feng et al., 2007; Steawart et al., 2009).

Clinical studies indicated that HTK preservation was associated with higher odds or early graft loss as compared to UW preservation with a more pronounced effect on allograft with cold ischemia time over 8 h, donor after cardiac death allografts and donors over 70 years (Steawart et al., 2009). As previously reported, HTK is not so efficient for longer periods of cold ischemia causing a higher incidence of delayed graft function (Olschewski et al., 2008; Straatsburg et al., 2002)

5.3 Gene therapy

Advances in molecular biology provide new opportunities to reduce liver I/R injury by using gene therapy. To suppress the ROS burst, SOD and catalase have been transfected by either adenovirus, liposomes or polyethyleneglycol (Fan et al., 1999; Selzner, 2003). To inhibit apoptosis, overexpression of Bag-1 and Bcl-2, mainly by using adenovirus, has been tested (Selzner, 2003) (see Fig. 3). To limit neutrophil recruitment and activation, reduction in ICAM-1 expression was obtained by using liposomes. Cytoprotective strategies based on expression of genes such as HO-1, anti-inflammatory cytokine IL-13 and interleukin-1 receptor antagonist (IL-1Ra) have been developed employing adenoviral or liposome vector (Casillas et al., 2006). Attempts have also been made to modulate the NFκB effect through adenoviral transfection of a mutant inhibitor of kappaB-alpha (IκBalpha), which would inhibit NFκB and ameliorate the hepatic inflammatory response to I/R (Fan et al., 1999; Casillas et al., 2006) (see Fig. 3). However, there are a number of problems inherent in gene therapy, for example, vector toxicity, difficulties in increasing transfection efficiencies and protein expression at the appropriate time and site, and the problem of obtaining adequate mutants (in the case of NFκB) due to controversy about NFκB activation (Chaisson et al., 2002; Somia & Verma, 2000). Although non-viral vectors (such as naked DNA and liposomes) are likely to present fewer toxic or immunological problems, they suffer from inefficient gene transfer (Somia & Verma, 2000). In addition, LT is an emergency procedure in most cases, which leaves very little time to pre-treat the donor with genetic approaches.

6. Directions for the future

New potential strategies that could be promissory in LT are now discussed. The present review will now centre on emerging protective strategies such as enrichments of UW solution and pharmacological treatments with favourable results in I/R injury but that up to now have not been tested in clinical LT. Moreover, we will discuss ischemic preconditioning taking into account the novel clinical reports that suggest the effectiveness of this surgical procedure in LT.

6.1 Pharmacological treatments and preservation solutions

6.1.1 Trimetazidine and AICAR

Trimetazidine (TMZ), which has been used as an anti-ischemic drug in the heart for over 35 years (Ikizler et al., 2003) reduced liver injury and improved liver regeneration and survival rate in partial hepatectomy under vascular occlusion (Casillas et al., 2006). TMZ has been used as an additive in UW solution to protect steatotic livers exposed to prolonged cold ischemia in an *ex vivo* model of hepatic ischemia (Ben Mosbah et al., 2006). This could be of interest since irreversible injury has been reported in liver grafts preserved in UW after prolonged cold ischemic periods (between 16 h to 24 h) (Ben Mosbah et al., 2006). Studies examining the underlying protective mechanisms of TMZ suggest that mitochondria, energy metabolism, oxidative stress and microcirculation might be important targets through which TMZ exerts its cytoprotective effect (Ben Mosbah et al., 2006; Ikizler et al., 2003). Interestingly, these mechanisms are responsible for the vulnerability of steatotic livers to I/R. Similarly to the benefits of TMZ, the addition of AMPK activators to UW solutions such as 5-amino-4-imidazole carboxamide riboside (AICAR), protected steatotic livers against their vulnerability to I/R. TMZ, by means of AMPK, increased NO, thus protecting steatotic livers against their vulnerability to I/R injury (Ben Mosbah et al., 2006, 2007; Carrasco et al., 2005). Taking these observations into account, TMZ and AICAR may constitute new additives to UW solution in steatotic liver preservation, whereas a combination of both seems unnecessary.

6.1.2 Modulators of renin-angiotensin system

Previous researches have observed an important role for the renin-angiotensin system (RAS), known for its regulation of blood pressure and fluid homeostasis, in both I/R injury and liver regeneration after partial hepatectomy (Ramalho et al., 2002; 2009). Furthermore, angiotensin-converting enzyme (ACE) inhibitors (captopril and enalapril) and angiotensin II (Ang-II) type 1 receptor blockers (losartan and candesartan) reduced inflammatory response associated with I/R injury (Araya et al., 2002). In addition, ACE inhibitors (lisinopril, captopril and enalaprilat) promoted liver regeneration after partial hepatectomy (Ramalho et al., 2002). Candesartan, a potent and long-lasting Ang-II type 1 receptor antagonist, up-regulated the hepatocyte growth factor (HGF), the most potent mitogen for mature hepatocytes (Araya et al., 2002). Steatotic livers against I/R. In conditions of partial hepatectomy under I/R, Angiotensin receptors (AT1R and AT2R) antagonists for steatotic livers improved regeneration in the remnant liver. AT1R antagonist, through NO inhibition, protected steatotic livers against oxidative stress and damage. The combination of AT1R and AT2R antagonists in steatotic livers showed stronger liver regeneration than either

antagonist used separately and also provided the same protection against damage as that afforded by AT1R antagonist alone. These results could be of clinical interest in liver surgery (Ramalho et al., 2009). BK seems to be a key mediator in the benefits of all the blockers of Ang II activity (ACE inhibitors, AT1R antagonists, and AT2R antagonists) in steatotic livers undergoing I/R (Casillas et al., 2008). In liver transplantation, Ang II is an appropriate therapeutic target only in non-steatotic livers. It was observed an upregulation of ACE2 in steatotic liver grafts, which was associated with decreased Ang II and high Ang-(1–7) levels. Ang-(1–7) receptor antagonist reduced necrotic cell death and increased survival in recipients transplanted with steatotic liver grafts. These results indicate a novel target for therapeutic interventions in liver transplantation within the RAS cascade, based on Ang- (1–7), which could be specific for this type of liver (Alfany et al., 2009). Further studies will be required to elucidate whether these strategies based on regulating RAS can be useful in hepatic I/R injury. ACE inhibitors are widely used in clinical practice. However, hepatotoxicity and cholestatic liver diseases have been reported under ACE inhibition (Casillas et al., 2008). Previous studies have indicated that losartan is as effective as captopril in its cardiovascular effects but has fewer adverse effects (Zhu et al., 2000). Thus, AT1R antagonists may be a safer protective pharmacologic strategy than ACE inhibitors for hepatic I/R injury.

6.1.3 Modulators of activating pro-survival kinase cascades, PI3K-Akt and Erk 1/2 pathway

Trophic factors such as insulin-like growth factor (IGF), EGF, cardiotrophin-1 and fibroblast growth factor (FGF) have been shown to protect against I/R injury through the activation of phosphatidylinositol-3-OH kinase (PI3K)-Akt and p42/p44 extra-cellular signal-regulated kinases (Erk 1/2). This pathway has been implicated in cellular survival, through recruitment of anti-apoptotic protection pathways. PI3K-Akt has been shown to increase NO, inhibit opening of the MPT pore, and activate protein kinase C (PKC) and mitochondrial Raf-1, which has been shown to phosphorylate and inactivate the pro-apoptotic factor, Bad. Activation of either the PI3K-Akt or the Erk 1/2 pathway inhibits the conformational change in Bax required for its translocation to the mitochondria. Moreover Erk 1/2 kinase activation has been shown to inhibit apoptosis, by inhibiting caspase 3 activation and Akt activation can suppress the mitochondrial apoptotic death pathway by inactivating caspase 9. Interestingly, PI3K-Akt is a cell signalling mechanism also involved in the benefits of liver ischemic preconditioning in isolated hepatocytes. The modulation of therapeutic targets such as the anti-apoptotic pro-survival PI3K-Akt and Erk 1/2 kinase cascades could open new perspectives for limiting I/R injury associated with LT (Casillas et al., 2006).

Cardiotrophin-1 (CT-1) and alpha-lipoic acid (LA) could be promising drugs against I/R injury associated with LT because their benefits on pro-survival kinase cascades. The pretreatment of isolated hepatocytes with the pro-apoptotic mediator transforming growth factor-beta stimulates CT-1 production. In addition, pretreatment with CT-1 protects rats against fulminant liver failure after subtotal hepatectomy. This protective effect was associated with reduced caspase-3 activity and activation of Erk1/2 and PI3K/Akt pathways (Bustos et al., 2003). Recent research points to the potential of preconditioning with LA for hepatic IRI, which is mediated via the PI3K/Akt pathway. However, neither

Bad nor eNOS phosphorylation was increased after LA pretreatment, suggesting a new mechanism by which LA exerts antinecrotic but not antiapoptotic action during hepatic I/R (Muller et al., 2003). This could be of special interest to protect steatotic liver grafts, given that necrosis rather than apoptosis is the predominant type of cell death in such cases.

The results, based on isolated perfused liver, indicated that the addition of EGF and IGF-I (separately or in combination) to UW reduced hepatic injury and improved function in both liver types. A combination of EGF and IGF-I resulted in hepatic injury and function parameters in both liver types similar to those obtained by EGF and IGF-I separately. EGF increased IGF-I, and both additives up-regulated AKT in both liver types. This was associated with glycogen synthase kinase-3β (GSK3β) inhibition in non-steatotic livers and peroxisome proliferator-activated receptor gamma (PPARγ) over-expression in steatotic livers. The benefits of EGF and IGF-I as additives in UW solution were also clearly seen in the LT model, because the presence of EGF and IGF-I (separately or in combination) in UW solution reduced hepatic injury and improved survival in recipients who underwent transplantation with steatotic and nonsteatotic liver grafts. Thus, EGF and IGF-I may constitute new additives to UW solution in steatotic and nonsteatotic liver preservation, whereas a combination of both seems unnecessary (Zaouali et al., 2010).

6.2 Antiapoptotic strategies

An interesting research in hepatic warm ischemia by Bailly-Maitre et al. has pointed to BAX inhibitor-1 (BI-1) as a regulator of the endoplasmic reticulum (ER) stress-mediated apoptosis pathway (Bailly et al., 2006). The results could lead to new strategies for reducing I/R injury associated with LT. Some mechanisms of ER stress-mediated apoptosis are briefly described below. During liver ischemia, hypoxia-induced ATP deficiency promotes the release of Ca^{2+} from ER to cytosol. The depletion of ER Ca^{2+} stores triggers downstream ER stress pathways that induce apoptosis. The pro-apoptotic Bcl-2 family members BAX and BAK, localized to the ER, also induce emptying of ER Ca^{2+} pools concomitantly with Ca^{2+} translocation into the mitochondria (Breckenridge et al., 2003). In addition, I/R initiates protein misfolding in the ER, which can activate a highly conserved unfolded protein response (UPR) signal transduction pathway. The UPR is characterized by coordinated activation of three ER transmembrane proteins, IRE1, PKR-like ER kinase (PERK) and activating transcription factor (ATF)-6. If the damage is so severe that homeostasis cannot be restored, ER stress signal transduction pathways ultimately initiate apoptosis (Oyadomari & Mori, 2004; Xu et al., 2005). The study by Bailly-Maitre indicated that the ER membrane protein BI-1 protects against apoptosis induced by ER stress. Compared to wild-type BI-1 mice, BI-1 knockout mice subjected to hepatic ischemia/reperfusion exhibited greater elevation in caspase-9 activity, more activation of IRE1, ATF6 and JNK, and greater increases in expression of CHOP and spliced X-box binding protein 1 (XBP-1) (Bailly et al., 2006). Thus, strategies aimed at modulating BI-1 as well as other component of ER stress-mediated apoptosis could protect not only against ER stress but also against the mitochondrial-dependent apoptosis pathway. In liver, the small molecule chemical chaperones, 4-PBA and Tauroursodeoxycholic acid (TUDCA) protect against I/R-induced ER stress-mediated cell death in non-steatotic livers undergoing ischemic conditions (Falasca et al., 2001; Vilatoba et al., 2005). 4-PBA reduced inflammatory response, apoptosis and mortality in non-steatotic livers undergoing total hepatic ischemia (Vilatoba et al., 2005). The addition of TUDCA to UW preservation solution protected non-steatotic livers, specifically sinusoidal lining cells and hepatocytes

against cold ischemia injury (Falasca et al., 2001). Recent studies indicated that PBA, and especially TUDCA, reduced inflammation, apoptosis and necrosis, and improved liver regeneration in both steatotic and non-steatotic livers in partial hepatectomy under vascular occlusion. Both compounds, especially TUDCA, protected both liver types against ER damage, as they reduced the activation of two of the three pathways of UPR (namely inositol-requiring enzyme and PKR-like ER kinase) and their target molecules caspase 12, c-Jun N-terminal kinase and C/EBP homologous protein-10. Only TUDCA, possibly mediated by extracellular signal-regulated kinase upregulation, inactivated glycogen synthase kinase-3β. This in turn, inactivated mitochondrial voltage-dependent anion channel, reduced Cyt c release from the mitochondria and caspase 9 activation and protected both liver types against mitochondrial damage (Ben Mosbah et al., 2010). Also, strategies aimed at modulating component of ER stress-mediated cell death could protect not only against ER stress but also against the mitochondrial-dependent apoptosis pathway. A recent study indicated that TUDCA reduced ER stress in steatotic liver transplantation. Further studies will be required to elucidate whether these chemical chaperones such as 4-PBA and TUDCA could be considered as useful strategies in clinical LT. They have been used for clinical treatment of urea cycle disorders, cholestatic liver diseases and cirrhosis (Ben Mosbah et al., 2010). Results of clinical trials have shown that 4-PBA has few side effects and is safe for patients since it is well tolerated at high dose for long periods of time (Özcan et al., 2006). TUDCA is a derivate of an endogenous bile acid, and it has been safely used as a hepatoprotective agent in humans with cholestatic liver diseases (Falasca et al., 2001).

Recently, autophagy has been described to be activated in stress conditions to ensure cell survival by limiting necrosis or apoptosis *in vivo*. Autophagy is a catabolic pathway triggered following various stress conditions, such as starvation or transient hypoxia, and aimed to restore adequate intracellular ATP and aminoacids levels and to eliminate damaged organelles (Degli et al., 2011). Autophagy has been shown to retard cell death by suppressing ER stress. Thus, the possibility that activation of autophagy may be involved in ER stress attenuation in steatotic livers, and that the modulation of autophagy and ER stress can have beneficial effects in clinical LT should not be discarded.

6.3 Omega-3 PUFAs

Manipulation of the chemical composition of hepatic lipids may evolve as a useful strategy to expand the donor pool and improve the outcome after LT. Macrosteatotic livers disclosed an abnormal omega-6: omega-3 PUFA ratio that correlates with a microcirculatory defect that enhanced reperfusion injury (El-Badry et al., 2007). Therefore, normalization of the Ω-6:Ω-3 FA ratio appears to be crucial for protection of the steatotic liver from reperfusion injury. Preoperative dietary omega-3 PUFAs protect macrosteatotic livers against reperfusion injury and might represent a valuable method to expand the live liver donor pool (El-Badry et al., 2007). Clavien *et al*., treated three live liver donors with moderate degrees of steatosis by oral administration of X-3 FAs. All donors showed a significant reduction of hepatic fatty infiltration within one month. Subsequently, LT was carried out for three candidates with uneventful outcomes for both donors and recipients. A very promising option to prevent post-transplant complications appears to be the use of a pretreatment with X- 3 FAs. However, the approach is only feasible in living donation since requires oral administration of X-3 FAs before organ procurement (McCormack et al., 2011).

Due to large inconsistencies in the qualitative and quantitative measurement of fat deposits in the liver, new techniques of assessment of steatosis are needed. Computerized programs have been developed to more objectively quantitate hepatic steatosis by determining the area occupied by lipid droplets in a given field of a liver section (El-Badry et al., 2009). However, these quantitative methods provide information only on the total amount of fat, omitting any data on the chemical composition of hepatic lipids. Therefore, novel and objective tools, such as measurement of the X-6 and X-3 FAs and prostanoid levels in liver biopsy samples, may help prediction of the magnitude of reperfusion injury (McCormack et al., 2011).

6.4 Adipocytokines derived from liver and/or adipose tissue

To date, adipose tissue has been considered the major site for endogenous adiponectin production, although there are other potential sources, including the liver (Massip-Salcedo et al., 2008; Neumeier et al., 2006). A recent study indicated that steatotic livers can generate adiponectin as a consequence of I/R (Massip-Salcedo et al., 2008). The role of adiponectin in hepatic I/R injury remains unclear. Adiponectin silent small interfering RNA (siRNA) treatment decreased oxidative stress and hepatic injury in steatotic livers. PPAR-α agonists as well as ischemic preconditioning (PC), through PPAR-α, inhibited mitogen-activated protein kinase expression following I/R. This in turn inhibited the accumulation of adiponectin in steatotic livers and reduced its negative effects on oxidative stress and hepatic injury (Massip-Salcedo et al., 2008). However, another study by Man et al., 2006 in small fatty grafts, adiponectin treatment exerted anti-inflammatory effects that down-regulated TNF-α mRNA and vasoregulatory effects that improved the microcirculation. Adiponectin anti-inflammatory effects also include the activation of cell survival signaling via the phosphorylation of Akt and the stimulation of NO production. Additionally, the studies by Man et al., 2006 showed the anti-obesity and proliferative properties of adiponectin in small fatty transplants. Thus, on the basis of the different results reported to date in hepatic I/R, it is difficult to discern whether we should aim to inhibit adiponectin, or administer adiponectin to protect steatotic livers against cold ischemia associated with transplantation.

Levels of adiponectin are reduced in obese subjects (Bugianesi et al., 2005; Targher et al., 2006; Weyer et al., 2001) and in experimental models of fatty livers, irrespective of the type of steatosis (induced by diet or alcohol) (Rogers et al., 2008; Xu et al., 2003). Indeed, in a cohort of 68 obese individuals, serum levels of adiponectin significantly predicted hepatic steatosis and hepatic damage (Schäffler et al., 2005; Targher et al., 2004). Research aimed at identifying prognostic factors in LT are both necessary and relevant. Further investigations will be required to elucidate whether measurements of adiponectin in serum, a non-invasive tool, might predict the severity of steatosis and liver damage and contribute to the identification of steatotic liver donors with a high risk for transplantation. The decision to implant or reject a steatotic liver is difficult due to the risk of impaired graft function or even failure after implantation. How much fat, and what types of fat, represent a significant risks for primary non-function of the graft remain under debate. The assessment of donor liver fat is a difficult task for the transplant team due to large inconsistencies in the qualitative and quantitative measurement of fat deposits in the liver (El-Badry et al., 2009; McCormack et al., 2011).

Retinol binding protein 4 (RBP4) is an adipokine synthesized by the liver, whose known function is to transport retinol in circulation. However, the role of RBP4 in the liver is largely unknown. A recent study indicated that steatotic liver grafts were found to be more vulnerable to the down-regulation of RBP4 and the over-expression of PPARγ. RBP4 treatment (through AMP-activated protein kinase (AMPK) induction) reduced PPARγ over-expression, thus protecting steatotic liver grafts against I/R injury associated with transplantation. In terms of clinical application, therapies based on RBP4 treatment and PPARγ antagonists might open new avenues for steatotic LT and improve the initial conditions of donor livers with low steatosis that are available for transplantation. (Casillas et al., 2011).

6.5 Surgical strategies

The response of hepatocyte to ischemia never ceases to be surprising. In fact, contrary to what might be expected, the induction of consecutive periods of ischemia to the liver does not provoque an additive effect in terms of the hepatocyte lesion. Murry et al. have reported that ischemic PC based on a brief period of ischemia followed by a short interval of reperfusion prior to a prolonged ischemic stress protects against I/R injury (Murry et al., 1986). The molecular basis for PC consists of a sequence of events: in response to the triggers of PC, a signal must be rapidly generated which is then transduced into an intracellular message leading to the amplification of the effector mechanism of protection (Cutrin et al., 2002; Serafin et al., 2004b). As in the pathophysiology of hepatic I/R, in the modulation of hepatic injury induced by IP there is a complex interaction between different cell types.

The present review is focused on some of the proposed mechanisms leading to the development of hepatocyte resistance to I/R injury following hepatic PC (see Fig. 4). Vasoactive substances such as adenosine, NO, bradykinin, etc, have been considered as the major players in triggering preconditioning (Cutrin et al., 2002). In addition to the extracellular mediators, PC involves activation of intracellular messengers such as PKC, AMPK, p38 MAPK, Ik kinase; signal transducer and activator of transcription-3 (STAT3) and transcription factors including NFκB and heat shock transcription factor 1 (HSF1) (Carini & Albano, 2003; Selzner, 2003) (see Fig. 4). The downstream consequences of these pathways could be cytoprotective by abrogation of cell death pathways, stimulating antioxidant and other cellular protective mechanisms including MnSOD and heat shock proteins (HSPs), and by initiating entry into the cell cycle (Cutrin et al., 2002; Selzner, 2003). The benefits of PC on energy metabolism, inflammatory mediators including ROS and TNF, mitochondrial dysfunction, KC activation, and microcirculatory disorders associated with I/R injury have also been described (Casillas et al., 2006; Massip-Salcedo et al., 2007). PC via AMPK activation, reduced the ATP depletion thus attenuating the accumulation of glycolytic intermediates and lactate production during hepatic sustained ischemia (Peralta et al., 2000b). The benefits of PC on oxidative stress could be explained by the induction of antioxidants, such as SOD and HSPs as well as by its effect on XDH/XOD (Carini & Albano, 2003; Casillas et al., 2006; Massip-Salcedo et al., 2007). PC reduced the accumulation of xanthine during ischemia and prevented the conversion of XDH to XOD, thus preventing the deleterious effect of this ROS generating system on liver (Fernández et al., 2002; Serafin et al., 2004b) (see Fig. 4). It is possible that NFkB and p38 MAPK-regulated transcription factors (ATF-2 and MEF2C) might be responsible for inducing the expression of protective

genes, including SOD. HSPs induced by PC might contribute to improve membrane potential and respiratory control in hepatic mitochondria, allowing a faster recovery of ATP on reoxygenation (Carini & Albano, 2003; Massip-Salcedo et al., 2007). The modulation of inflammatory response by hepatic PC has been also reported in different experimental models of warm and cold hepatic ischemia. PC reduces neutrophil accumulation, the generation of different cytokines and interleukins including TNF and IL-1 (Casillas et al., 2006; Cutrin et al., 2002; Massip-Salcedo et al., 2007). The benefits of PC were also observed on hepatic microcirculation by inhibiting the effects of different vasoconstrictor mediators such as ETs, thus ameliorating sinusoidal perfusion and microvascular dysfunction (Peralta et al., 1996; Peralta et al., 1999a). The benefits of PC regulating Ang II and adipocytokines such as adiponectin and RBP4 have been also reported in hepatic I/R. PC, through PPARα inhibits adiponectin accumulation in steatotic livers and adiponectin-worsening effects on oxidative stress and hepatic injury in hepatic resactions (Massip-Salcedo et al., 2008). In liver transplantation PC, which increases RBP4 levels, reduced PPARγ levels and hepatic injury in steatotic livers (Casillas et al., 2011). As ER stress activates an adaptive response to injury, modulating ER stress before transplantation by PC could improve the grafted organ viability (see Fig. 4). Along these lines, it has been proposed that induction of ER chaperones, particularly of BiP, underlies the phenomenon of PC in the heart, in which exposure to a transient episode of brief ischemia provides subsequent protection from a

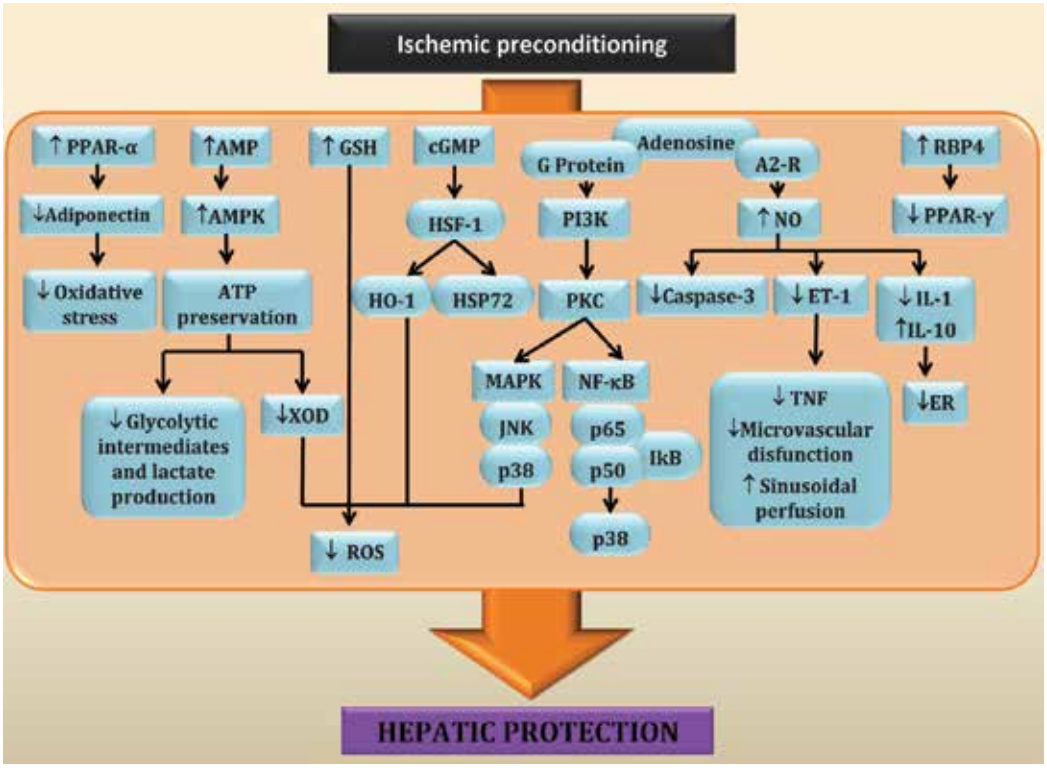


Fig. 4. Molecular basis of the ischemic preconditioning protection. (Carini & Albano, 2003; Casillas et al., 2006; Cutrin et al., 2002; Fernández et al., 2002; Massip-Salcedo et al., 2007, 2008; Peralta et al., 1996, 1999a, 2000b; Selzner, 2003; Serafin et al., 2004b)

sustained ischemic challenge (Kim et al., 2008). It is tempting to speculate that PC activates the UPR, particularly the adaptive and pro-survival aspects of ER stress (Pallet et al., 2009).

Since the effectiveness of PC was first described, numerous efforts have been made to find strategies capable of mimicking its beneficial effects. One of these strategies is known as heat shock preconditioning, in which the organ or the whole body is temporarily exposed to hyperthermia prior to hepatic ischemia. Chemical preconditioning with either doxorubicine, atrial natriuretic peptide or oxidants decreases hepatic injury in several experimental models of I/R. However, their possible clinical application seems limited owing to difficulties in implementing them in clinical practice, toxicity problems and the side-effects that have been identified (Casillas et al., 2006; Massip-Salcedo et al., 2007; Peralta et al., 1999a).

The benefits of PC observed in experimental models of hepatic warm and cold ischemia created the need for human trials of PC. To date, PC has been successfully applied in human liver resections in both steatotic and non-steatotic livers. The effectiveness of PC in hepatic surgery was first reported by Clavien et al., 2003, but unfortunately, in this study, it proved ineffective in elderly patients. It is well known that the impact of cold ischemia on organ function becomes even more significant as the age of the donor increases (Busuttil & Tanaka, 2003). Recent research indicates that melatonin prevents oxidative stress and inflammatory response in hepatocytes from elderly rats and this could improve the viability of liver grafts from elderly donors and increase the effectiveness of PC (Castillo et al., 2005).

Prevention of post-hepatectomy liver insufficiency by PC, particularly in patients with cirrhotic or steatotic livers has also been demonstrated (Nuzzo et al., 2004). A clinical study by Koneru and colleagues showed no effects of PC on cadaveric donor livers compared with controls. However, the study consisted of clamping the hepatic vessels for a period of 5 min, and as the authors concluded, that may be insufficient to obtain a beneficial effect from PC (Koneru et al., 2005). Another clinical study carried out by Azoulay and colleagues using the model of cadaveric whole liver transplantation showed that PC based on 10 min of ischemia was associated with better tolerance to ischemia. However, this was at the price of decreased early function (Azoulay et al., 2005). Beginning this year, Jassem and colleagues concluded that 10 min of preconditioning was effective to protect cadaveric donor allografts from cold ischemia, reduced inflammatory response and resulted in better graft function (Jassem et al., 2006). Further randomised clinical studies are necessary to confirm whether PC is appropriate for LT in clinical practice. The potential applications of PC in human LT are numerous. PC also has the potential to increase the number of organs suitable for LT since it can improve the outcome for marginal grafts that would not otherwise have been transplanted. Its benefits to reduce the vulnerability of steatotic grafts to I/R injury have also been reported in different experimental studies of LT (Carrasco et al., 2005; Fernández et al., 2004). Interestingly, the effectiveness of PC in clinical practice in major liver hepatectomy opens up new possibilities in living donor liver transplantation, since the ischemia period is similar in both surgical procedures. Moreover, PC increases liver regeneration, the most critical aspect to be considered in living donor liver transplantation (Franco et al., 2004). Again, PC may also have a role in the transplantation of small grafts whose pathophysiology overlaps with I/R injury. In fact, a study published by Barrier et al., 2005 has shown the benefits of PC in transplantation from living human liver donors. PC is easy to apply, inexpensive and does not require the use of drugs with potential side effects.

One disadvantage of PC is that it requires a period of pre-ischemic manipulation for organ protection.

7. Conclusion and perspectives

The hope of finding new surgical and pharmacological therapeutic applications provides a strong impetus to identify the mechanisms responsible for the failure of fatty livers. We must continue conducting researches attempting to improve the outcomes of LT using fatty liver grafts. Before a complete definition of a successful therapeutic strategy based on regulating hepatic I/R injury is stated, several additional points need to be addressed. The effects of the new potential protective strategies (TMZ, AICAR, RAS modulators, PI3K and ERK1/2 modulators, anti-apoptotic strategies, omega-3 PUFA, adiponectin, RBP4 and PC) on the pathways involved in the inflammatory process and lipid metabolism have only just been mapped. The success of these protective strategies might depend on the surgical procedure. Moreover, the response of different type of liver to these treatments might differ and involve different signal transduction pathways that are at present marginally understood. Whether the above-mentioned approaches can be translated into as viable options in the clinical practice remain unknown, but further researches are required to optimize the their use (e.g. dose, pharmacokinetics…etc). Such approaches have the potential to increase the number of organs suitable for transplantation, since they may improve the outcomes of marginal grafts that would not otherwise have been used.

8. Acknowledgment

Elias-Miró M. and Jiménez-Castro M.B. contributed equally to this work

9. References

Alfany-Fernández, I., Casillas-Ramírez, A., Bintanel-Morcillo, M., Brosnihan, K., Ferrario, C., Serafin, A., Rimola, A., Rodés, J., Rosellò-Catafau J. & Peralta C. (2009). Therapeutic targets in liver transplantation: angiotensin II in nonsteatotic grafts and angiotensin-(1-7) in steatotic grafts. Am J Transplant, Vol.9, No3, pp. 439-451

Amersi, F., Dulkanchainun, T., Nelson, S., Farmer, D., Kato, H., Zaky, J., Melinek, J., Shaw, G., Kupiec-Weglinski, J., Horwitz, L., Horwitz, M. & Busuttil, R. (2001). A novel iron chelator in combination with a P-selectin antagonist prevents ischemia/reperfusion injury in a rat liver model. Transplantation, Vol. 71, No. 1, pp. 112–118

Araya, J., Tsuruma, T., Hirata, K., Yagihashi, A. & Watanabe, N. (2002). TCV-116, an angiotensin II type 1 receptor antagonist, reduces hepatic ischemia-reperfusion injury in rats. Transplantation, Vol. 73, No. 4, pp. 529–534

Azoulay, D., DelGaudio, M., Andreani, P., Ichai, P., Sebag, M., Adam, R., Scatton, O., Min, B., Delvard, V., Lemoine, A., Bismuth, H. & Castaing, D. (2005). Effects of 10 minutes of ischemic preconditioning of the cadaveric liver on the graft's preservation and function: the ying and the yang. Annals of Surgery, Vol. 242, No. 1, pp. 133–139

Bailly-Maitre, B., Fondevila, C., Kaldas, F., Droin, N., Luciano, F., Ricci, J., Croxton, R., Krajewska M., Zapata J., Kupiec.Weglinski J., Farmer, D. & Reed, J. (2006).

Cytoprotective gene bi-1 is required for intrinsic protection from endoplasmic reticulum stress and ischemia-reperfusion injury. Proc Natl Acad Sci USA, Vol. 103, No. 8, pp. 2809-2814

Banga, N., Homer-Vanniasinkam, S., Graham, A., Al-Mukhtar, A., White, S. & Prasad, K. (2005). Ischaemic preconditioning in transplantation and major resection of the liver. Br J Surg, Vol. 92, No. 5, pp. 528-538

Barrier, A., Olaya, N., Chiappini. F., Roser, F., Scatton, O., Artus, C., Franc, B., Dudoit, S., Flahault, A., Debuire, B., Azoulay, D. & Lemoine, A. (2005). Ischemic preconditioning modulates the expression of several genes, leading to the overproduction of IL-1Ra, iNOS, and Bcl-2 in a human model of liver ischemia-reperfusion. FASEB J, Vol. 19, No. 12, pp. 1617–1626

Ben Mosbah, I., Casillas-Ramírez, A., Xaus, C., Serafín, A., Roselló-Catafau, J. & Peralta, C. (2006). Trimetazidine: Is it a promising drug for use in steatotic grafts?. World J. Gastroenterol, vol. 12, No. 6, pp. 908-914

Ben Mosbah, I., Massip-Salcedo, M., Fernández-Monteiro, I., Xaus, C., Bartrons, R., Boillot, O., Roselló-Catafau, J. & Peralta C. (2007). Addition of adenosine monophosphate-activated protein kinase activators to University of Wisconsin solution: a way of protecting rat steatotic livers. Liver Transpl, Vol. 13, No. 3, pp. 410-425

Ben Mosbah, I., Alfany-Fernández, I., Martel, C., Zaouali, M., Bintanel-Morcillo, M., Rimola, A., Rodés, J., Brenner, C., Roselló-Catafau, J. & Peralta C. (2010). Endoplasmic reticulum stress inhibition protects steatotic and non-steatotic livers in partial hepatectomy under ischemia-reperfusion. Cell Death Dis, Vol. 1, pp. e52

Bilzer, M. & Gerbes, A. (2000). Preservation injury of the liver: mechanisms and novel therapeutic strategies. J Hepatol, Vol. 32, pp. 508–515

Bradham, C., Schemmer, P., Stachlewitz, R., Thurman, R. & Brenner, D. (1999). Activation of nuclear factor-kappaB during orthotopic liver transplantation in rats is protective and does not require Kupffer cells. Liver Transplantation and Surgery, Vol. 5, No. 4, pp. 282–293

Breckenridge, D., Germain, M., Mathai, J., Nguyen, M. & Shore G. (2003). Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene, Vol. 22, pp. 8608-8618

Bugianesi, E., Pagotto, U., Manini, R., Vanni, E., Gastaldelli, A., de Iasio, R., Gentilcore, E., Natale, S., Cassader, M., Rizzetto, M., Pasquali, R. & Marchesini, G. (2005). Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab, Vol. 90, No. 6, pp. 3498-3504

Bustos, M., Beraza, N., Lasarte, J., Baixeras, E., Alzuguren, P., Bordet, T. & Prieto J. (2003). Protection against liver damage by cardiotrophin-1: a hepatocyte survival factor up-regulated in the regenerating liver in rats. Gastroenterology, Vol. 125, pp. 192-201

Busuttil, R. & Tanaka, K. (2003). The utility of marginal donors in liver transplantation. Liver Transpl, Vol. 9, No. 7, pp. 651–663

Caldwell-Kenkel, J., Currin, R., Tanaka, Y., Thurman, R. & Lemasters, J. (1989). Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology, Vol. 10, pp. 292–299.

Caraceni, P., Bianchi, C., Domenicali, M., Maria Pertosa, A., Maiolini, E., Parenti Castelli, G., Nardo, B., Trevisani, F., Lenaz, G. & Bernardi, M. (2004). Impairment of

mitochondrial oxidative phosphorylation in rat fatty liver exposed to preservation-reperfusion injury. J Hepatology, Vol. 41, No. 1, pp. 82–88

Caraceni, P., Domenicali, M., Vendemiale, G., Grattagliano, I., Pertosa, A., Nardo, B., Morselli-Labate, A.m Trevisani, F., Palasciano, G., Altomare, E. & Bernardi, M. (2005). The reduced tolerance of rat fatty liver to ischemia reperfusion is associated with mitochondrial oxidative injury. J Surg Res, Vol. 124, No. 2, pp. 160–168

Carini, R. & Albano, E. (2003). Recent insights on the mechanisms of liver preconditioning. Gastroenterology, Vol. 125, No. 5, pp. 1480–1491

Carini, R., Grazia De Cesaris, M., Splendore, R., Baldanzi, G., Nitti, M., Alchera, E., Filigheddu, N., Domenicotti, C., Pronzato, M., Graziani, A. & Albano, E. (2004). Role of phosphatidylinositol 3-kinase in the development of hepatocyte preconditioning. Gastroenterology, Vol. 127, No. 3, pp. 914-923

Carrasco-Chaumel, E., Rosello-Catafau, J., Bartrons, R., Franco-Gou, R., Xaus, C., Casillas, A., Gelpí, E., Rodés, J. & Peralta, C. (2005). Adenosine monophosphate-activated protein kinase and nitric oxide in rat steatotic liver transplantation. J Hepatol, Vol. 43, No. 6, pp. 997–1000

Casillas-Ramírez, A., Ben Mosbah, I., Ramalho, F., Rosello-Catafau, J. & Peralta, C. (2006). Past and future approaches to ischemia-reperfusion lesion associated with liver transplantation. Life Sci, Vol. 79, pp. 1881–1894

Casillas-Ramírez, A., Amine-Zaouali, M., Massip-Salcedo, M., Padrissa-Altés, S., Bintanel-Morcillo, M., Ramalho, F., Serafín, A., Rimola, A., Arroyo, V., Rodés, J., Roselló-Catafau, J. & Peralta, C. (2008). Inhibition of angiotensin II action protects rat steatotic livers against ischemia-reperfusion injury. Crit Care Med, Vol. 36, No. 4, pp. 1256-1266

Casillas-Ramírez, A., Alfany-Fernández, I., Massip-Salcedo, M., Juan, M., Planas, J., Serafin, A., Pallas, M., Rimola, A., Rodes, J. & Peralta, C. (2011). Retinol-Binding protein 4 and peroxisome proliferator-activated receptor-{gamma} in steatotic liver transplantation. J Pharmacol Exp Ther, Apr 12. Vol. 338, No. 1, pp. 143-153

Castillo, C., Salazar, V., Ariznavarreta, C., Vara, E. & Tresguerres, J. (2005). Effect of melatonin administration on parameters related to oxidative damage in hepatocytes isolated from old Wistar rats. Journal of Pineal Research, Vol. 38, No. 4, pp. 240–246

Chaisson, M., Brooling, J., Ladiges, W., Tsai, S. & Fausto, N. (2002). Hepatocytespecific inhibition of NF-kappaB leads to apoptosis after TNF treatment, but not after partial hepatectomy. Journal of Clinical Investigation, Vol. 110, No. 2, pp. 193–202

Chavin, K., Fiorini, R., Shafizadeh, S., Cheng, G., Wan, C., Evans, Z., Rodwell, D., Polito, C., Haines, J., Baillie, G. & Schmidt, M. (2004). Fatty acid synthase blockade protects steatotic livers from warm ischemia reperfusion injury and transplantation. American Journal of Transplantation, Vol. 4, No. 9, pp. 1440–1447

Chavin, K., Yang, S., Lin, H., Chatham, J., Chacko, V., Hoek, J., Walajtys-Rode, E., Rashid, A., Chen, C., Huang, C., Wu, T., Lane, M. & Diehl, A. (1999). Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. Journal of Biological Chemistry, Vol. 274, No. 9, pp. 5692–5700

Cheng, G., Polito, C., Haines, J., Shafizadeh, S., Fiorini, R., Zhou, X., Schmidt, M. & Chavin, K. (2003). Decrease of intracellular ATP content downregulated UCP2 expression in

mouse hepatocytes. Biochemical and Biophysical Research Communications, Vol. 308, No. 3, pp. 573–580

Chung, H., Pae, H., Choi, B., Billiar, T. & Kim, Y. (2001). Nitric oxide as a bioregulator of apoptosis. Biochemical and Biophysical Research Communications, Vol. 282, No. 5, pp. 1075–1079

Clavien, P., Harvey, P. & Strasberg, S. (1992). Preservation and reperfusion injuries in liver allografts. An overview and synthesis of current studies. Transplantation, Vol.53, No.5, pp. 957-978

Clavien, P., Selzner, M., Rudiger, H., Graf, R., Kadry, Z., Rousson, V. & Jochum, W. (2003). A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Annals of Surgery, Vol. 238, No. 6, pp. 843–852

Cutrin, J., Perrelli, M., Cavalieri, B., Peralta, C., Rosello-Catafau, J. & Poli, G. (2002). Microvascular dysfunction induced by reperfusion injury and protective effect of ischemic preconditioning. Free Radical Biology and Medicine, Vol. 33, No. 9, pp. 1200–1208

Degli, D., Sebagh, M., Pham, P., Reffas, M., Poüs, C., Brenner, C., Azoulay, D. & Lemoine, A. (2011). Ischemic preconditioning induces autophagy and limits necrosis in human recipients of fatty liver grafts, decreasing the incidence of rejection episodes. Cell Death Dis, Vol. 2, pp. e1-11

El-Badry, A., Breitenstein, S., Jochum, W., Washington, K., Paradis, V., Rubbia-Brandt, L., Puhan, M., Slankamenac, K., Graf, R. & Clavien, P. (2009). Assessment of hepatic steatosis by expert pathologists: the end of a gold standard. Ann Surg, Vol. 250, No. 5, pp, 691–697

El-Badry, A., Moritz, W., Contaldo, C., Tian, Y., Graf, R. & Clavien, P. (2007). Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. Hepatology, Vol. 45, No. 4, pp. 855-863

Ellett, J., Evans, Z., Atkinson, C., Schmidt, M., Schnellmann, R. & Chavin, K. (2009). Toll-like receptor 4 is a key mediator of murine steatotic liver warm ischemia/reperfusion injury. Liver Transpl, Vol. 15, pp. 1101-1109

Esfandiari, F., You, M., Villanueva, J., Wong, D., French, S. & Halsted, C. (2007). S-adenosylmethionine attenuates hepatic lipid synthesis in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res, Vol. 31, pp. 1231-1239

Essani, N., Fisher, M., Simmons, C., Hoover, J., Farhood, A. & Jaeschke, H. (1998). Increased P-selectin gene expression in the liver vasculature and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. Journal of Leukocyte Biology, Vol. 63, No. 3, pp. 288–296

Falasca, L., Tisone, G., Palmieri, G., Anselmo, A., Di Paolo, D., Baiocchi, L., Torri, E., Orlando, G., Casciani, C. & Angelico, M. (2001). Protective role of tauroursodeoxycolate during harvesting and cold storage of human liver: a pilot study in transplant recipients. Transplantation, Vol. 71, pp. 1268-1276

Fan, C., Zwacka, R. & Engelhardt, J. (1999). Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med, Vol. 77, No. 8, pp. 577-592

Farhood, A., McGuire, G., Manning, A., Miyasaka, M., Smith, C. & Jaeschke, H. (1995). Intercellular adhesion molecule 1 (ICAM-1) expression and its role in neutrophil-induced ischemia-reperfusion injury in rat liver. Journal of Leukocyte Biology, Vol. 57, No. 3, pp. 368–374

Farmer, D., Yersiz, H., Ghobrial, R., McDiarmid, S., Gornbein, J., Le, H., Schlifke, A., Amersi, F., Maxfield, A., Amos, N., Restrepo, G., Chen, P., Dawson, S. & Busuttil, R. (2001). Early graft function after pediatric liver transplantation: comparison between in situ split liver grafts and living-related liver grafts. Transplantation, Vol. 72, No. 11, pp. 1795-1802

Feng, L., Zhao, N., Yao, X., Sun, X., Du, L., Diao, X., Li, S., & Li, Y. (2007). Histidine-tryptophan-ketoglutarate solution vs. University of Wisconsin solution for liver transplantation: a systematic review. Liver Transpl, Vol. 13, No. 8, pp. 1125-1136

Fernández, L., Heredia, N., Grande, L., Gómez, G., Rimola, A., Marco, A., Gelpi, E., Rosello-Catafau, J. & Peralta, C. (2002). Preconditioning protects liver and lung damage in rat liver transplantation: role of xanthine/xanthine oxidase. Hepatology, Vol. 36, No. 3, pp. 562–572

Fernández, L., Carrasco-Chaumel, E., Serafín, A., Xaus, C., Grande, L., Rimola, A., Roselló-Catafau, J. & Peralta, C. (2004). Is ischemic preconditioning a useful strategy in steatotic liver transplantation? Am J Transplant, Vol. 4, No. 6, pp. 888-899

Franco-Gou, R., Peralta, C., Massip-Salcedo, M., Xaus, C., Serafín, A. & Roselló-Catafau, J. (2004). Protection of reduced-size liver for transplantation. Am J Transplant, Vol. 4, No. 9, pp. 1408-1420

Gao, W., Bentley, R., Madden, J. & Clavien, P. (1998). Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. Hepatology, Vol. 27, pp. 1652–1660

Genaro, A., Hortelano, S., Álvarez, A., Martínez, C. & Bosca, L. (1995). Splenic B lymphocyte programmed cell death is prevented by nitric oxide release through mechanisms involving sustained Bcl-2 levels. Journal of Clinical Investigation, Vol. 95, No. 4, pp. 1884–1890

Ghobrial, I., Witzig, T. & Adjei, A. (2005). Targeting apoptosis pathways in cancer therapy. CA: A Cancer Journal for Clinicians, Vol. 55, No. 3, pp. 178–194

Grattagliano, I., Vendemiale, G. & Lauterburg, B. (1999). Reperfusion injury of the liver: role of mitochondria and protection by glutathione ester. Journal of Surgical Research, Vol. 86, No. 1, pp. 2–8

Hassan-Khabbar, S., Cottart, C., Wendum, D., Vibert, F., Clot, J., Savouret, J., Conti, M. & Nivet-Antoine, V. (2008). Postischemic treatment by trans-resveratrol in rat liver ischemia-reperfusion: a possible strategy in liver surgery. Liver Transpl, Vol. 14, No. 4, pp. 451-459

Hong, F., Radaeva, S., Pan, H., Tian, Z., Veech, R. & Gao, B. (2004). Interleukin 6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. Hepatology, Vol. 40, No. 4, pp. 933-941

Huet, P., Nagaoka, M., Desbiens, G., Tarrab, E., Brault, A., Bralet, M. & Bilodeau, M. (2004). Sinusoidal endothelial cell and hepatocyte death following cold ischemia-warm reperfusion of the rat liver. Hepatology, Vol. 39, No. 4, pp. 1110–1119

Iimuro, Y., Nishiura, T., Hellerbrand, C., Behrns, K., Schoonhoven, R., Grisham, J. & Brenner, D. (1998). NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. Journal of Clinical Investigation, Vol. 101, No. 4, pp. 802–811

Ijaz, S., Yang, W., Winslet, M. & Seifalian, A. (2003). Impairment of hepatic microcirculation in fatty liver. Microcirculation, Vol. 10, pp. 447–556

Ikizler, M., Dernek, S., Sevin, B. & Kural, T. (2003). Trimetazidine improves recovery during reperfusion in isolated rat hearts after prolonged ischemia. Anadolu Kardiyoloji Dergisi, Vol. 3, No. 4, pp. 303–308

Imamura, H., Sutto, F., Brault, A. & Huet, P. (1995). Role of Kupffer cells in cold ischemia/reperfusion injury of rat liver. Gastroenterology, Vol. 109, No. 1, pp. 189–197

Jaeschke, H. (1996). Preservation injury: mechanisms, prevention and consequences. J Hepatol, Vol.25, No.5, pp. 774-780

Jaeschke H. (1998). Mechanisms of reperfusion injury after warm ischemia of the liver. J Hepatobiliary Pancreat Surg, Vol.5, No.4, pp.402–408

Jaeschke, H. (2003). Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol, Vol. 284, pp. G15–26

Jaeschke, H., Farhood, A., Fisher, M. & Smith, C. (1996). Sequestration of neutrophils in the hepatic vasculature during endotoxemia is independent of beta 2 integrins and intercellular adhesion molecule-1. Shock, Vol. 6, No. 5, pp. 351–356

Jaeschke, H. & Lemasters, J. (2003). Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology, Vol. 125, No. 4, pp. 1246–1257

Jaeschke, H. & Mitchell, J. (1989). Mitochondria and xanthine oxidase both generate reactive oxygen species in isolated perfused rat liver after hypoxic injury. Biochemical and Biophysical Research Communications, Vol. 160, No. 1, pp. 140–147

Jassem, W., Fuggle, S., Cerundolo, L., Heaton, N. & Rela, M. (2006). Ischemic preconditioning of cadaver donor livers protects allografts following transplantation. Transplantation, Vol. 81, No. 2, pp. 169–174

Jeon, B., Yeom, D. & Lee, S. (2001). Protective effect of allopurinol on hepatic energy metabolism in ischemic and reperfused rat liver. Shock, Vol. 15, No. 2, pp. 112–117

Kang, J., Koh, E. & Lee, S. (2011). Melatonin protects liver against ischemia and reperfusion injury through inhibition of toll-like receptor signaling pathway. J Pineal Res, Vol. 50, pp. 403-411

Kayawake, S., Narbaitz, R. & Kako, K. (1982). Effects of chloroquine and nifedipine on the phospholipid content and enzyme activity in the subcellular fraction of ischemic rat liver. Basic Research in Cardiology, Vol. 77, No. 2, pp. 140–157

Kim, I., Xu, W. & Reed, J. (2008). Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov, Vol. 7, pp. 1013-1030

Kohli, V., Selzner, M., Madden, J., Bentley, R. & Clavien, P. (1999). Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation, Vol. 67, pp. 1099–1105

Koneru, B., Fisher, A., He, Y., Klein, K., Skurnick, J., Wilson, D., de la Torre, A., Merchant, A., Arora, R. & Samanta, A. (2005). Ischemic preconditioning in deceased donor liver transplantation: a prospective randomized clinical trial of safety and efficacy. Liver Transplant, Vol. 11, No. 2, pp. 196–202

Kubes, P., Payne, D. &Woodman, R. (2002). Molecular mechanisms of leukocyte recruitment in postischemic liver microcirculation. American Journal of Physiology: Gastrointestinal and Liver Physiology, Vol. 283, No. 1, pp. G139–G147

Kukan, M. & Haddad, P. (2001). Role of hepatocytes and bile duct cells in preservation-reperfusion injury of liver grafts. Liver Transpl, Vol. 7, pp. 381–400

Kumamoto, Y., Suematsu, M., Shimazu, M., Kato, Y., Sano, T., Makino, N., Hirano, K., Naito, M., Wakabayashi, G., Ishimura, Y. & Kitajima, M. (1999). Kupffer cell-independent acute hepatocellular oxidative stress and decreased bile formation in post-cold-ischemic rat liver. Hepatology, Vol. 30, No. 6, pp. 1454-1463

Lentsch, A., Kato, A., Yoshidome, H., McMasters, K. & Edwards, M. (2000). Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology, Vol. 32, No. 2, pp. 169-173

Man, K., Zhao, Y., Xu, A., Lo, C., Lam, K., Ng, K., Ho, J., Sun, C., Lee, T., Li, X. & Fan, S. (2006). Fat-derived hormone adiponectin combined with FTY720 significantly improves small-for-size fatty liver graft survival. Am J Transplant, Vol. 6, No. 3, pp. 467-476

Malhi, H., Gores, G. & Lemasters, J. (2006). Apoptosis and necrosis in the liver: a tale of two deaths? Hepatology, Vol. 43, No. 2 Suppl 1, PP. S31–S44

Massip-Salcedo, M., Casillas-Ramírez, A., Franco-Gou, R., Bartrons, R., Ben Mosbah, I., Serafin, A., Roselló-Catafau, J. & Peralta C. (2006). Heat shock proteins and mitogen-activated protein kinases in steatotic livers undergoing ischemia-reperfusion: some answers. Am J Pathol, Vol. 168, No. 5, pp. 1474–1485

Massip-Salcedo, M., Roselló-Catafau, J., Prieto, J., Avila, M. & Peralta, C. (2007). The response of the hepatocyte to ischemia. Liver Int, Vol. 27, pp. 6-16

Massip-Salcedo, M., Zaouali, M., Padrissa-Altés, S., Casillas-Ramírez, A., Rodés, J., Roselló-Catafau, J. & Peralta, C. (2008). Activation of peroxisome proliferator-activated receptor-alpha inhibits the injurious effects of adiponectin in rat steatotic liver undergoing ischemia-reperfusion. Hepatology, Vol. 47, pp 461-472

McCormack, L., Dutkowski, P., El-Badry, A. & Clavien, P. (2011). Liver transplantation using fatty livers: Always feasible? J Hepatol, Vol. 54, No. 5, pp. 1055-1062

McCuskey, R., Urbaschek, R. & Urbaschek, B. (1996). The microcirculation during endotoxemia. Cardiovascular Research, Vol. 32, No. 4, pp. 752–763

McKeown, C., Edwards, V., Phillips, M., Harvey, P., Petrunka, C. & Strasberg, S. (1988). Sinusoidal lining cell damage: the critical injury in cold preservation of liver allografts in the rat. Transplantation, Vol. 46, No. 2, pp. 178–191

Metzger, J., Dore, S. & Lauterburg, B. (1988). Oxidant stress during reperfusion of ischemic liver: no evidence for a role of xanthine oxidase. Hepatology, Vol. 8, No. 3, pp. 580–584

Muller, C., Dunschede, F., Koch, E., Vollmar, A. & Kiemer, A. (2003). Alpha-lipoic acid preconditioning reduces ischemia-reperfusion injury of the rat liver via the PI3-kinase/Akt pathway. Am J Physiol Gastrointest Liver Physiol, Vol. 285, pp. G769-778

Murry, C., Jennings, R. & Reimer, K. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation, Vol. 74, pp. 1124–1136

Nakano, S., Inada, Y., Masuzaki, H., Tanaka, T., Yasue, S., Ishii, T., Arai, N., Ebihara, K., Hosoda, K., Maruyama, K., Yamazaki, Y., Shibata, N. & Nakao, K. (2007). Bezafibrate regulates the expression and enzyme activity of 11beta-hydroxysteroid dehydrogenase type 1 in murine adipose tissue and 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab, Vol. 292, No. 4, pp. 1213-1222

Natori, S., Selzner, M., Valentino, K., Fritz, L., Srinivasan, A., Clavien, P. & Gores, G. (1999). Apoptosis of sinusoidal endothelial cells occurs during liver preservation injury by a caspase-dependent mechanism. Transplantation, Vol. 68, No. 1, pp. 89–96

Neumeier, M., Hellerbrand, C., Gäbele, E., Buettner, R., Bollheimer, C., Weigert, J., Schäffler, A., Weiss, T., Lichtenauer, M., Schölmerich, J. & Buechler, C. (2006). Adiponectin and its receptors in rodent models of fatty liver disease and liver cirrhosis. World J Gastroenterol, Vol. 12, No. 34, pp. 5490-5494

Noack, K., Bronk, S., Kato, A. & Gores, G. (1993). The greater vulnerability of bile duct cells to reoxygenation injury than to anoxia. Implications for the pathogenesis of biliary strictures after liver transplantation. Transplantation, Vol. 56, pp. 495-500

Nuzzo G, Giuliante F, Vellone M, De Cosmo, G., Ardito, F., Murazio, M., D'Acapito, F. & Giovannini, I. (2004). Pedicle clamping with ischemic preconditioning in liver resection. Liver Transpl, Vol. 10, No. 2 Suppl 1, pp. S53–S57

Olschewski, P., Hunold, G., Eipel, C., Neumann, U., Schöning, W., Schmitz, V., Vollmar, B., Neuhaus, P., & Puhl, G. (2008). Improved microcirculation by low-viscosity histidine-tryptophan-ketoglutarate graft flush and subsequent cold storage in University of Wisconsin solution: results of an orthotopic rat liver transplantation model. Transpl Int, Vol. 21, No. 12, pp. 1175-1180

Oyadomari, S. & Mori M. (2004). Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ, Vol. 11, pp. 381-389

Özcan, U., Yilmaz, E., Ozcan, L., Furushashi, M., Vaillancourt, E., Smith, R., Görgün, C. & Hotamisligil, G. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science, Vol. 313, pp. 1137-1140

Pallet, N., Fougeray, S., Beaune, P., Legendre, C., Thervet, E. & Anglicheau, D. (2009). Endoplasmic reticulum stress: an unrecognized actor in solid organ transplantation. Tansplantation, Vol. 15, pp. 605-613

Peralta, C., Closa, D., Hotter, G., Gelpi, E., Prats, N. & Rosello-Catafau, J. (1996). Liver ischemic preconditioning is mediated by the inhibitory action of nitric oxide on endothelin. Biochem Biophys Res Commun, Vol. 229, pp 264–70

Peralta, C., Leon, O., Xaus, C., Prats, N., Jalil, E., Planell, E., Puig-Parellada, P., Gelpi, E. & Rosello-Catafau, J. (1999a). Protective effect of ozone treatment on the injury associated with hepatic ischemia-reperfusion: antioxidant-prooxidant balance. Free Radical Research, Vol. 31, No. 3, pp. 191–196

Peralta, C., Prats, N., Xaus, C., Gelpi, E. & Rosello-Catafau, J. (1999b). Protective effect of liver ischemic preconditioning on liver and lung injury induced by hepatic ischemia-reperfusion in the rat. Hepatology, Vol. 30, No. 6, pp. 1481–1489

Peralta, C., Bartrons, R., Riera, L., Manzano, A., Xaus, C., Gelpí, E. & Roselló-Catafau, J. (2000a). Hepatic preconditioning preserves energy metabolism during sustained ischemia. Am J Physiol Gastrointest Liver Physiol, Vol. 279, No. 1, pp. G163–G171

Peralta, C., Bulbena, O., Bargalló, R., Prats, N., Gelpí, E. & Roselló-Catafau, J. (2000b). Strategies to modulate the deleterious effects of endothelin in hepatic ischemia-reperfusion. Transplantation, Vol. 70, No. 12, pp. 1761-1770

Peralta, C., Rull, R., Rimola, A., Deulofeu, R., Rosello-Catafau, J., Gelpi, E. & Rodes, J. (2001a). Endogenous nitric oxide and exogenous nitric oxide supplementation in hepatic ischemia-reperfusion injury in the rat. Transplantation, Vol. 71, No. 4, pp. 529–536

Peralta, C., Fernandez, L., Panes, J., Prats, N., Sans, M., Pique, J., Gelpi, E. & Rosello-Catafau, J. (2001b). Preconditioning protects against systemic disorders associated with hepatic ischemia-reperfusion through blockade of tumor necrosis factor-induced P-selectin up-regulation in the rat. Hepatology, Vol. 33, No. 1, pp. 100–113

Pesonen, E., Linder, N., Raivio, K., Sarnesto, A., Lapatto, R., Hockerstedt, K., Makisalo, H. & Andersson, S. (1998). Circulating xanthine oxidase and neutrophil activation during human liver transplantation. Gastroenterology, Vol. 114, No. 5, pp. 1009–1115

Polyak, M., Arrington, B., Kapur, S., Stubenbord, W. & Kinkhabwala, M. (2000). Glutathione supplementation during cold ischemia does not confer early functional advantage in renal transplantation. Transplantation, Vol. 70, No. 1, pp. 202–205

Ramalho, F., Ramalho, L., Castro-e-Silva Junior, O., Zucoloto, S. & Correa, F. (2002). Effect of angiotensin-converting enzyme inhibitors on liver regeneration in rats. Hepatogastroenterology, Vol. 49, No. 47, pp. 1347–1351

Ramalho, F., Alfany-Fernandez, I., Casillas-Ramírez, A., Massip-Salcedo, M., Serafín, A., Rimola, A., Arroyo, V., Rodés, J., Roselló-Catafau, J. & Peralta, C. (2009). Are angiotensin II receptor antagonists useful strategies in steatotic and nonsteatotic livers in conditions of partial hepatectomy under ischemia-reperfusion? J Pharmacol Exp Ther, Vol. 329, No. 1, pp. 130-140

Reddy, S., Zilvetti, M., Brockmann, J., McLaren, A. & Friend, P. (2004). Liver transplantation from non-heart-beating donors: current status and future prospects. Liver Transpl, Vol. 10, No. 10, pp. 1223–1232

Reynaert, H., Geerts, A. & Henrion, J. (2005). Review article: the treatment of non-alcoholic steatohepatitis with thiazolidinediones. Aliment Pharmacol Ther, Vol. 22, pp. 897-905

Rogers, C., Ajmo, J. & You, M. (2008). Adiponectin and alcoholic fatty liver disease. Life, Vol. 60, pp. 790-797

Sasaki, H., Matsuno, T., Tanaka, N. & Orita, K. (1996). Activation of apoptosis during the reperfusion phase after rat liver ischemia. Transplant Proc, Vol. 28, pp. 1908–1909

Schäffler, A., Schölmerich, J. & Büchler, C. (2005). Mechanisms of disease: adipocytokines and visceral adipose tissue-emerging role in nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol, No. 2, pp. 273-280

Schauer, R., Bilzer, M., Kalmuk, S., Gerbes, A., Leiderer, R., Schildberg, F. & Messmer K. (2001). Microcirculatory failure after rat liver transplantation is related to Kupffer cell-derived oxidant stress but not involved in early graft dysfunction. Transplantation, Vol. 72, pp. 1692-1699

Schmidt, R. (2010). Hepatic organ protection: from basic science to clinical practice. World J Gastroenterol, Vol. 16, No. 48, pp. 6044-6045

Seifalian, A., Piasecki, C., Agarwal, A. & Davidson, B. (1999). The effect of graded steatosis on flow in the hepatic parenchymal microcirculation. Transplantation, Vol. 68, pp. 780-784

Selzner, M., Rüdiger, H., Sindram, D., Madden, J. & Clavien, P. (2000). Mechanisms of ischemic injury are different in the steatotic and normal rat liver. Hepatology, Vol. 32, No. 6, pp. 1280-1288

Selzner, N., Rudiger, H., Graf, R. & Clavien, P. (2003). Protective strategies against ischemic injury of the liver. Gastroenterology Vol. 125, pp. 917–936

Serafin, A., Rosello-Catafau, J., Prats, N., Xaus, C., Gelpi, E. & Peralta, C. (2002). Ischemic preconditioning increases the tolerance of fatty liver to hepatic ischemia-reperfusion injury in the rat. American Journal of Pathology, Vol. 161, No. 2, pp. 587–601

Serafin, A., Rosello-Catafau, J., Prats, N., Gelpi, E., Rodes, J. & Peralta, C. (2004a). Ischemic preconditioning affects interleukin release in fatty livers of rats undergoing ischemia/reperfusion. Hepatology, Vol. 39, No. 3, pp. 688–698

Serafin, A., Fernandez-Zabalegui, L., Prats, N., Wu, Z., Rosello-Catafau, J. & Peralta, C. (2004b). Ischemic preconditioning: tolerance to hepatic ischemia-reperfusion injury. Histol Histopathol, Vol. 19, pp. 281–289

Shah, V. & Kamath, P. (2003). Nitric oxide in liver transplantation: pathobiology and clinical implications. Liver Transpl, Vol. 9, pp. 1–11

Soltys, K., Dikdan, G. & Koneru, B. (2001). Oxidative stress in fatty livers of obese Zucker rats: rapid amelioration and improved tolerance to warm ischemia with tocopherol. Hepatology, Vol. 34, No. 1, pp. 13–18

Somia, N. & Verma, I. (2000). Gene therapy: trials and tribulations. Nature reviews: Genetics, Vol. 1, No. 2, pp. 91–99

Stewart, Z., Cameron, A., Singer, A., Montgomery, R., & Segev, D. (2009). Histidine-Tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. Am J Transplant, Vol. 9, No. 2, pp. 286-293

Straatsburg, I., Abrahamse, S., Song, S., Hartman, R., & Van Gulik, T. (2002). Evaluation of rat liver apoptotic and necrotic cell death after cold storage using UW, HTK, and Celsior. Transplantation, Vol. 4, No. 4, pp. 458-464

Takahashi, Y., Ganster, R., Gambotto, A., Shao, L., Kaizu, T., Wu, T., Yagnik, G., Nakao, A., Tsoulfas, G., Ishikaea, T., Okuda, T., Geller, D. & Murase, N. (2002). Role of NF-kappaB on liver cold ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol, Vol. 283, No. 5, pp. G1175-1184

Takei, Y., Marzi, I., Kauffman, F., Currin, R., Lemasters, J. & Thurman, R. (1990). Increase in survival time of liver transplants by protease inhibitors and a calcium channel blocker, nisoldipine. Transplantation, Vol. 50, No. 1, pp. 14–20

Targher, G., Bertolini, L., Scala, L., Poli, F., Zenari, L. & Falezza, G. (2004). Decreased plasma adiponectin concentrations are closely associated with nonalcoholic hepatic steatosis in obese individuals. Clin Endocrinol (Oxf), Vol. 61, pp. 700-703

Targher, G., Bertolini, L., Rodella, S., Zoppini, G., Scala, L., Zenari, L., & Falezza, G. (2006). Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. Clin Endocrinol (Oxf), Vol. 64, pp. 679-683

Teoh, N. & Farrell, G. (2003). Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol, Vol. 18, No. 8, pp. 891-902

Teoh, N., Leclercq, I., Pena, A. & Farell, G. (2003). Low-dose TNF-alpha protects against hepatic ischemia-reperfusion injury in mice: implications for preconditioning. Hepatology, Vol. 37, No. 1, pp. 118–128

Teoh, N., Williams, J., Hartley, J., Yu, J., McCuskey, R. & Farrell, G. (2010). Short-term therapy with peroxisome proliferation-activator receptor-alpha agonist Wy-14,643 protects murine fatty liver against ischemia-reperfusion injury. Hepatology, Vol. 51, No. 3, pp. 996-1006

Terada, K., Kaziro, Y. & Satoh, T. (2000). Analysis of Ras-dependent signals that prevent caspase-3 activation and apoptosis induced by cytokine deprivation in hematopoietic cells. Biochemical and Biophysical Research Communications, Vol. 267, No. 1, pp. 449–455

Tian, Y., Jochum, W., Georgiev, P., Moritz, W., Graf, R. & Clavien, P. (2006). Kupffer cell-dependent TNF-alpha signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse. Proc Natl Acad Sci USA, Vol. 103, pp. 4598-4603

Tolba, R., Putz, U., Decker, D., Dombrowski, F. & Lauschke, H. (2003). L-carnitine ameliorates abnormal vulnerability of steatotic rat livers to cold ischemic preservation. Transplantation, Vol. 76, No. 12, pp. 1681–1686

Vajdova, K., Graf, R. & Clavien, P. (2002). ATP-supplies in the cold-preserved liver: a long-neglected factor of organ viability. Hepatology, Vol. 36, pp. 1543–1552

Vilatoba, M., Eckstein, C., Bilbao, G., Smyth, C., Jenkins, S., Thompson, J., Eckhoff, D. & Contreras, J. (2005) Sodium a-phenylbutyrate protects against liver ischemia reperfusion injury by inhibition of endoplasmic reticulum-stress mediated apoptosis. Surgery, Vol. 138, pp. 342-351

Vollmar, B., Glasz, J., Menger, M. & Messmer, K. (1995). Leukocytes contribuye to hepatic ischemia/reperfusion injury via intercellular adhesion molecule-1-mediated venular adherence. Surgery, Vol. 117, No. 2, pp. 195–200

Wan, C., Wang, C., Liu, T., Cheng, R. & Wang, H. (2008). Alleviation of ischemia/reperfusion injury in ob/ob mice by inhibiting UCP-2 expression in fatty liver. World J Gastroenterol, Vol. 14, No. 4, pp. 590-594

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. & Tataranni, P. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab, Vol. 86, pp. 1930-1935

Wiezorek, J., Brown, D., Kupperman, D. & Brass, C. (1994). Rapid conversion to high xanthine oxidase activity in viable Kupffer cells during hypoxia. J Clin Invest, Vol. 94, pp. 2224-2230

Xu, A., Wang, Y., Keshaw, H., Xu, L., Lam, K. & Cooper, G. (2003). The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest, Vol. 112, pp. 91-100

Xu, C., Bailly-Maitre, B. & Reed, J. (2005). Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest, Vol. 115, pp. 2656-2664

Yin, X. (2000). Bid, a critical mediator for apoptosis induced by the activation of Fas/TNF-R1 death receptors in hepatocytes. Journal of Molecular Medicine, Vol. 78, No. 4, pp. 203–211

Yonezawa, K., Tolba, R., Wetter, A., Yamamoto, Y., Yamaoka, Y. & Minor, T. (2005). L-carnitine could not improve hepatic warm ischemia-reperfusion injury despite ameliorated blood flow. Journal of Surgical Research, Vol. 125, No. 1, pp. 16–22

Yoshinari, D., Takeyoshi, I., Kobayashi, M., Koyama, T., Iijima, K., Ohwada, S., Matsumoto, K. & Morishita, Y., 2001. Effects of a p38 mitogen-activated protein kinase inhibitor as an additive to University ofWisconsin solution on reperfusion injury in liver transplantation. Transplantation, Vol. 72, No. 1, 22–27

Zaouali, M., Padrissa-Altés, S., Ben Mosbah, I., Alfany-Fernandez, I., Massip-Salcedo, M., Casillas-Ramirez, A., Bintanel-Morcillo, M., Boillot, O., Serafin, A., Rimola, A., Rodés, J., Roselló-Catafau, J. & Peralta, C. (2010). Improved rat steatotic and nonsteatotic liver preservation by the addition of epidermal growth factor and insulin-like growth factor-I to University of Wisconsin solution. Liver Transpl, Vol. 16, No. 9, pp. 1098-111

Zhong, Z., Connor, H., Mason, R., Qu, W., Stachlewitz, R., Gao, W., Lemasters J. & Thurman R. (1996). Destruction of Kupffer cells increases survival and reduces graft injury after transplantation of fatty livers from ethanol-treated rats. Liver Transpl Surg, Vol. 2, No. 383-387

Zhu, B., Sun, Y., Sievers, R., Browne, A., Pulukurthy, S., Sudhir, K., Lee, R., Chou, T., Chatterjee, K. & Parmley, W. (2000). Comparative effects of pretreatment with captopril and losartan on cardiovascular protection in a rat model of ischemia-reperfusion. J Am Coll Cardiol, Vol. 35, No. 3, pp. 787-795

3

The Influences of Nitric Oxide on Liver Ischemia-Reperfusion Injury

Erik Stoltenberg, Alexander A. Vitin,
Bradley E. Hansen and John D. Lang, Jr.
University of Washington, Seattle,
Department of Anesthesiology and Pain Medicine
USA

1. Introduction

Ischemia-reperfusion injury (IRI) is a series of multifaceted cellular events that takes place on the resumption of oxygen delivery to the affected organ after a period of hypoxia. IRI occurs in the liver during procedures that are associated with vascular inflow obstruction followed by restoration of blood flow, particularly during orthotopic liver transplantation. IRI can result in major hepatocellular damage.

In the last decade, nitric oxide (NO) has been shown to have various protective effects on cells during IRI. NO was first described as endothelium derived relaxing factor (EDRF), and it was described as being released from vascular endothelium to induce smooth muscle vasorelaxation. Since that time, much more has been elucidated about the role of NO in biological systems. NO has been demonstrated to inhibit oxidative stress, cytokine release, leukocyte endothelial adhesion and apoptosis (Phillips, Toledo, Lopez-Neblina, Anaya-Prado, & Toledo-Pereyra, 2009). On a cellular-signaling level, NO effects are mediated via redox-sensitive sites, and include: inhibition of protein kinase C, activation of tyrosine kinase, inactivation of NF-kB and activation of G proteins (Y. M. Kim, de Vera, Watkins, & Billiar, 1997). Previous studies have demonstrated that a reduction of NO during hepatic IRI, generally via a reduction in endothelial nitric oxide synthase (eNOS) activity, leads to liver injury (Köken & Inal, 1999). Inhaled NO or NO donor drugs are novel treatments that have been used clinically to attenuate liver IRI (Zaky, Siriussawakul, Tostenrud, Pauldine, & J. Lang, 2009). This review will discuss the pathophysiology of liver involvement during IRI and the clinical use of NO and its sister compounds in ameliorating the impact of liver IRI.

2. An overview of hepatic ischemia reperfusion injury

As noted above, the pathophysiology of IRI is multifactorial and involves a multitude of oxidative and cellular mechanisms. Briefly, hepatic IRI can be described as a two phase process with early (acute) and late (sub-acute) injury (Fan, Zwacka, & Engelhardt, 1999; Zwacka, Zhang, Zhou, Halldorson, & Engelhardt, 1998b). The distinction is particularly important because potential theraputic targets (i.e. methods of increasing NO in the hepatic

micro-envirnoment) may have different effects on these two phases. Early injury is mediated by a rapid change in the biochemical redox state of the tissue to a more oxidative one. It occurs within 5 minutes, and is not associated with leukocyte infiltration. Following the acute state is an increase in endothelial cell adhesion molecules, chemokines and cytokines. These molecules then herald the late phase characterized by a significant infiltration of polymorphonuclear neutrophils, further release of a reactive oxygen species (ROS) and extensive inflammation and tissue injury.

NO plays a significant role in the acute phase of IRI, as this phase is associated with a rapid decrease in available NO. This decrease occurs either by depressed production by eNOS in sinusoidal endothelial cells (SECs), increased degradation by ROS, or both. The ROS implicated are chiefly O2•- (superoxide, see next paragraph), but also include hydrogen peroxide (H_2O_2). In the last few years, the implicated enzyme responsible for production of ROS has shifted from hepatoctye xanthine oxidase to NADPH oxidase in Kuppfer cells or mitochondrial sources of ROS (Hines & Grisham, 2011).

The term "reactive oxygen species" in the context of hepatic IRI primarily refers to superoxide. Two studies that incorporated manganese superoxide dismutase (MnSOD) – an enzyme which degrades superoxide – into liver tissue showed attenuation of IRI (He et al., 2006; Zwacka et al., 1998a). Therefore superoxide itself seems important in IRI. The mechanism by which superoxide imparts its damage is somewhat unclear, but it is known that membrane lipid peroxidation is associated with oxidative damage. Perhaps more importantly, damage by superoxide to mitochondrial membrane proteins and therefore ATP generating capacity and may a more important mechanism in IRI(Madesh & Hajnóczky, 2001; Moon et al., 2008).

3. Nitric oxide biochemistry

NO is a highly reactive molecule with other free radical species and possesses an extremely short half-life (Rubbo, Darley-Usmar, & Freeman, 1996). NO is produced endogenously or delivered exogenously where it can react with a variety of cellular targets resulting in vasorelaxation, enhanced neuronal transmission, reduced apoptosis, inhibition of neutrophil aggregation and adhesion, and modulation of vascular smooth muscle proliferation.

NO synthesis is dependent on the enzyme nitric oxide synthase (NOS). NOS catalyzes the net reaction:

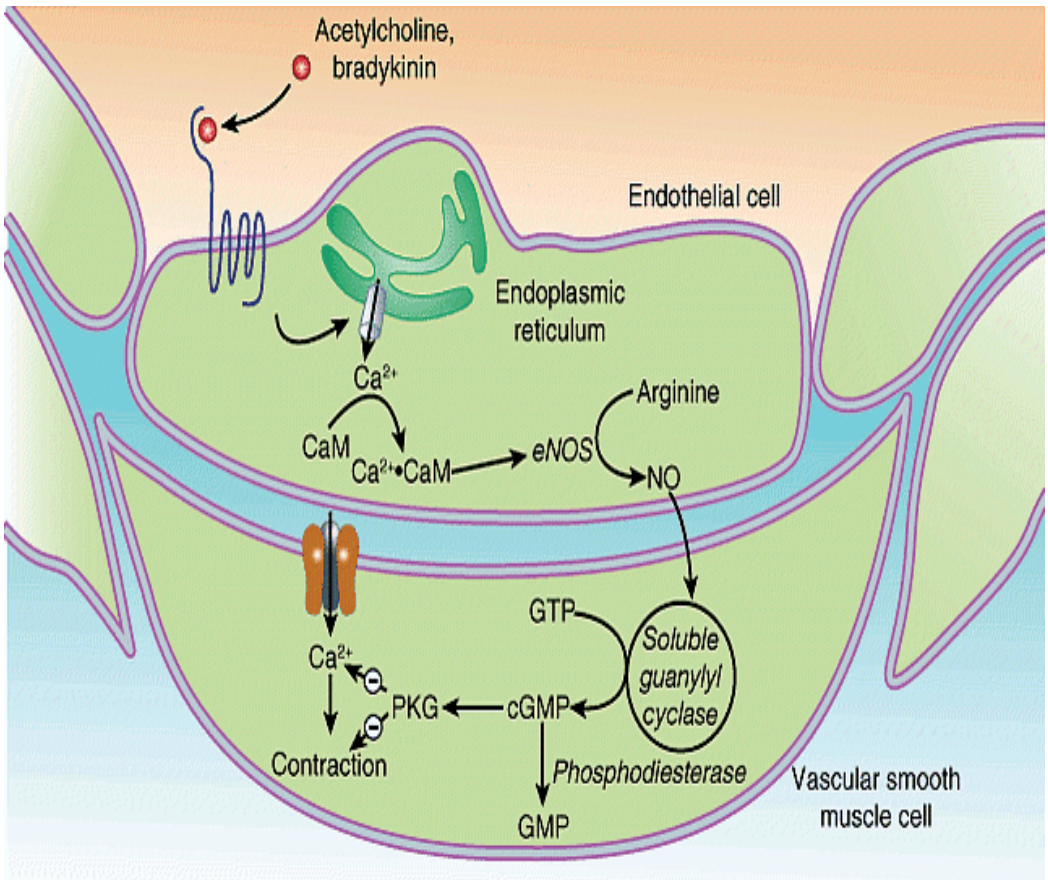
$$\text{L-Arginine} + \text{NADPH} + O_2 = \text{Citrulline} + \text{Nitric oxide} + \text{NADP}^+ \quad (1)$$

(adapted from Alderton, Cooper, & Knowles, 2001)

This complex enzyme system generates NO from the terminal nitrogen atom of L-arginine in the presence of NADPH and dioxygen. NOS binds flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH_4) and calmodulin from L-arginine and oxygen by a family of three NO synthases (NOS), all of which are expressed in a variety of cell types.

Three distinct isoforms are known: 1) Neuronal NOS (NOS I), is produced in central and peripheral nerves and is pivotal in neuronal transmission and cell-to-cell communication

within the central nervous system. 2) Inducible NOS (NOS II is induced by an inflammatory stimulus such as a microbe (Parul Tripathi, Prashant Tripathi, Kashyap, & Singh, 2007). Unlike the other types of NOS (I and III), NOS II is not constitutive and is independent of calcium regulation. While NOS II is expressed by immune cells such as neutrophils and macrophages, it is also present in other cell lines including hepatocytes. Endothelial NOS (NOS III), is constitutively expressed by endothelial cells and is critical for the regulation of vascular function, more specifically vasorelaxation.



(Modified, with permission from http://www.wiley.com/college/boyer)

Fig. 1. Mechanisms of smooth muscle relaxation. NO diffuses across the muscle cell membrane and binds to guanylyl cyclase. Guanylyl cyclase catalyzes the synthesis of cyclic GMP from GTP. cGMP activates a cGMP-dependent protein kinase which stimulates the uptake of calcium by the endoplasmic reticulum of the muscle cell. The reduced levels of cytoplasmic calcium cause the muscle cell to relax. As a consequence of muscle cell relaxation, vasodilation occurs. PKG – protein kinase G

The generation of NO leads to several actions that promote smooth muscle relaxation. First, activation of guanylate cyclase raises the level of intracellular cGMP which in turn inhibits the entry of calcium into the cell thereby inducing smooth muscle relaxation. Second,

activation of K^+ channels leads to cellular hyperpolarization and relaxation. Finally, stimulation of cGMP-dependent protein kinase activates of myosin light chain phosphatase leading to dephosphorylation of myosin light chains resulting in smooth muscle relaxation. NOSs are related but encoded by distinct genes. Classically, the ability of NO to elicit vasorelaxation is due to its ability to increase intracellular levels of cyclic guanosine monophosphate (cGMP) through the activation of soluble guanylate cyclase (sGC). cGMP–dependent protein kinases in turn decrease the sensitivity of myosin to calcium-induced contraction and lower intracellular calcium by activation of calcium-sensitive potassium channels and inhibits the release of calcium from the sacroplasmic reticulum. Mechanisms of smooth muscle relaxation are shown on Figure 1.

4. Brief review of the pathophysiology of IRI

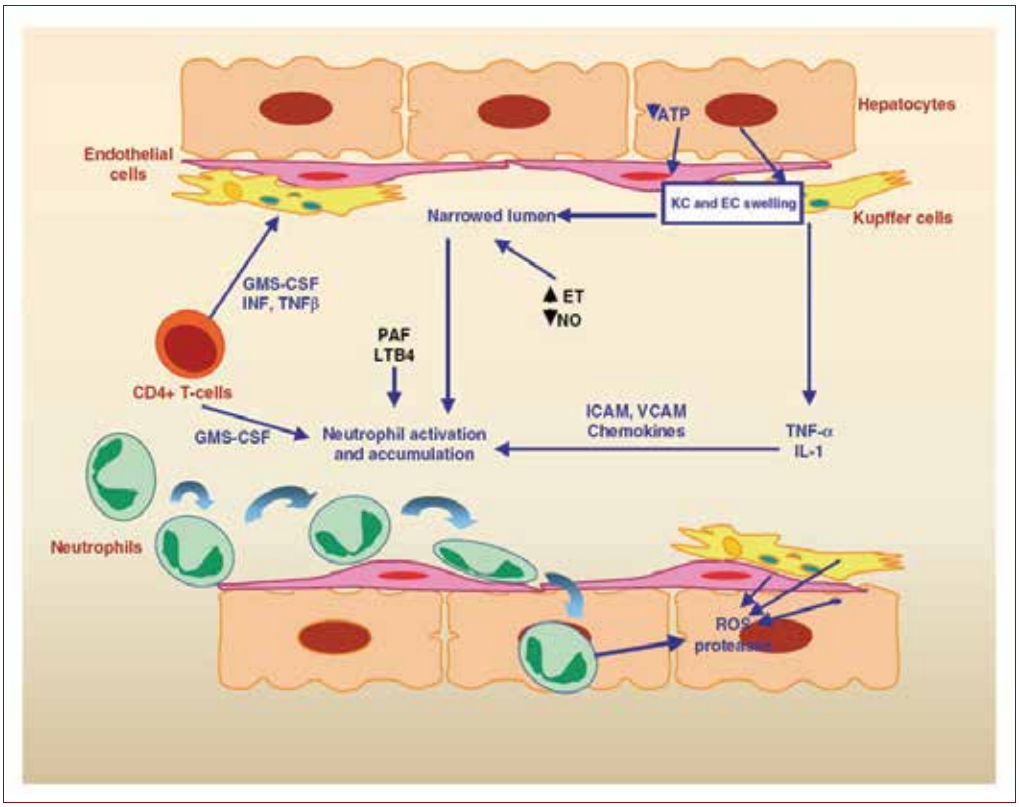


Fig. 2. Multifaceted mechanism are involved in the causation of hepatic ischemia-reperfusion injury (IRI). Kupffer and endothelial cells produce cytokines and chemokines, recruiting neutrophils that further accentuate injury. EC, endothelial cell. NO (nitric oxide) is decreased as a result of IRI allowing for decreased perfusion and exaggerated injury (Massip-Salcedo, Roselló-Catafau, Prieto, Avíla, & Peralta, 2007). KC, Kuppfer cell. ATP, adenosine triphosphate. TNF, tumor necrosis factor. IL, interleukin. ICAM, intercellular adhesion molecule. PAF, platelet activation factor. LTB4, leukotrien B4. GMS-CSF, granulocyte macrophage colony stimulating factor. INF, interferon. ROS, reactive oxygen species. (Slighty modified with permission of Dr. Joan Rosello-Catafau, Barcelona, Spain)

During the ischemic phase, anaerobic metabolism ensues and produces an inadequate amount of high-energy phosphates which are fundamental to most cellular functions. Low levels of high-energy phosphates affects a myriad of cellular functions: homeostasis, signaling interactions, cellular proliferation and processing of the apoptotic death cycle. Adenosine triphosphate (ATP) depletion impairs the sodium/potassium ATPase (Na+/K+- ATPase) function, resulting in an impairment of the efflux of sodium from the cell. Additionally, toxic metabolites – which are generated during ischemia – attract free water into ischemic cells and organelles leading to the formation of cellular edema (Jennings, Shen, Hill, Ganote, & Herdson, 1978). If the ischemic insult lasts greater than 24 hours, it is likely that ATP-synthase activity becomes irreversible after blood restoration, leading to cellular necrosis, apoptosis or necroapoptosis (Sammut et al., 2000). Ischemia also causes an increased expression of adhesion molecules that leads to endothelial cell and neutrophil adhesion resulting in vascular studding and occlusion (Yadav et al., 1998). Furthermore, disequilibrium between NO and endothelin (ET) induces vasoconstriction and subsequent microcirculatory failure even though blood circulation has been re-established (Montalvo-Jave, Escalante-Tattersfield, Ortega-Salgado, Piña, & Geller, 2008). Reestablishment of blood flow will serve to amplify inflammation with consequent injury that is highly variable but dependent on numerous variables that include the extent of mediators produced (i.e. reactive oxygen species), the degree of endothelial and neutrophil adhesive responses and the degree of Kuppfer cell activation.

5. Principal participants in liver IRI

5.1 Sinusoidal endothelial cells (SEC)

Injury to these cells is initiated during cold ischemia whereby Ca^{2+}-ATPase results in the accumulation of intracellular calcium (Bigelow & Thomas, 1987). Following this event, a series of actions occur making the endothelium more susceptible to platelet adhesion and reduced sinusoidal flow.

5.2 Kupffer cells

Kupffer cells are crucial in liver injury orchestration. Metabolic alterations of these cells occur during no-flow ischemia leading to the formation of reactive oxygen species during early reperfusion (Jaeschke, Bautista, Spolarics, & Spitzer, 1991). Additionally, at the onset of reperfusion Kupffer cells undergo further activation by toll-like receptor 4 signaling and/or by complement. Subsequently, Kupffer cells release pro-inflammatory cytokines such as TNF-α and Interleukin-1 which themselves can perpetuate inflammatory injury by leukocyte activation.

While major participants in the promotion of injury, during cold ischemia they undergo intracellular energetic bioenergetic perturbations that reduce ATP stores due to mitochondrial dysfunction and predispose these cells to injury during reperfusion (Kamiike et al., 1988).

5.3 Leukocytes and lymphocytes

As a result of IRI, cellular adhesion molecules (ie, intracellular adhesion molecule-1 or ICAM-1, vascular adhesion molecule-1 or VCAM-1), selectins and integrins are activated and upregulated on the surface of endothelial cells, neutrophils and platelets. The activated

neutrophils adhere to endothelial cells at the initial stages of reperfusion, and subsequently transmigrate the endothelium where they continue to orchestrate tissue injury. The accumulation of activated neutrophils contributes to microcirculatory disturbances both locally and remotely. Activated neutrophils release reactive oxygen species, specifically superoxide radical ($O_2^{-}\bullet$), proteases and various cytokines (Teoh & Farrell, 2003). Monocytes and macrophages are also activated shortly following reperfusion (Ysebaert et al., 2000). Recent studies propose an important role for lymphocytes, especially $CD4^+$ T cells, in augmenting injury responses after IRI. However, lymphocytes may also play a protective role, but this is probably dependent on cell type and time course of injury (Ysebaert et al., 2000).

5.4 Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

During periods of ischemia, ROS and RNS are generated which can promote intracellular damage. Due to electron transport chain alterations, mitochondrial dysfunction ensues leading to reductions in ATP production and with subsequent loss of inner membrane stability resulting in mitochondrial swelling and rupture. With the reintroduction of oxygen during reperfusion, ROS are produced due to reactions of oxygen introduced during reperfusion and possible xanthine oxidase (or mitochondrial sources of ROS). ROS serve to stimulate other cell lines including Kupffer cells to produce proinflammatory cytokines (Diesen & Kuo, 2011). The major ROS are hydroxyl radical ($OH\bullet$) and hydrogen peroxide.(H_2O_2). Reactions of ROS such as $O_2^{-}\bullet$ with NO yield products such as peroxynitrite ($ONOO^-$), a RNS which can be an extremely aggressive oxidant.

5.5 Cytokines

Cytokines play a vital role in IRI, both by inducing and sustaining the inflammatory response, and by modulating IRI severity. Tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are the two cytokines most commonly implicated in liver IRI. TNF-α is a pleiotropic cytokine generated by various different cell types in response to inflammatory and immunomodulatory stimuli. TNF-α modulates leukocyte chemotaxis and activation, and induces ROS production in Kupffer cells (Colletti et al., 1996). Additionally, IL-1 is known to promote production of ROS, induce TNF-α synthesis by Kupffer cells and induce neutrophil recruitment (Kato, Gabay, Okaya, & Lentsch, 2002).

5.6 Complement

The complement system also contributes significantly to IRI and is composed of approximately thirty soluble and membrane-bound proteins. This system can be stimulated in three pathways: (1) the antibody-dependent classical pathway, (2) the alternative pathway, or (3) the mannose-binding lectin pathway (Qin & Gao, 2006). When activated, complement acts as a membrane-attacking complex that stimulates the production of proinflammatory cytokines and chemotactic agents. Furthermore, it can regulate adaptive immunity (Boros & Bromberg, 2006).

6. The influence of endogenous NO on liver IRI

Damage to the liver due to IRI is a culmination of inflammatory cross talk with the principal participants mentioned previously. Injury due to ischemia and reperfusion is the main cause

of liver injury in response to vascular clamping during hepatic procedures such as hepatectomy and liver transplantation. This insult on the liver results in disturbances of the sinusoidal microcirculation and the generation of a variety of mediators such as reactive oxygen species, cytokines, activation of chemokines and other cell signaling molecules previously mentioned.

Hepatic IRI can cause severe hepatocellular injury that contributes to morbidity and mortality after liver surgery. As briefly mentioned previously, reductions of NO during liver IRI occur and are associated with increased liver injury (Köken & Inal, 1999). This is now appreciated to be due to decreases in NO steady-state production resulting from low concentrations of eNOS. This event coupled with NO inactivation due to reactions with abundant ROS, such as $O_2^{-}\bullet$, results in reduced NO bioavailability. The consequences of this reduced bioavailability include but are not exclusive to increased oxidative stress, increased apoptosis, increased leukocyte adhesion, increased microcirculatory tone, and perturbed mitochondrial function. Interestingly, restoration with of NO to more "physiologic" concentrations serves to diminish the liver ischemia injury via countering the adverse actions mentioned previously. Other studies have demonstrated findings that are consistent with the premise that eNOS is crucial for minimizing injury during liver IRI. For example, liver injury was less in wild type mice compared to eNOS knockouts (eNOS $^{-/-}$), in addition to the findings that agents given to increase eNOS expression or donate NO afford greater liver IRI protection (Duranski et al., 2006; Katsumi, Nishikawa, Yamashita, & Hashida, 2008). It is also well established that the NO concentrations during various inflammatory states are significantly increased by increased expression of inducible nitric oxide synthase or iNOS. However, the influence of iNOS and its true contribution in conferring liver protection deserves additional studies. In a rat model of liver IRI, iNOS expression was significantly increased as per increases in iNOS RNA at 1 and 5 hrs (Hur et al., 1999). This is consistent with other studies measuring iNOS expression of conditions of liver IRI. In a porcine model of IRI, intraportal injection of the selective iNOS inhibitor, aminoguanidine was demonstrated to decrease injury (M Isobe et al., 2000). In an intriguing study, NOS knockout mice (iNOS $^{-/-}$) exposed to warm liver IRI demonstrated a much greater magnitude of injury compared to wild type mice. Interestingly, even though injury was greater in the iNOS knockout mice, little to no iNOS RNA was detectable in the wild type mice. It would appear for now, the true influence of iNOS's influence on liver injury during IR remains unclear.

A number of other endogenous NO-mediated mechanisms thought to confer protection have been published. For example, NO has been shown to inhibit caspase proteases via *S*-nitrosylation, thereby inhibiting apoptosis (Maejima, Adachi, Morikawa, Ito, & Mitsuaki Isobe, 2005). This appears to be somewhat concentration dependent. Low physiological concentrations of NO may inhibit apoptosis. In contrast, higher concentrations may lead to the formation of toxic reactive nitrogen species such as $ONOO^{-}$ or reactive oxygen species which lead to cell necrosis and apoptosis (P. K. Kim, Zamora, Petrosko, & Billiar, 2001). Other published mechanisms of NO-mediated protection include inhibition of nuclear factor kappa B (Marshall, Hess, & Stamler, 2004), reversible inhibition of mitochondrial complex I, and decreased mitochondrial calcium accumulation (Burwell & Brookes, 2008). As to be expected, controversy exists concerning "if" and "how" NO exerts cellular protection. For instance, in a study by Jaeschke *et al* (Jaeschke et al., 1991), administration of a NO synthase inhibitor did not attenuate or accentuate liver injury during the initial reperfusion period.

Inhibition of NO was observed not to influence neutrophil migration to the injured sites. While this contradicts a number of other studies, based on their findings, the authors concluded that NO availability was unlikely to be involved in the post-ischemic oxidant stress and reperfusion injury (Jaeschke, Schini, & Farhood, 1992). Nevertheless, the majority of published literature has demonstrated the beneficial effects of NO during liver IRI. These conflicting results might be explained by the fact that the mechanism of NO-mediated protection varies depending on cell type, quantities supplied, laboratory methods applied, timing and duration of NO exposure. Here, we summarize some key studies studying endogenous NO and NOS in hepatic IRI Table 1.

Species	Experimental Methods	Ischemic Time	NO or NOS Effects	Reference
Pigs	Aminoguanidine, 5 min before ischemia	120 min	NO derived from iNOS, antioxidant	(M Isobe et al., 2000)
Dogs	FK 409, 30 min before ischemia and15 min before and to 45 min after reperfusion	60 min	NO, improve hepatic microcirculation	(Aiba et al., 2001)
Rats	L-arginine, 7 days before IRI	60 min	NO, antioxidant	(Chattopadhyay et al., 2008)
Rats	L-NAME 60 min before ischemia	30 min	NO, antioxidant	(Köken & Inal, 1999)
Mouse	- Gadolinium chloride 24h before ischemia - L-nitroarginine (L-NAME) methyl ester 15 min prior to ischemia.	45 min	NO derived from eNOS, antioxidant, suppresses Kupffer cell function, regulated basal hepatic blood flow, but not affects blood flow after reperfusion, attenuated neutrophils infiltration.	(Hines et al., 2005)
Rats	- L-arginine or Sodium nitroprusside or L-Name prior to ischemia	60 min	NO, improve peripheral liver blood flow after reperfusion, cytoprotective	(Nilsson, Delbro, Wallin, & Friman, 2001)
Male Rats	- Arginine or L-NAME or 8-bromo guanosine 3′5′-cyclic monophosphate or rat atrial natriuretic peptide (ANP 1-28) 30 min before ischemia	45 min	NO, antioxidant, antiproinflammatory cytokines, improves microcirculation by the cGMP pathway, Inhibit neutrophils infiltration and platelet aggregation.	(Cottart et al., 2003)

Table 1. Effect of endogenous NO and NOS on liver IRI

7. Efficacy of exogenous NO, nitrite anion and NO donor administration in attenuating hepatic IRI

7.1 Inhaled nitric oxide (iNO)

Inhaled NO was approved by the U.S. Food and Drug Administration in December 1999, for the treatment of Persistent Hypertension of the newborn. Over the last decade, the primary advantage of iNO was seen to be its ability to selectively decrease pulmonary vascular resistance with minimal effects on systemic blood pressure; however, there is currently much interest in exploring its other benefits, including its antioxidant properties and its cytoprotective abilities (Zaky et al., 2009). In many animal studies, iNO decreased infarct size and left ventricular dysfunction after ischemia-reperfusion injury, increased coronary artery patency after thrombosis, increased blood flow in brain, kidney and peripheral vasculature, decreased leukocyte adhesion in bowel during ischemia reperfusion, and decreased platelet aggregation (McMahon & Doctor, 2006). Date *et al* reported the use of iNO in 15 out of 32 patients who suffered from immediate severe allograft dysfunction with iNO at 20 to 60 ppm. The mortality was significantly lower in the iNO group (7% and 24%, respectively). The gross benefits reported were that iNO improves oxygenation, decreases pulmonary artery pressure, shortens the period of postoperative mechanical ventilation, and reduces airway complications and mortality (Date et al., 1996). Likewise, a recent retrospective study also presented an improvement of overall respiratory functions. The authors encouraged the administration of iNO for the prevention and treatment of early graft failure in lung transplant recipients (Yerebakan, Ugurlucan, Bayraktar, Bethea, & Conte, 2009). Varadarajan *et al* were the first group to study the relationship between NO metabolism and IRI in human liver transplantation (Varadarajan et al., 2004). From their study, they concluded that reduced bioavailability of eNOS contributed to IRI one hour after portal reperfusion. On the other hand, iNOS did not contribute to early IRI after human liver transplantation. Clinical and mechanistic reports on therapeutic use of iNO demonstrating well beyond vascular relaxation, subsequently inactivated by oxy-ordeoxyhemoglobin in the red blood cells. iNO has various positive effects on extrapulmonary systems. However, how iNO mediates extrapulmonary effects remains unclear. Evidence supporting of stable forms of iNO is probably strongest for *S*-nitrosothiol (SNOs) and nitrite (McMahon & Doctor, 2006). In a prospective, blinded, placebo-controlled study, iNO at 80 ppm was administered to patients undergoing orthotropic liver transplantation (J. D. Lang et al., 2007). Many advantages were reported in the iNO group, including reduced platelet transfusion, an improvement in the rate at which liver function was restored post-transplantation on, and a decrease in the length of hospital stay. Most interesting was the finding of an approximated 75% reduction of hepatocellular apoptosis in patients treated with iNO (J. D. Lang et al., 2007). Possible biochemical intermediates of iNO including plasma and red blood cell nitrate, nitrite, S-nitrosothiols, C- or N-nitrosamines and red blood cell ferrous nitrosylhemoglobin. In this study, a detailed analysis indicated that the most likely candidate transducer of iNO on liver IRI was nitrite.

7.2 iNO delivery systems

An iNO delivery system should allow for constant and accurate measurements of NO and nitrogen dioxide [NO_2] concentration in inspired gas as well as minimize the contact time between oxygen and NO in order to decrease the feasibility of producing high NO_2

concentrations. The measurement of iNO and NO_2 concentrations can be undertaken using chemiluminescence or electrochemical devices. There are some drawbacks of chemiluminescence devices such as cost, the need for a relatively high sample volume, noise and maintenance difficulties (Mupanemunda & Edwards, 1995). However, an electrochemical analyzer is relatively insensitive, and these measurements may be affected by pressure, humidity, temperature and the presence of other gases in the environment (Macrae et al., 2004). The delivery system should display the pressure of iNO in the cylinder and should have a backup power supply to avoid sudden discontinuation of iNO. Inhaled NO is usually supplied in nitrogen at various concentrations. The gas mixture concentration should be sampled downstream of the input port just proximal to the patient manifold. iNO also can be administered via nasal cannula, oxygen mask and oxygen hood (Ambalavanan, St John, Carlo, Bulger, & Philips, 2002). Finally, the exhausted gas should be scavenged by passing it through carbon and filters, soda lime or activated charcoal (Ambalavanan, El-Ferzli, Roane, Johnson, & Carlo, 2009).

7.3 Potential toxicities during inhalation

In the presence of high concentrations of O_2, NO oxidizes to nitrogen dioxide (NO_2). NO_2 reacts with the alveolar lining fluid to form nitric acid. NO dissolves in the alveolar lining fluid reacts with O_2^- yielding $OONO,^-$ then decomposes into a hydroxyl anion (Pryor & Squadrito, 1995). Nitration of tyrosine residues of proteins is used as a marker of oxidative stress (Ischiropoulos, 1998). The rate at which NO is oxidized to NO_2 depends on the square of NO concentration and fractional concentration of oxygen to which it is exposed. The Occupational and Health Administration recommend 5 ppm/8 hr/24 hour interval as the upper safe limit of human exposure (Fullerton & McIntyre, 1996). In order to protect against NO_2 toxicity, iNO should be given with the least possible O_2 concentration. Inhaled NO and NO_2 concentrations should be monitored, exhaled gases should be scavenged and a soda lime canister should be placed in the inspiratory limb of the breathing circuit.

7.4 Nitrite

The simple molecule nitrite had been thought to be just an index of NO production for decades (Köken & Inal, 1999). Recently, a number of evidence suggests that nitrite is a pro-mediator of NO homeostasis. Administration of nitrite at near physiological concentrations (<5 μg) leads to vasodilatation in animals and human studies (Fullerton & McIntyre, 1996). Gladwin *et al* observed that nitrite was metabolized across the peripheral circulation. In addition, nitrite caused an increase in peripheral forearm blood flow when 80 ppm iNO was administered (Shiva & Gladwin, 2009). Under distinct conditions such as hypoxia and acidosis, nitrite can be reduced to NO by a number of deoxyhemeproteins (hemoglobin, myoglobin, neuroglobin and cytoglobin), enzymes (cytochrome P_{450} and xanthine oxidoreductase), and components of the mitochondrial electron transport chain (Zaky et al., 2009). Since nitrite can be converted back to NO during hypoxia nitrite therefore is expected to be utilized during ischemia reperfusion injury. Furthermore, nitrite shows more potential benefits than NO in terms of safety and ease of administration. In other words, nitrite concentrations administered need only a small dose in order to increase plasma and tissue nitrite level several-fold. Routes of administration are oral, intravenous injection or infusion, intraperitoneal, nebulizer or topical (Duranski et al., 2005). Nitrite has now been

demonstrated to have cytoprotective effects in animal models of ischemia reperfusion in organs. Duranski *et al* evaluated the effects of nitrite therapy in vivo murine models of hepatic and myocardial ischemia reperfusion injury and showed that nitrite was associated with cytoprotective effects. In the setting, nitrite reduced cardiac infarct size by 67% and limited elevations of liver enzymes in a dose-dependent manner. They also demonstrated that nitrite was reduced to NO regardless of eNOS and heme oxygenase-1 enzyme activities (Duranski et al., 2005). The exact mechanisms of how nitrite protects against the particular condition are being explored, but it appears that the benefit is mediated through the modulation of mitochondrial function by involving the posttranslational *S*-nitrosation of complex I to attenuate reperfusion oxygen radical generation and prevents cytochrome-C release (Shiva & Gladwin, 2009).

7.5 NO donor drugs

Since nitric oxide is not considered to be an ideal gas for the treatment of ischemia reperfusion injury, NO donor drugs are now being explored as an alternative to the parent compound. Novel drugs have been developed and used for the delivery of NO in order to compensate for the very short half-life of NO in vivo. However, there are only two types of NO donor drugs that are currently used clinically: organic nitrates and sodium nitroprusside. Organic nitrates are the most commonly used NO donor drugs treatment for coronary artery disease and congestive heart failure because the drugs produce clear clinical responses through their vasodilatory effects. Preparations of drugs include: slow release oral forms, ointments, transdermal patches, nebulizers and traditional intravenous forms. The main limitation of organic nitrates is the induction of drug tolerance with prolonged continuous use. NO release from nitroglycerin is likely via the enzyme mitochondrial aldehyde dehydrogenase (Yang, Chen, Kong, Xu, & Lou, 2007). On the other hand, the mechanism of NO release from sodium nitroprusside is more complex as demonstrated by

Model	Drugs	Outcomes	Reference
Canine liver IRI	FK-409,	Promote hepatic tissue blood flow, decrease serum Endothelin-1, cytoprotection	(Aiba et al., 2001)
Isolated hepatocytes	S-nitroso-N-acetylpenicillamine (SNAP)	- drug induced the expression of heat shock protein 70 mRNA and protein resulting in cytoprotection from TNFα	(Y. M. Kim et al., 1997)
Murine liver IRI	Sodium nitroprusside	- Promote hepatic tissue blood flow after reperfusion - cytoprotection	(Nilsson et al., 2001)
Murine liver IRI	PEG-poly SNO-BSA, a sustained release of NO	- Decreased neutrophils accumulation - Prevented the excessive production of iNOS	(Katsumi et al., 2008)
Murine liver IRI	Macromolecule S-nitrosothiols	Prevented hepatocellular injury	(Katsumi et al., 2009)

Table 2. Nitric oxide donors

Yang et al in a murine model of hepatic IRI. Sodium nitroprusside is thought to down-regulate the mRNA expression of several enzymes related to hepatic injury (Katsumi et al., 2008). Lastly, enhanced eNOS activation affords hepatoprotection during IRI and serves as yet another potential treatment option. Interestingly, liver preservation solutions supplemented with the agents trimetazidine (TMZ), 5-amino-4-imidazole carboxamide riboside (AICAR) or activated protein C (APC) have demonstrated allograft protection during conditions of cold ischemia (Katsumi, Nishikawa, Yasui, Yamashita, & Hashida, 2009). Below, we summarize other novel NO donor drugs in Table 2.

8. Conclusion

Ischemia reperfusion injury is a well-defined threat to the liver during periods of interruption and restoration of oxygen delivery as occurs in certain procedures as hepatic resections and orthotopic liver transplantations. Relative NO deficiency is central in the pathogenesis of this injury. Replacing NO per se either by inhalation, nitrate anion or via donor drugs represents a novel means in ameliorating IRI. Further randomized controlled drugs are needed to evaluate this therapy in patients undergoing operative procedures causing IRI.

9. References

Aiba, M., Takeyoshi, I., Ohwada, S., Kawashima, Y., Iwanami, K., Sunose, Y., Yamada, T., et al. (2001). Novel nitric oxide donor (FK409) ameliorates liver damage during extended liver resection with warm ischemia in dogs *Journal of the American College of Surgeons*, *193*(3), 264–271.

Alderton, W. K., Cooper, C. E., & Knowles, R. G. (2001). Nitric oxide synthases: structure, function and inhibition *The Biochemical journal*, *357*(Pt 3), 593–615.

Ambalavanan, N., El-Ferzli, G. T., Roane, C., Johnson, R., & Carlo, W. A. (2009). Nitric oxide administration using an oxygen hood: a pilot trial *PloS one*, *4*(2), e4312. doi:10.1371/journal.pone.0004312

Ambalavanan, N., St John, E., Carlo, W. A., Bulger, A., & Philips, J. B. (2002). Feasibility of nitric oxide administration by oxygen hood in neonatal pulmonary hypertension *Journal of perinatology : official journal of the California Perinatal Association*, *22*(1), 50–56. doi:10.1038/sj.jp.7210652

Bigelow, D. J., & Thomas, D. D. (1987). Rotational dynamics of lipid and the Ca-ATPase in sarcoplasmic reticulum. The molecular basis of activation by diethyl ether *The Journal of biological chemistry*, *262*(28), 13449–13456.

Boros, P., & Bromberg, J. S. (2006). New cellular and molecular immune pathways in ischemia/reperfusion injury *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *6*(4), 652–658. doi:10.1111/j.1600-6143.2005.01228.x

Burwell, L. S., & Brookes, P. S. (2008). Mitochondria as a target for the cardioprotective effects of nitric oxide in ischemia-reperfusion injury *Antioxidants & redox signaling*, *10*(3), 579–599. doi:10.1089/ars.2007.1845

Chattopadhyay, P., Verma, N., Verma, A., Kamboj, T., Khan, N. A., & Wahi, A. K. (2008). L-arginine protects from pringle manoeuvere of ischemia-reperfusion induced liver injury *Biological & pharmaceutical bulletin*, *31*(5), 890–892.

Colletti, L. M., Kunkel, S. L., Walz, A., Burdick, M. D., Kunkel, R. G., Wilke, C. A., & Strieter, R. M. (1996). The role of cytokine networks in the local liver injury following hepatic ischemia/reperfusion in the rat *Hepatology (Baltimore, Md.)*, *23*(3), 506–514. doi:10.1002/hep.510230315

Cottart, C. H., Nivet-Antoine, V., Do, L., Al-Massarani, G., Descamps, G., Xavier-Galen, F., & Clot, J.-P. (2003). Hepatic cytoprotection by nitric oxide and the cGMP pathway after ischaemia-reperfusion in the rat *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society*, *9*(2), 57–63.

Date, H., Triantafillou, A. N., Trulock, E. P., Pohl, M. S., Cooper, J. D., & Patterson, G. A. (1996). Inhaled nitric oxide reduces human lung allograft dysfunction *The Journal of thoracic and cardiovascular surgery*, *111*(5), 913–919.

Diesen, D. L., & Kuo, P. C. (2011). Nitric oxide and redox regulation in the liver: part II. Redox biology in pathologic hepatocytes and implications for intervention *The Journal of surgical research*, *167*(1), 96–112. doi:10.1016/j.jss.2009.10.006

Duranski, M. R., Greer, J. J. M., Dejam, A., Jaganmohan, S., Hogg, N., Langston, W., Patel, R. P., et al. (2005). Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver *The Journal of clinical investigation*, *115*(5), 1232–1240. doi:10.1172/JCI22493

Duranski, M. R., Elrod, J. W., Calvert, J. W., Bryan, N. S., Feelisch, M., & Lefer, D. J. (2006). Genetic overexpression of eNOS attenuates hepatic ischemia-reperfusion injury *American journal of physiology. Heart and circulatory physiology*, *291*(6), H2980–6. doi:10.1152/ajpheart.01173.2005

Fan, C., Zwacka, R. M., & Engelhardt, J. F. (1999). Therapeutic approaches for ischemia/reperfusion injury in the liver *Journal of molecular medicine (Berlin, Germany)*, *77*(8), 577–592.

Fullerton, D. A., & McIntyre, R. C. (1996). Inhaled nitric oxide: therapeutic applications in cardiothoracic surgery *The Annals of thoracic surgery*, *61*(6), 1856–1864. doi:10.1016/0003-4975(96)00046-X

He, S.-Q., Zhang, Y.-H., Venugopal, S. K., Dicus, C. W., Perez, R. V., Ramsamooj, R., Nantz, M. H., et al. (2006). Delivery of antioxidative enzyme genes protects against ischemia/reperfusion-induced liver injury in mice *Liver Transplantation*, *12*(12), 1869–1879. doi:10.1002/lt.21001

Hines, I. N., Harada, H., Flores, S., Gao, B., McCord, J. M., & Grisham, M. B. (2005). Endothelial nitric oxide synthase protects the post-ischemic liver: potential interactions with superoxide *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie*, *59*(4), 183–189. doi:10.1016/j.biopha.2005.03.011

Hines, I. N., & Grisham, M. B. (2011). Divergent roles of superoxide and nitric oxide in liver ischemia and reperfusion injury. *Journal of clinical biochemistry and nutrition*, *48*(1), 50–56. doi:10.3164/jcbn.11-016FR

Hur, G. M., Ryu, Y. S., Yun, H. Y., Jeon, B. H., Kim, Y. M., Seok, J. H., & Lee, J. H. (1999). Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB *Biochemical and biophysical research communications*, *261*(3), 917–922. doi:10.1006/bbrc.1999.1143

Ischiropoulos, H. (1998). Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species *Archives of Biochemistry and Biophysics*, *356*(1), 1–11. doi:10.1006/abbi.1998.0755

Isobe, M, Katsuramaki, T., Kimura, H., Nagayama, M., Matsuno, T., Yagihashi, A., & Hirata, K. (2000). Role of inducible nitric oxide synthase on hepatic ischemia and reperfusion injury *Transplantation proceedings*, *32*(7), 1650–1652.

Jaeschke, H., Bautista, A. P., Spolarics, Z., & Spitzer, J. J. (1991). Superoxide generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia *Free radical research communications*, *15*(5), 277–284.

Jaeschke, H., Schini, V. B., & Farhood, A. (1992). Role of nitric oxide in the oxidant stress during ischemia/reperfusion injury of the liver *Life sciences*, *50*(23), 1797–1804.

Jennings, R. B., Shen, A. C., Hill, M. L., Ganote, C. E., & Herdson, P. B. (1978). Mitochondrial matrix densities in myocardial ischemia and autolysis *Experimental and molecular pathology*, *29*(1), 55–65.

Kamiike, W., Burdelski, M., Steinhoff, G., Ringe, B., Lauchart, W., & Pichlmayr, R. (1988). Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation *Transplantation*, *45*(1), 138–143.

Kato, A., Gabay, C., Okaya, T., & Lentsch, A. B. (2002). Specific role of interleukin-1 in hepatic neutrophil recruitment after ischemia/reperfusion *The American journal of pathology*, *161*(5), 1797–1803. doi:10.1016/S0002-9440(10)64456-2

Katsumi, H., Nishikawa, M., Yamashita, F., & Hashida, M. (2008). Prevention of hepatic ischemia/reperfusion injury by prolonged delivery of nitric oxide to the circulating blood in mice *Transplantation*, *85*(2), 264–269. doi:10.1097/TP.0b013e31815e902b

Katsumi, H., Nishikawa, M., Yasui, H., Yamashita, F., & Hashida, M. (2009). Prevention of ischemia/reperfusion injury by hepatic targeting of nitric oxide in mice *Journal of controlled release : official journal of the Controlled Release Society*, *140*(1), 12–17. doi:10.1016/j.jconrel.2009.07.013

Kim, P. K., Zamora, R., Petrosko, P., & Billiar, T. R. (2001). The regulatory role of nitric oxide in apoptosis *International immunopharmacology*, *1*(8), 1421–1441.

Kim, Y. M., de Vera, M. E., Watkins, S. C., & Billiar, T. R. (1997). Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-alpha-induced apoptosis by inducing heat shock protein 70 expression *The Journal of biological chemistry*, *272*(2), 1402–1411.

Köken, T., & Inal, M. (1999). The effect of nitric oxide on ischemia-reperfusion injury in rat liver *Clinica chimica acta; international journal of clinical chemistry*, *288*(1-2), 55–62.

Lang, J. D., Teng, X., Chumley, P., Crawford, J. H., Isbell, T. S., Chacko, B. K., Liu, Y., et al. (2007). Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *The Journal of clinical investigation*, *117*(9), 2583–2591. doi:10.1172/JCI31892

Macrae, D. J., Field, D., Mercier, J.-C., Møller, J., Stiris, T., Biban, P., Cornick, P., et al. (2004). Inhaled nitric oxide therapy in neonates and children: reaching a European consensus In *Intensive care medicine* (Vol. 30, pp. 372–380). Presented at the Intensive care medicine. doi:10.1007/s00134-003-2122-3

Madesh, M., & Hajnóczky, G. (2001). VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release *The Journal of cell biology*, *155*(6), 1003–1015. doi:10.1083/jcb.200105057

Maejima, Y., Adachi, S., Morikawa, K., Ito, H., & Isobe, Mitsuaki. (2005). Nitric oxide inhibits myocardial apoptosis by preventing caspase-3 activity via S-nitrosylation *Journal of molecular and cellular cardiology*, *38*(1), 163–174. doi:10.1016/j.yjmcc.2004.10.012

Marshall, H. E., Hess, D. T., & Stamler, J. S. (2004). S-nitrosylation: physiological regulation of NF-kappaB *Proceedings of the National Academy of Sciences of the United States of America*, *101*(24), 8841–8842. doi:10.1073/pnas.0403034101

Massip-Salcedo, M., Roselló-Catafau, J., Prieto, J., Avíla, M. A., & Peralta, C. (2007). The response of the hepatocyte to ischemia *Liver international : official journal of the International Association for the Study of the Liver*, *27*(1), 6–16. doi:10.1111/j.1478-3231.2006.01390.x

McMahon, T. J., & Doctor, A. (2006). Extrapulmonary effects of inhaled nitric oxide: role of reversible S-nitrosylation of erythrocytic hemoglobin *Proceedings of the American Thoracic Society*, *3*(2), 153–160. doi:10.1513/pats.200507-066BG

Montalvo-Jave, E. E., Escalante-Tattersfield, T., Ortega-Salgado, J. A., Piña, E., & Geller, D. A. (2008). Factors in the pathophysiology of the liver ischemia-reperfusion injury *The Journal of surgical research*, *147*(1), 153–159. doi:10.1016/j.jss.2007.06.015

Moon, K.-H., Hood, B. L., Mukhopadhyay, P., Rajesh, M., Abdelmegeed, M. A., Kwon, Y.-I., Conrads, T. P., et al. (2008). Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion *Gastroenterology*, *135*(4), 1344–1357. doi:10.1053/j.gastro.2008.06.048

Mupanemunda, R. H., & Edwards, A. D. (1995). Treatment of newborn infants with inhaled nitric oxide *Archives of disease in childhood. Fetal and neonatal edition*, *72*(2), F131–4.

Nilsson, B., Delbro, D., Wallin, M., & Friman, S. (2001). Protective effect of nitric oxide and prostaglandin E(2) in ischemia/reperfusion injury of the liver *Transplantation proceedings*, *33*(4), 2518–2520.

Phillips, L., Toledo, A. H., Lopez-Neblina, F., Anaya-Prado, R., & Toledo-Pereyra, L. H. (2009). Nitric oxide mechanism of protection in ischemia and reperfusion injury *Journal of investigative surgery : the official journal of the Academy of Surgical Research*, *22*(1), 46–55. doi:10.1080/08941930802709470

Pryor, W. A., & Squadrito, G. L. (1995). The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide *The American journal of physiology*, *268*(5 Pt 1), L699–722.

Qin, X., & Gao, B. (2006). The complement system in liver diseases *Cellular & molecular immunology*, *3*(5), 333–340.

Rubbo, H., Darley-Usmar, V., & Freeman, B. A. (1996). Nitric oxide regulation of tissue free radical injury *Chemical research in toxicology*, *9*(5), 809–820. doi:10.1021/tx960037q

Sammut, I. A., Burton, K., Balogun, E., Sarathchandra, P., Brooks, K. J., Bates, T. E., & Green, C. J. (2000). Time-dependent impairment of mitochondrial function after storage and transplantation of rabbit kidneys *Transplantation*, *69*(7), 1265–1275.

Shiva, S., & Gladwin, M. T. (2009). Nitrite mediates cytoprotection after ischemia/reperfusion by modulating mitochondrial function *Basic research in cardiology*, *104*(2), 113–119. doi:10.1007/s00395-009-0009-3

Teoh, N. C., & Farrell, G. C. (2003). Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection *Journal of gastroenterology and hepatology*, *18*(8), 891–902.

Tripathi, Parul, Tripathi, Prashant, Kashyap, L., & Singh, V. (2007). The role of nitric oxide in inflammatory reactions *FEMS immunology and medical microbiology*, *51*(3), 443–452. doi:10.1111/j.1574-695X.2007.00329.x

Varadarajan, R., Golden-Mason, L., Young, L., McLoughlin, P., Nolan, N., McEntee, G., Traynor, O., et al. (2004). Nitric oxide in early ischaemia reperfusion injury during human orthotopic liver transplantation. *Transplantation*, *78*(2), 250–256.

Yadav, S. S., Howell, D. N., Gao, W., Steeber, D. A., Harland, R. C., & Clavien, P. A. (1998). L-selectin and ICAM-1 mediate reperfusion injury and neutrophil adhesion in the warm ischemic mouse liver *The American journal of physiology*, *275*(6 Pt 1), G1341–52.

Yang, S.-L., Chen, L.-J., Kong, Y., Xu, D., & Lou, Y.-J. (2007). Sodium nitroprusside regulates mRNA expressions of LTC4 synthesis enzymes in hepatic ischemia/reperfusion injury rats via NF-kappaB signaling pathway *Pharmacology*, *80*(1), 11–20. doi:10.1159/000102595

Yerebakan, C., Ugurlucan, M., Bayraktar, S., Bethea, B. T., & Conte, J. V. (2009). Effects of inhaled nitric oxide following lung transplantation *Journal of cardiac surgery*, *24*(3), 269–274. doi:10.1111/j.1540-8191.2009.00833.x

Ysebaert, D. K., De Greef, K. E., Vercauteren, S. R., Ghielli, M., Verpooten, G. A., Eyskens, E. J., & De Broe, M. E. (2000). Identification and kinetics of leukocytes after severe ischaemia/reperfusion renal injury *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*, *15*(10), 1562–1574.

Zaky, A., Siriussawakul, A., Tostenrud, R., Pauldine, R., & Lang, J. (2009). Clinical Update on Theraputic Use of Nitric Oxide. *Contemporary Critical Care*, *7*(6), 1–12.

Zwacka, R. M., Zhou, W., Zhang, Y., Darby, C. J., Dudus, L., Halldorson, J., Oberley, L., et al. (1998a). Redox gene therapy for ischemia/reperfusion injury of the liver reduces AP1 and NF-kappaB activation *Nature medicine*, *4*(6), 698–704.

Zwacka, R. M., Zhang, Y., Zhou, W., Halldorson, J., & Engelhardt, J. F. (1998b). Ischemia/reperfusion injury in the liver of BALB/c mice activates AP-1 and nuclear factor kappaB independently of IkappaB degradation *Hepatology (Baltimore, Md.)*, *28*(4), 1022–1030. doi:10.1002/hep.510280417

Part 3

Immunology and Liver Allograft Rejection

4

Immunology of Liver Transplantation

Ling Lu, Jianhua Rao, Guoqiang Li,
Xiaofeng Qian, Beicheng Sun and Xuehao Wang
Liver Transplantation Center, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

1. Introduction

The past four decades have witnessed the evolution of liver transplantation exploration procedures, and whilst they had witnessed a high mortality and morbidity they now serve as a successful therapeutic measure for end-stage liver disease. Nowadays, one year and five years survival for elective cases are often in excess of 85%-88% and 70%-75% and with an excellent quality of life (Annals of Hepatology, 2010). The remarkable success of liver transplantation is due largely to the development of immunosuppressive regimens that are highly effective at protecting allografts from acute rejection, and which ensure their survival in most cases. Interestingly, early liver transplantation studies with out-bred swine demonstrated that a high percentage of recipients maintained their graft in the absence of immunosuppression (Calne R Y et al., 1969). Subsequently, the spontaneous tolerance of liver allografts was also shown in liver transplantation in several allogeneic rat strain combinations and in most allogeneic mouse strain combinations (Farges O et al., 1995; Dresske B et al., 2002). As such, and compared with other solid-organ transplants, liver allografts have long been considered to be immunologically privileged, as manifest by an absence of hyperacute rejection despite a positive T cell cross-match, a low incidence of graft loss due to chronic rejection, and the potential for hepatocyte regeneration after tissue injury. Finally, in clinical transplantation, there is increasing evidence that some liver transplant recipients who cease taking immunosuppressive drugs maintain allograft function. Despite this special status, the liver can display destructive immunologic processes, since acute liver allograft rejection occurs in approximately 50% to 75% of liver transplant recipients (although in the majority of cases it is readily reversed with immunosuppressive approaches tailored to treat cellular rejection) (covered in a separate CAQ corner). Immunosuppressive drugs, however, also produce significant toxic effects that increase patient morbidity and mortality (Lechler R I et al., 2005; Sayegh M H et al., 2004). Moreover, the current immunosuppressive regimens do not prevent the development of chronic rejection, which is a major cause of graft loss. Most studies have also shown that a variety of autoimmune diseases with unknown aetiologies target the liver, including primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, and biliary atresia (Duclos-Vallee J C et al., 2009; Schramm C et al., 2010; Guichelaar MM et al., 2003). As such, compared with other solid-organ transplants, liver transplantation is complex, having a sometimes paradoxical interaction with the host immune system. Understanding

these mechanisms is important, as it aids in the understanding of the clinical features of rejection and – hence – in making an early diagnosis and delivering appropriate treatment. Knowledge of these mechanisms is also critical in developing strategies to minimise rejection and in developing new drugs and treatments that blunt the effects of the immune system on transplanted organs, thereby ensuring the longer survival of these organs. The next chapter will elaborate on various aspects of liver transplantation immunology.

2. Types of grafts

Grafts of different species can cause different degrees of immune response to recipients. Generally, the greater the difference between the species, the more likely that it is that there will be a stronger immunological rejection. Accordingly, this section will discuss the types of graft. Liver grafts often mainly consist of four types: autograft, isograft, allograft and xenograft (Figure 1). The definitions and features of all grafts will be now be described in detail and the effects of different grafts on the body's immune system also will be introduced.

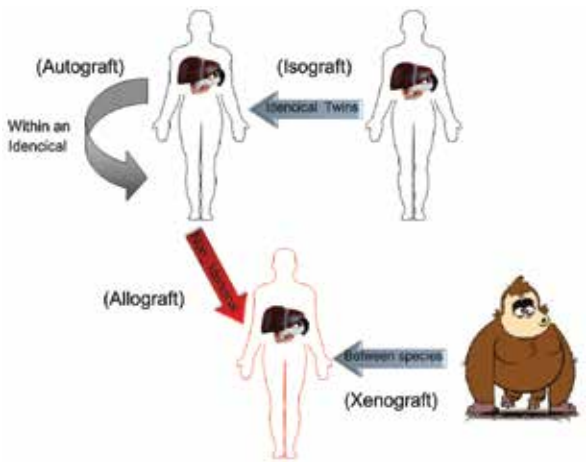


Fig. 1. Types of grafts

2.1 Autograft and isograft

Autograft means 'self-tissue' and refers to where on organ is transferred from one body site to another within the same individual. In recent years, auto-liver transplantation has been operated in some centres, but the number does not exceed one hundred because the operation technique is very complex and difficult. The autograft is a promising graft and has two advantages: (1) the graft is a tissue or organ already belong to the recipient who has spontaneous tolerance to the graft and so may avoid taking immunosuppressive drugs; (2) liver shortage is rapidly becoming a major restricting factor on the development of liver transplantation, and autografts avoid this problem. An isograft is a tissue or organ which is transferred between genetically identical individuals, e.g., liver transplantation between identical twins or grafts between mice of the same in-bred strain. This graft has the same advantages as the autograft, and it is not necessary to apply immunosuppressive regimens to the recipients. In particular, with the development of living donor liver transplantation,

this is an excellent graft. Nevertheless, the isograft is fairly uncommon and far from universal.

2.2 Allograft

An allograft is a tissue or organ which has been transferred between genetically different members of the same species. Allografts account for many human transplants, including those from cadaveric, living-related and living-unrelated donors. It is also called an allogeneic graft or a homograft. With regard to liver transplantation, most grafts are allografts. The graft may cause acute rejection and chronic rejection, and as such surgeons have to select the optimal match so as to reduce the occurrence of rejection by the ABO group and the HLA system.

2.3 Xenograft

A xenograft is a tissue or organ which has been transferred between different species. Donor shortage imposes the main restricting factor on liver transplantation and the continual growth of transplantation has led to significant organ shortages for a long time. At present in China – which is a country with a high incidence of liver disease – almost 3,000,000 patients develop some degree of liver cirrhosis, and about 10% of patients deteriorate to end-stage liver cirrhosis or liver cancer. As such, clinicians are often faced with the difficult prospect of rationing organs. Furthermore, liver transplantation in urgent cases is usually delayed – occasionally with fatal outcomes – until a suitable liver becomes available. Using animals as liver donors is a theoretically attractive solution, because it offers a potentially inexhaustible source of liver. This is of particular relevance for patients with fulminant liver failure who require urgent transplantation. Another potential use of xenografts lies in the fact that some animals are immune to certain viruses which may re-infect a human liver transplant (hepatitis being a case in point). Xenografts have been applied in the clinical region. Starzl's team transplanted two baboon livers into human subjects at Pittsburgh (Starzl et al., 1993). Although they did so with good graft function (and this may well result in the further development of this approach) the graft is confronted with some problems. The first is a problem of theory, such that only about fifty-percent of people support xenotransplantation. The second – and the biggest – problem is one of rejection, since xenotransplantation often causes hyperacute rejection and leads to graft dysfunction.

3. Immunological basis of allograft rejection

With regard to liver transplantation, grafts mainly originate from different members of the human species. The genetically encoded immunologically mediated barrier to transplantation was recognised and defined over the course of the last century. The immunological study of transplantation has played a pivotal role in the development of clinical transplantation. Although the first successful liver transplant was between identical twins, the development of transplantation as an important facet of modern medical therapy required the introduction of immunosuppression so as to prevent and treat the rejection of allografts (Liu L U et al., 2002; Yoshizawa A et al., 2006; Braillon A, 2009). The process of rejection is very complicated and has been shown to be caused by transplantation antigens, including major histocompatibility antigens, minor histocompatibility antigens and other

alloantigens. In addition, infiltrating leucocytes also launch the process, and it exhibits specificity and memory and is prevented by lymphocyte depletion (Gowans J L, 1962). The major histocompatibility complex (MHC) was identified as encoding the dominant transplantation antigens, and these were shown to be identical to serologically defined human leucocyte antigens (HLA), and subsequently to the elements responsible for the self-restriction of immunological responses to conventional antigens. The molecular and cellular basis of graft rejection will be described in the next section (Figure 2).

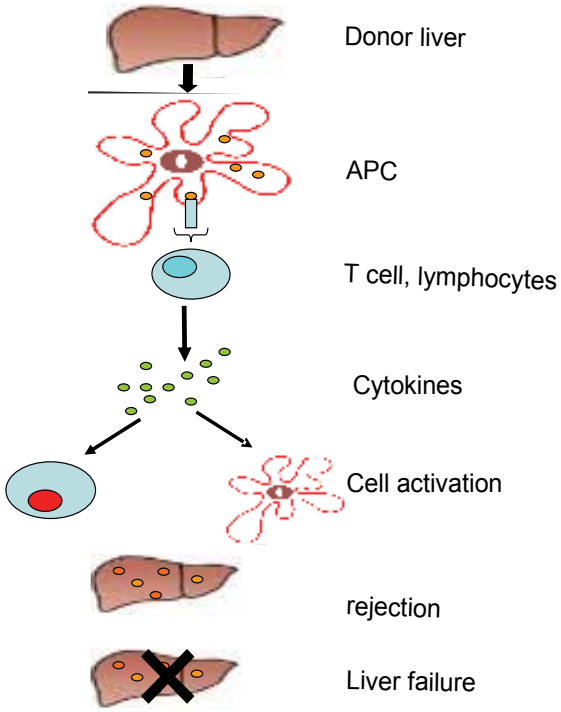


Fig. 2. The evolution of the immune response after liver transplantation. MHC, major histocompatibility complex; TCR, T cell receptor; APC, antigen presenting cell; IFN, interferon; TNF, tumour necrosis factor

3.1 Cell-mediated rejection of allografts

After liver transplantation, antibody-mediated, hyperacute vasculitic rejection can occur in individuals with preformed antibodies against the donor's MHC class I–encoded antigens. Under most other circumstances, acute allograft rejection is initiated by the large number of recipient T cells that recognise donor alloantigens (Stefanova I et al., 2003). Thus, the transplantation of MHC histoincompatible tissues elicits a strong, cytopathic, T cell-dependent immune response to donor tissues. By the T cell-dependent pathway to rejection, graft alloantigens are processed by specialised antigen presenting cells (APCs). Graft MHC molecules are internalised by donor and recipient APCs (Figure 3), following intracellular processing, and MHC peptide fragments are presented to the recipient's T cells (Watschinger B, 1995; Afzali B et al., 2008). Antigen presentation involves the engagement of these peptide antigenic fragments within a groove on the MHC molecules of the APC

surface. Acute cellular rejection is the best-characterised graft-specific form of immune rejection. Clinically apparent acute cellular rejection is defined by an often-sudden deterioration in allograft function; biopsy analysis of the transplanted tissue shows infiltration by host T cells and other mononuclear leucocytes and signs that these infiltrating cells have damaged the graft. Despite the routine use of immunosuppressive therapy, acute rejection is not rare. Studies show that CD4 and CD8 T cells both participate in acute rejection, although the rejection response is mediated primarily by CD4 T cells. CD4 T cells are activated by the above direct and indirect pathways, and primarily mediate the rejection response (Watschinger B., 1995). Although CD4 T cells are important in rejection, many activated CD8 T cells infiltrate the transplant tissue at the time of rejection, along with other mononuclear leucocytes (Strom TB et al., 1975). The cells of the innate immune system, such as natural killer (NK) cells, are also present in allografts during rejection. NK cells can recognise alloantigens because they constitutively express inhibitory receptors that are specific for self-MHC class I antigens; in addition, cytokines secreted by activated CD4 or CD8 T cells can promote the activation of NK cells, which can initiate and aggravate the rejection response (Dollinger MM et al., 1998).

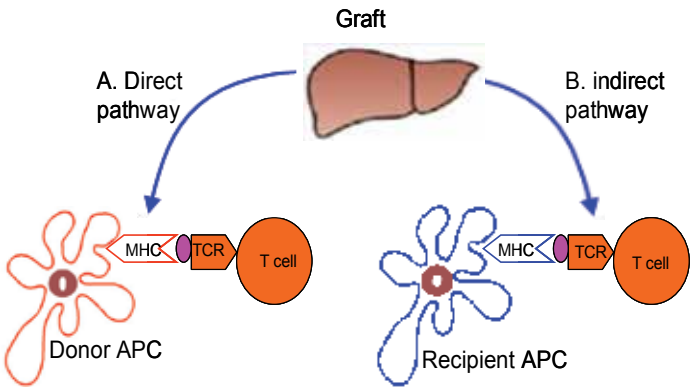


Fig. 3. Pathways of alloantigen presentation. (A) In the direct pathway, recipient T cells recognise intact allogeneic MHC molecules on the surface of donor APCs. The direct pathway is responsible for the large proportion of T cells that have reactivity against alloantigens due to the cross-reactivity of the T cell receptor (TCR) with self and foreign MHC molecules. (B) In the indirect pathway, recipient APCs trafficking through the allograft phagocytose allogeneic material are shed by donor cells (mostly peptides derived from allogeneic MHC molecules) and presented to the T cells on recipient MHC molecules

3.2 Humoral-mediated rejection of allografts

The humoral immune response is also important in the mediation of allograft rejection. The production of anti-donor MHC antibodies is associated with acute and chronic graft damage, usually in the form of graft vasculopathy. These antibodies can damage the graft by activating complement and mononuclear cells with Fc receptors that recognise the heavy chain of antibodies. Thus, Fc receptor–expressing leucocytes can be activated by antibody-coated donor cells. Anti-donor antibodies can also directly inhibit signalling cascades within endothelial cells (Li F et al., 2009). Humoral-mediated rejection of allografts is often observed following kidney, heart and lung transplantation, but liver allografts appear to

recover in relation to the development of humoral-mediated rejection. Most transplant organs manifest insidious and inexorable dysfunction as time passes. Although this process was formerly called 'chronic rejection', it is not clear that donor-specific immune rejection is the sole or even the primary cause in many conditions (Seetharam A et al., 2010). Pathology analysis often reveals fibrosis and atrophy in the absence of infiltration by T cells and other mononuclear leucocytes. Potential additional causes for chronic allograft failure include viral infection, recurrence of the original disease and drug toxicity. In general, humoral-mediated rejection of allografts is relatively uncommon in liver transplantation.

3.3 Memory T cell mediated rejection of allografts

Following T cell activation and proliferation, homeostasis of the adaptive immune system is restored by cell death – via "neglect" – of most antigen-specific T cells. A small number of T cells, however, survive and become long-lasting memory cells that ensure protective immunity against pathogens. Memory T cells can be divided into central memory and effector memory subsets, based on their circulation pattern and functional responsiveness. With regard to organ transplantation, upon re-exposure to donor antigens donor-reactive memory T cells are more sensitive to antigens, function more rapidly, produce effector cytokines, survive longer than naïve T cells and directly or indirectly produce cytolytic effects on the transplanted tissue (Ku C C et al., 2000; Sallusto F et al., 2000; Garcia S et al., 1999 & Barber DL et., 1999). Central memory T cells are responsible for recall antigen responses, and effector memory T cells survey peripheral tissues and immediately respond to invading pathogens (Sallusto F et al., 2004). As a consequence of continuous exposure to foreign antigens, memory T cells accumulate with time and represent approximately 50% of the total T cell pool in adults. Recipients who have not received a transplanted graft can still generate donor-reactive T cells, which can appear through immunisation by direct exposure to alloantigens via pregnancy or blood transfusion (Bingaman A W et., 2002). Furthermore, donor-reactive memory T cells can be generated in the absence of alloantigen exposure through heterologous immunity. Some memory T cells are therefore primed by an antigenic pathogen-derived peptides and cross-react with allogeneic peptides presented by the self or the donor MHC molecules. Alloreactive naïve T cells can acquire a memory phenotype and generate a substantial pool of donor-reactive memory T cells after transplantation, even when a recipient is under immunosuppressive therapy. Furthermore, the use of antibodies that deplete host T cells can amplify this phenomenon by inducing homeostatic T cell proliferation in response to lymphopenia (Wu Z et al., 2004). Because of their capacity to rapidly generate effector immune responses upon rechallenge, memory T cells appear to be particularly efficient at mediating allograft rejection (Zheng X X et al., 1999 & Schenk A D et al., 2008). In addition, memory T cells are less sensitive than naïve T cells to many immunosuppressive strategies. Compared with conventional T cells, memory T cells are less sensitive to T cell-depleting antibodies and therapeutics that block the CD28 and CD154 co-stimulatory signallers which inhibit the mammalian target of rapamycin (Pearl J P et al., 2005; Vu M D et al., 2006; Adams A B et al., 2003 & Araki K et al., 2009). The effects of memory T cells on the allograft response have been well delineated in animal models of allograft tolerance, wherein the generation of memory T cells by pre-sensitisation, heterologous immunity or homeostatic proliferation prevents the graft-protecting effects of most tolerising therapeutic strategies (Koyama I et al., 2007 & Valujskikh A et al., 2002). In contrast to human recipients, animals live in the protected environments of transplantation

laboratories and do not usually contain substantial numbers of memory T cells. This is one of the reasons that may explain the difficulties of translating into the clinic the results of protocols capable of creating allograft tolerance in rodent models. But the results cannot be applied in clinical conditions. Given the lower efficacy of conventional immunosuppressive drugs on activated memory lymphocytes, it is not surprising that memory T cells also exert harmful effects in clinical transplantation. In transplant studies, it is clearly understood that memory T cells – however they are generated – pose a significant barrier to inducing tolerance to allografts (Chalasani G et al., 2002; Zhai Y et al., 2002 & Adams AB et al., 2003). Thus, a better understanding of how to target this cell population and the designing novel of therapies that inhibit these cells would be beneficial.

3.4 Co-stimulatory pathways and the immunology of allografts

Evidently, T cells must receive two distinct but coordinated signals in order to achieve optimal activation. The first signal is provided by the TCR engagement with recognition of peptide/ MHC I or II on APCs, and the second signal is achieved by the interaction of co-stimulatory molecules on the T cells and their ligands on APCs. The importance of co-stimulation was found through experimental models in which its inhibition was achieved by some means, Signal 1 in the absence of signal 2 – as likely occurs in the liver – leads to a state of T cell non-responsiveness (or anergy) in which T cells can recognise cognate antigens through the TCR, but fail to mount a functional response upon reencounters with the antigen. So, there have been significant efforts to inhibit or block co-stimulatory pathways as a means of achieving allograft tolerance. There are two co-stimulatory pathways that are important in the generation of a complete T cell response are CD28/B7 and CD40/CD154 in the co-stimulatory field.

The role of CD28 has perhaps been that most intensively investigated in the co-stimulatory field. CD28 represents the prototypical T cell co-stimulatory molecule. In humans, CD28 is expressed on 90% of CD4 T cells and 50% of CD8 T cells; moreover, ligands for CD28, B7-1 (CD80) and B7-2 (CD86) are found on a variety of APCs including DCs, B cells and macrophages. The expression of CD86 is greater than for CD80 on APCs, although CD80 expression is enhanced during APC activation. The expression of CD80 and CD86 has been examined by immunohistochemistry or real-time polymerase chain reaction in livers following transplantation (Kwekkeboom J et al., 2003). CD80 was expressed only sporadically on normal liver but was present on at least 25% of the Kupffer cells in 45% of the transplanted livers. CD86 was found on the majority of Kupffer cells in all transplanted liver tissue and in normal liver tissue. The effect of ligation of CD28 by either CD86 or CD80 appears to be increased cytokine synthesis and proliferation by various intracellular signalling. Immunohistochemical analysis of CD86 expression in biopsies of liver recipients demonstrated an association with the increased expression of CD86 in the graft during severe acute cellular rejection (Bartlett A S et al., 2003). CTLA4 (CD152) is a CD28-related protein that binds to CD86 and CD80. Whereas CD28 delivers a positive co-stimulatory signal to T cells, CD152 delivers a negative signal that attenuates T cell function. CD152 expression is enhanced after T cell activation, and it has a higher affinity for CD86 or CD80 than does CD28; it has been proposed that the physiologic function of CD152 is to downregulate T cell responses. Therefore, specific activation of CD152 could potentially yield immunoinhibitory function and achieve allograft tolerance, but this ideal approach has been reached by the lack of suitable reagents.

The CD40/CD154 co-stimulatory pathway is a second important co-stimulatory pathway that is critical in the immune response of allotransplantation. CD40 is mainly expressed on APCs (including DCs, B cells, and macrophages) but it can also be expressed on nonimmune cells (including endothelial cells, mast cells, platelets and epithelial cells). However, CD154 is mainly expressed on CD4 T cells following activation, and to a lesser extent on NK cells, B cells, and CD8 T cells. CD154 combines with CD40, which is critical for the activation of DCs, B cells, and macrophages. In DCs, CD40 upregulates interleukin12 (IL-12) production, and in macrophages it results in the production of various proinflammatory cytokines. CD154 was also detected on Kupffer cells and on sinusoidal macrophages in livers during chronic rejection, but not in stable liver allografts or normal liver (Gaweco A S et al., 1999).

The most widely-used measure to block CD28-B7 interactions has been CTLA-immunoglobulin (Ig). In the orthotopic rat liver transplantation model, repeated administration of CTLA-Ig – beginning with CTLA-Ig in combination with donor splenocytes – leads to extended graft survival of >100 days, whereas the delayed administration of CTLA4-Ig alone or donor splenocytes alone did not (Neumann U P, et al., 2002). In recent years, many studies have shown that B7 cross-linking on APCs by CTLA4-Ig induces indoleamine 2, 3-dioxygenase (IDO), which itself inhibits local T cell activation (Mellor A L et al., 2003; Li W et al., 2009). Gene therapy approaches to deliver CTLA4-Ig to liver allografts have been successfully used in some animal experiments. Adenoviral-mediated gene delivery of CTLA4-Ig through ex vivo perfusion of cold preserved livers resulted in indefinite survival of rat liver allografts and in the generation of donor-specific unresponsiveness (Olthoff K et al., 1998). An interesting report suggests that CD154/CD40 interaction plays a role in promoting dendritic cell-maturation in the absence of CD4+CD25+ regulatory lymphocytes, whilst these cells promote the maintenance of immaturity (Serra P et al., 2003; Misra N et al., 2004). This accounts for the importance of DC activation, not only by innate immune mechanisms but also by activated T cells. The efficacy of anti-CD154 in a rat liver allograft model not only prolongs allograft survival but it was also associated with fewer complications (Bartlett AS et al., 2002). These roles underline the significant beneficial effects of CTLA4-Ig and anti-CD154 in pre-clinical models of transplantation; however, its clinical application has a long way to go for liver transplantation.

4. Classification and effector mechanisms of allograft rejection

Allograft rejection mainly involves host-versus-graft reaction in liver transplantation, which is the rejection of the transplant by the recipient's body. The recipient's lymphocyte mediated reactions to allogeneic or xenogeneic cells – acquired as a graft or otherwise –lead to damage and/or the destruction of the grafted cells. The graft rejection has been divided into three groups: hyperacute rejection, acute rejection and chronic rejection (Table 1).

Type of rejection	Time taken	Cause
Hyperacute	Minutes-hours	Pre-existing anti-donor antibodies and complement activation
Acute	Days – weeks	Primary activation of T cells
Chronic	Months – years	Causes unclear: antibodies, slow cellular reactions, immune complexes, recurrence of disease.

Table 1. Different types of graft rejection

4.1 Hyperacute rejection

Hyperacute rejection often occurs within minutes to hours after the host blood vessels are anastomosed to graft vessels. The rejection is mediated by pre-existing antibodies specific to the graft antigens (including ABO blood type antigens, VEC antigens and HLA antigens). Furthermore, these different antigens can activate the complement of the host and lead to damage to the endothelial cell. Studies have reported that the process is often accompanied with platelets activation and results in thrombosis and vascular occlusion (Fiane A E et al., 1999). In addition, the massive recruitment of neutrophils occurs, followed by rapid inflammation after transplantation. The pathological changes of hyperacute rejection are thrombotic occlusion of the graft vasculature ischemia, denaturation and necrosis (Figure 4). This rejection is relatively rare in liver transplantation.

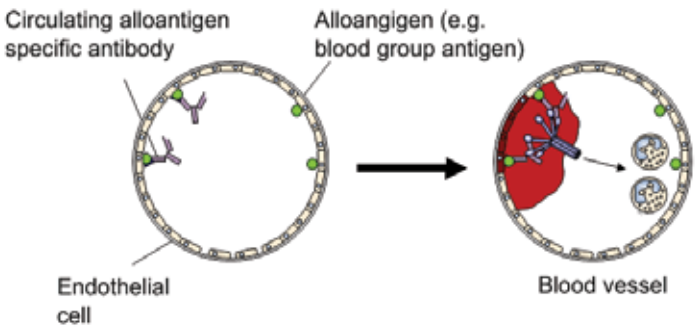


Fig. 4. Hyperacute rejection: complement activation, endothelial damage, inflammation, thrombosis and vascular occlusion

4.2 Acute rejection

Acute rejection occurs within days and up to three months after transplantation (80-90% of cases occur within one month). The rejection occurs due to donor HLA interaction with the host T cells, creating a cascade of immune responses initiated by that interface. After a solid organ transplant, there is an immunological milieu of activity. The mechanisms of the process involve abundant immune factors, such as humoral and/or cellular mechanisms (Figure 5). Antibodies can injure the graft by activating complement and mononuclear cells with Fc receptors that recognise alloantigens on the endothelial cell, resulting in vasculitis. Cytotoxic T cells (CD8+) will recognise alloantigens on an antigen presenting cell (APC) by direct presentation on the donor tissue and endothelial cells, which promotes the apoptosis of transplanted tissue. It has been shown that CD8+ cells alone are sufficient for the mediation of acute allograft rejection, but with the help of CD4+ cytokines secretion – such as IL-2 – clonal expansion and the expression of cytotoxic attack molecules will be upregulated (Kreisel D et al., 2002). The Fas/Fas ligand (FasL) pathway is another death-inducing pathway which is utilised by CD8+ cells. Whereas FasL is specifically induced upon CD8+ cells' activation, Fas is ubiquitously expressed on lymphoid and non-lymphoid tissue, including the liver. The Fas/FasL pathway is thought to play an important role in a variety of hepatic pathologies, and there is evidence that this pathway is also active during liver allograft rejection (Tannapel A et al., 1999; Ogura Y et al., 2001). Delayed hypersensitivity also has an important role in acute rejection, being initiated by alloantigen-

primed CD4+ cells specific to the donor class II (Carrodeguas L et., 1999). CD4+ cells release IFN-γ by re-exposure to specific alloantigens, a proinflammatory cytokine that can cause the activation of macrophages and the subsequent release of a variety of inflammatory mediators. These inflammatory mediators can augment the cellular anti-graft response or else can cause direct tissue damage. The acute rejection relatively occurs after liver transplantation is rare. But it has been a challenging process to try to unravel the participation of specific effector pathways and their interrelationships in the acute rejection of liver transplantation. The pathological features of acute rejection are acute vasculitis and parenchymal cell necrosis, along with the infiltration of lymphocytes and marophages (Figure 6).

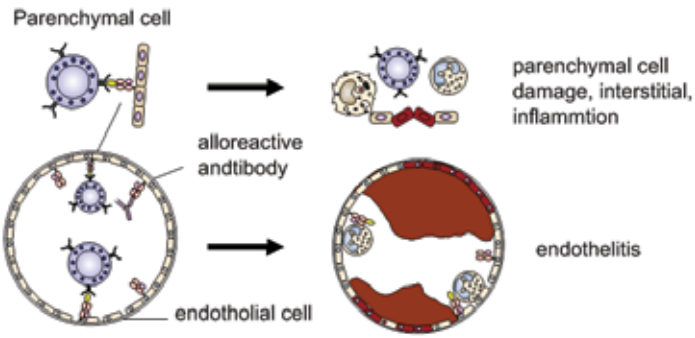


Fig. 5. Acute rejection: parenchymal cell damage and endotheliitis

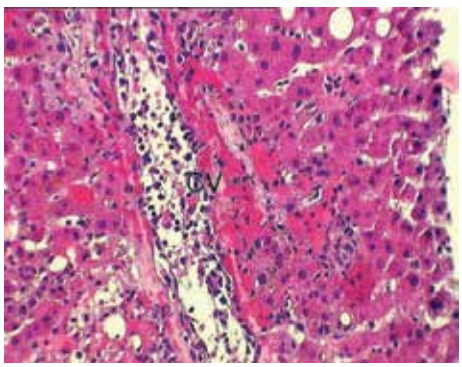


Fig. 6. A higher magnification of the previous photomicrograph details the subendothelial localisation of lymphocytes and the slight extension of the infiltrate into the perivenular hepatic parenchyma (http://tpis.upmc.com/TPIShome/)

4.3 Chronic rejection

Chronic rejection is less well-defined than either hyperacute or acute rejection, developing months or years after acute rejection reactions have subsided. Chronic rejection is an indolent but progressive form of allograft injury that is usually irreversible and which eventually results in the failure of most vascularised solid organ allografts. It is the single most significant obstacle to morbidity-free long-term survival. By five years after transplantation, it affects as many as 30-50% of heart, lung, pancreas and kidney allograft recipients, but only 4-8% of patients who undergo liver replacement (Demetris, A J et al.,

1997). Liver allografts differ from other solid organs in that chronic rejection is potentially reversible. This feature has been mainly attributed to its unique immunobiological privilege and the regenerative capacity of the process. Livers with chronic rejection have a decreased number of bile ducts on biopsy. This is referred to as "vanishing bile duct syndrome" (Demetris A et al., 2000). Chronic rejection is characterised by vasculopathy, fibrosis and a progressive loss of organ function. It is probably caused by multiple factors, viz., antibodies as well as lymphocytes (Figure 7). Chronic rejection may be mediated by a low-grade, persistent, delayed hypersensitivity response in which activated macrophages secrete mesenchymal cell-growth factors. Of potential importance are the persistent viral infections which induce cellular immune responses which in turn may synergise with donor-specific alloreactive T cells within the allograft. Chronic rejection may also reflect chronic ischemia secondary to the injury of blood vessels by antibody or cell-mediated mechanisms. Vascular occlusion may also occur as a result of smooth muscle cell proliferation in the intimae of arterial walls.

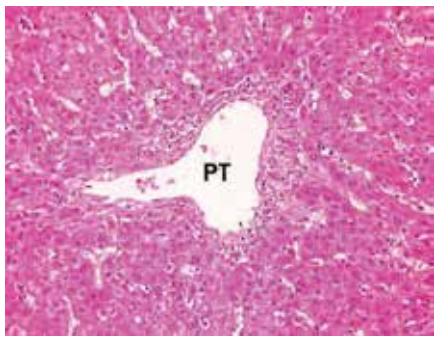


Fig. 7. Severe or very late-stage chronic rejection can result in the loss of the small branches of the hepatic artery, in addition to the loss of bile ducts. Note the lack of bile ducts and lack of hepatic artery branches in this portal tract (http://tpis.upmc.com/TPIShome/)

5. Prevention and treatment of allograft rejection

Allograft rejection is prevented by graft selection before transplantation, such as ABO blood group and HLA matching. Treatment of allograft rejection refers to immunosuppressive therapy, involving an immunosuppressive drugs selection and regimen, molecular therapy and cellular therapy. These related factors will be briefly described in this section.

5.1 Graft selection

The majority of liver transplant centres regard blood group compatibility as the primarily immunological selection criterion. A liver from a donor with a compatible ABO and Rh blood group is easy and feasible, with well-documented reports of this being performed in urgent situations (Gordon R D et al., 1986). In recent years, many transplantation centres have also carried out the operation with ABO-incompatible grafts, and the outcomes of ABO-incompatible liver transplantations have been similar to that of blood-type-matched transplantations in some centres. However, infection is the major cause of morbidity and mortality after ABO-incompatible liver transplantation (Tanabe M et al., 2010). At present, the transplantation of compatible but not identical livers is common practice, especially for

recipients with the less common blood groups. Interestingly, the results of ABO identical grafts were slightly better than the ABO compatible but non-identical grafts (Gugenheim J et al., 1990). An occasional complication with compatible, non-identical grafts is the occurrence of allograft rejection, due to the immunocompetent passenger lymphocytes within the transplanted liver producing antibodies against the recipient erythrocytes. It is well-established that renal transplantation in the presence of donor-specific cytotoxic antibodies – demonstrated by a positive cross-match – will result in rapid graft loss. However, the liver behaves in a totally contrary manner. In addition, the major histocompatibility antigens have a well-documented role in renal transplantation. However, early studies of liver transplantation in pigs implied that the liver may be a privileged organ exhibiting minimal rejection, with some grafts surviving without immunosuppression. This special feature prompted surgeons to ignore HLA-matching in patient selection for donor shortages. Retrospective data has not shown any clear survival advantages associated with good HLA-matching (Navarro V et al., 2006). Interestingly, some studies suggest that there is a clear disadvantage with certain aspects of HLA-matching. The largest series from Pittsburgh, involving more than 500 transplants, concludes that overall graft survival is actually reduced in grafts matched for HLA (Markus BH et al., 1988).

5.2 Immunosuppressive therapy

The liver is a privileged organ with a lower incidence of rejection than other organs, but immunosuppressive regimens are nonetheless required to control the alloreactive T-lymphocyte response after transplantation. In the 1990s, acute liver rejection occurred in up to 60% [1] of patients, without compromising graft or and patient survival (Neuberger J, 1999). Since 2000, the incidence of acute liver rejection has decreased to 15% of recipients. The incidence of chronic rejection is also declining, and most centres report current rates of 4% to 8%, whereas in the 1990s, rates of 15% to 20% were observed (Neuberger J, 1999).This decrease correlated with the use of new immunosuppressive drugs and improvements in treatment-management.

Over the last three decades, the number and types of immunosuppressive agents available to clinicians have increased considerably. The immunosuppressive therapy used in liver transplantation includes agents such as corticosteroids, calcineurin inhibitor (CNI), antimetabolites, inhibitors of TOR, and monoclonal and polyclonal antibodies which have different patterns of action (Figure 9) (Beaudreuil S et al., 2007). Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex. Corticosteroids are involved in a wide range of physiological systems, including stress-response, immune-response and the regulation of inflammation. The drugs are hydrophobic, which enables them to enter the cell by membrane diffusion. They then form complexes with cytosolic receptors, leading to their translocation to the nucleus where they bind to glucocorticoid-response elements in the promoter regions of cytokine genes, thereby blocking T cell-mediated cytokine expression. Thus, corticosteroids have been a mainstay of treatment during the early days after transplantation, but as immunosuppressive agents they are often accompanied by many side-effects within a few years. Calcineurin inhibitor is the first routinely employed immunosuppressive agent, including cyclosporine A (CyA) and tacrolimus (FK-506). CyA selectively inhibits T lymphocyte proliferation by forming a complex with cyclophilin. This complex can inhibit the calcium and calmodulin-dependent phosphatase calcineurin.

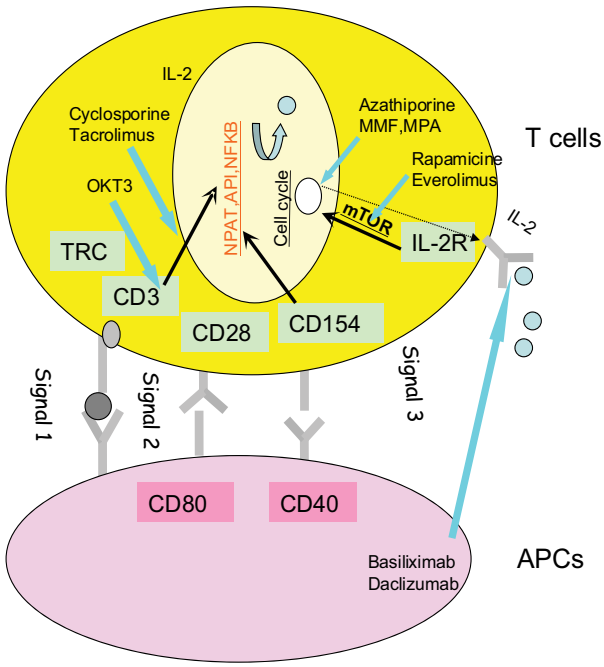


Fig. 9. Pattern of T cell activation and targeting of mainly immunosuppressors. Signals 1, 2 and 3 for T cell activation are shown with a number. The drug targets used in tolerance protocols are shown with blue arrow

Calcineurin is a key enzyme involved in controlling the transcription of IL-2 and other cytokines (Friman S et al., 1996). Therefore, impairing IL-2 transduction has a profound effect on the immune process of rejection by inhibiting calcineurin. However, the CyA metabolism is complex in liver transplant patients. Because it is metabolised primarily in the intestine and the liver, it increases the burden on the liver and even results in liver failure. Fk506 is very similar in action to CyA, but it is substantially more potent. It acts by binding to the FK-binding protein 12. The complex formed inhibits calcineurin, which regulates the transcription of the genes encoding IL-2, IL-3, IL-4, IL-8, as well as various chemotactic factors (Komolmit P et al., 1999). The side-effects of Fk506 are similar to those of CyA. In clinical practice, given the initial impairment of liver function and the frequent renal failure observed in the postoperative period, physicians should delay the administration of CyA or FK-506, with no impact on the outcome of liver transplantation or the occurrence of allograft rejection. Antimetabolites were not initially used in liver transplantation. Mycophenolate mofetil (MMF) – as a new antimetabolite molecule – has been shown to inhibit T and B cell proliferation, making it possible to reduce the rate of acute rejection in renal transplantation. These antimetabolites can be used together with an antibody against the IL2 receptor, delaying the introduction of CINs. These findings rapidly led to the use of these drugs in liver transplantation. Combination therapy with tacrolimus and MMF may significantly reduce the incidence of acute liver allograft rejection, allow a significant reduction in tacrolimus dosage, and decrease the incidence of nephrotoxicity (Eckhoff D E et al., 1998). In addition, the side-effects of MMF were relatively few. Inhibitors of TOR mainly include Rapamicine and Everolimus. Rapamicine is a macrocyclic triene antibiotic that is

structurally similar to tacrolimus. It forms a complex with the FK506-binding protein but it does not inhibit calcineurin. The complex blocks the cytokine response to T cell and B cell activation, preventing cell cycle progression and proliferation. Its principal side-effects are leukopenia, thrombocytopenia, high serum cholesterol and triglyceride levels, anaemia, lymphocele, wound dehiscence and mouth ulcers (Levitsky J., 2011). The biggest advantage of Rapamicine is associated with the lack of any significant nephrotoxicity (Vivarelli M et al., 2010). Compared with Rapamicine, Everolimus has greater bioavailability and a shorter half-life. The antibodies used in transplantation may be monoclonal or polyclonal. At present, monoclonal antibodies primarily include IL-2R antibodies and anti-CD52 antibodies. Two humanised IL-2R antibodies have been put on the market: basiliximab and daclizumab, which inhibit T cell proliferation by the competitive antagonism of IL-2-induced T cell proliferation, and they are accompanied with very few side-effects. OK3 is also currently the most widely-used monoclonal antibody, which binds to part of the T cell receptor (CD3) complex. The major impact of OK3 has been in the reversal of steroid-resistant, acute rejection (Cosimi A B et al., 1981). Polyclonal antibodies are IgG fractions from animals inoculated with human lymphocytes, thymocytes or cultured lymphoblast. Polyclonal antibodies have more profound and long-lasting biological depleting effects than other antibodies (Rebellato L M). However, polyclonal antibodies often induce the over-suppression of the immune system, increasing the risk of infectious diseases, lymphoproliferative syndrome and tumours.

There are significant variations in the regimens for immunosuppressive therapy used by different liver transplant centres. In general, most regimens include corticosteroids plus one calcineurin inhibitor, such as CyA or FK506. Anti-proliferative agents are often used in the first few months, with the patients also receiving bitoherapy with low doses of CIN and steroids. In liver transplantation, physicians tend to withdraw steroids within a few years due to their many side-effects. In addition, a study compared two groups of patients, one given induction therapy based on anti-thymocyte globulin (ATG) and FK506 without steroids, and the other treated with FK506, MMF and steroids. A graft survival of one-and-a-half years was 89% in both groups. However, rejection-rates were significantly lower in the group that was treated without steroids than in the group that was treated with steroids (Eason JD et al., 2001). Calcineurin inhibitor treatment often caused renal failure by nephrotoxicity. Studies have shown that up to 21% of patients were found to have developed chronic renal failure within five years of receiving a non renal transplant (Ojo AO et al.,2003). A recent report has shown that Sirolimus-based immunosuppressive therapy is a safe, effective replacement agent for primary immunosuppressive therapy in liver transplant recipients with FK506-related chronic renal insufficiency (Yang YJ et al., 2008). Furthermore, the addition of MMF to the regimen, and the reduction of the dose of calcineurin inhibitor by more than 50%, has been shown to improve renal function.

5.3 Prospective of using recipients T regulatory cell

In fact, the immunosuppression regimens used in liver transplantation were historically derived from those used in renal transplantation. Immunosuppressive regimens are required to control the allogeneic response in clinical liver transplantation, but they may also lead to severe complications, such as infectious diseases, cancers, cardiovascular diseases and – for treatments involving calcineurin inhibitors – chronic renal insufficiency.

T regulatory cells (Tregs), a subset of CD4+CD25+Foxp3+ lymphocytes, have the functional ability to suppress alloimmune responses both *in vitro* and *in vivo*. Increasing evidence from animal transplant research shows that Tregs can play a key role in promoting immunological unresponsiveness to allograft transplants (Pilat N et al., 2010; Webster KE et al., 2009). Regulatory T cells are the key cell-types in the induction of immune tolerance, and so the modulation of such cells may provide new strategies in creating transplant tolerance. However, there are several challenges to translating Tregs into the clinic. Tregs only account for about 5-10% of the total CD4+ T cells in the periphery, the limitation of cell number restricted the clinical application. There are a number of studies demonstrating the functional instability of Tregs *in vivo*, which can become IL-17 producing T effector cells in the presence of IL-6 (Yang XO et al., 2008). Furthermore, T effector cells activated under inflammatory conditions are highly resistant to Tregs-mediated suppression (Korn T et al., 2007). Concerning the Tregs, there are two broad approaches to the use of Tregs to promote transplant tolerance. The first is to expand Tregs *in vitro* and then apply expanded Tregs as a cell therapy *in vivo*. The advantage of this approach is that antigen-specific Tregs can be created *in vitro* using donor antigens. The second approach is to selectively and specifically stimulate *in vivo*, by taking advantage of fundamental differences between the biology of Tregs and T effector cells. In general, Tregs are a promising substance for the achievement of transplant tolerance.

6. Conclusion

The remarkable success of liver transplantation over the last four decades is due largely to the development of immunosuppressive regimens that are highly effective in protecting allografts from acute rejection, and that ensure their survival with a high quality of life in most cases. However, current immunosuppressive regimens do not prevent the development of chronic rejection, which constitutes a major cause of graft loss. In addition, these regimens may also lead to severe complications. This chapter mainly describe s the basic concepts of transplant immunology, the immunological basis of allograft rejection and the prevention and treatment of allograft rejection. In general, the immunological system of liver transplantation is very complex, and allograft tolerance has been well-established in experimental transplantation models; however, clinical operational tolerance will need to be further developed. Fortunately, Tregs may constitute a promising substance for achieving clinical operational tolerance by various modes of analysis. Furthermore, the clinical assessment of tolerance has been limited to laboratory-based evaluations of liver function and immunosuppressive agents' levels, and more precise clinical assessments should have been well-established.

7. References

Abstract section. VI meeting of the Mexican Association of Hepatology. Annals of Hepatology, 2010; 9(2): 225-246.

Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, Kaech SM, Wherry EJ, Onami T, Lanier JG, Kokko KE, Pearson TC, Ahmed R, Larsen CP (2003) Heterologous immunity provides a potent barrier to transplantation tolerance. J Clin Invest 111:1887–1895.

Afzali B, Lombardi G, Lechler RI. Pathways of major histocompatibility complex allorecognition. Curr Opin Organ Transplant 2008;13:438–444.

Araki K, Turner AP, Shaffer VO, et al. mTOR regulates memory CD8 T cell differentiation. Nature 2009;460:108–112.

Barber DL, Wherry EJ, Ahmed R. Cutting edge: rapid in vivo killing by memory CD8 T cells. J Immunol 2003;171:27–31.

Bartlett AS, McCall JL, Ameratunga R, Yeong ML, Gane E, Munn SR. Analysis of intragraft gene and protein expression of the co-stimulatory molecules, CD80, CD86 and CD154, in orthotopic liver transplant recipients. Am J Transplant 2003;3: 1363-1368.

Bartlett AS, McCall, Ameratunga R, Howden B, Yeong M-L, Benjamin CD, et al. Co-stimulatory blockade prevents early rejection, promotes lymphocyte apoptosis and inhibits the upregulation of inter-graft interleukin 6 in an orthotopic liver transplant model in the rat. Liver Transpl 2002;8:458-468.

Beaudreuil S, Samuel D, Rouas-Freiss N, Durrbach A. New aspect of immunosuppressive treatment in liver transplantation. How could you induce tolerance in liver transplantation? Transpl Immunol. 2007 Feb;17(2):98-107.

Bingaman AW, Farber DL. Memory T cells in transplantation: generation, function, and potential role in rejection. Am J Transplant 2004; 4:846–852.

Braillon A. Liver transplantation: from guidelines to randomized controlled trials. Ann Surg. 2010 Oct;252(4):705-6; author reply 706. PubMed PMID: 20881778.

Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, et al. Induction of immunological tolerance by porcine liver allografts. Nature 1969;223:472-476.

Carrodeguas L, Orosz CG, Waldman WJ, Sedmak DD, Adams PW, VanBuskirk AM. Trans-vivo analysis of human delayed-type hypersensitivity reactivity. Hum Immunol 1999;60:640-651.

Chalasani G, Dai Z, Konieczny BT, Baddoura FK, Lakkis FG (2002) Recall and propagation of allospecific memory T cells independent of secondary lymphoid organs. Proc Natl Acad Sci USA 99:6175–6180.

Cosimi AB, Burton RC, Colvin RB, Goldstein G, Delmonico FL, LaQuaglia MP, Tolkoff-Rubin N, Rubin RH, Herrin JT, Russell PS. Treatment of acute renal allograft rejection with OKT3 monoclonal antibody. Transplantation. 1981 Dec;32(6):535-9.

Demetris, A.J., Murase, N., Lee, R.G., Randhawa, P., Zeevi, A., et al. Chronic rejection. A general overview of histopathology and pathophysiology with emphasis on liver, heart and intestinal allografts. Ann Transplant 1997;2(2):27-44.

Demetris A, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. Hepatology. 2000 Mar;31(3):792-9

Dollinger MM, Howie SE, Plevris JN, Graham AM, Hayes PC, Harrison DJ. Intrahepatic proliferation of 'naïve' and 'memory' T cells during liver allograft rejection: primary immune response within the allograft. FASEB J. 1998 Aug;12(11):939-47.

Dresske B, Lin X, Huang DS, Zhou X, Fändrich F. Spontaneous tolerance: experience with the rat liver transplant model. Hum Immunol. 2002 Oct;63(10):853-61.

Duclos-Vallee JC, Sebagh M. Recurrence of autoimmune disease, primary sclerosing cholangitis, primary biliary cirrhosis, and autoimmune hepatitis after liver transplantation. Liver Transpl. 2009 Nov;15 Suppl 2:S25-34.

Eason JD, Loss GE, Blazek J, Nair S, Mason AL. Steroid-free liver transplantation using rabbit anti-thymocyte globulin induction: results of a prospective randomized trial. Liver Transplant 2001;7(8):693–7.

Eckhoff DE, McGuire BM, Frenette LR, Contreras JL, Hudson SL, Bynon JS. Tacrolimus (FK506) and mycophenolate mofetil combination therapy versus tacrolimus in adult liver transplantation. Transplantation. 1998 Jan 27;65(2):180-7.

Farges O, Morris PJ, Dallman MJ. Spontaneous acceptance of rat liver allografts is associated with an early downregulation of intragraft interleukin-4 messenger RNA expression. Hepatology. 1995 Mar;21(3):767-75.

Fiane AE, Videm V, Johansen HT, Mellbye OJ, Nielsen EW, Mollnes TE. C1-inhibitor attenuates hyperacute rejection and inhibits complement, leukocyte and platelet activation in an ex vivo pig-to-human perfusion model. Immunopharmacology 1999 May;42(1-3):231-43.

Friman S, Backman L. A new microemulsion formulation of cyclosporine: pharmacokinetic and clinical features. Clin Pharmacokinet 1996;30 (3):181–93.

Garcia S, DiSanto J, Stockinger B (1999) Following the development of a CD4 T cell response in vivo: from activation to memory formation. Immunity 11:163–171.

Gaweco AS, Wiesner RH, Yong S, Krom R, Porayko M, Chejfec G, et al. CD40L (CD154) expression in human liver allografts during chronic ductopenic rejection. Liver Transpl Surg 1999;5:1-7.

Gordon RD, Iwatsuki S, Esquivel CO, Tzakis A, Todo S, Starzl TE. Liver transplantation across ABO blood groups. Surgery. 1986 Aug;100(2):342-8.

Gowans JL. The fate of parental strain small lymphocytes in F1 hybrid rats. Ann N Y Acad Sci. 1962 Oct 24;99:432-55.

Gugenheim J, Samuel D, Reynes M, Bismuth H. Liver transplantation across ABO blood group barriers. Lancet. 1990 Sep 1;336(8714):519-23.

Guichelaar MM, Benson JT, Malinchoc M, Krom RA, Wiesner RH, Charlton MR. Risk factors for and clinical course of non-anastomotic biliary strictures after liver transplantation. Am J Transplant. 2003 Jul;3(7):885-90.

Komolmit P, Davies MH. Tacrolimus in liver transplantation. Expert Opin Investig Drugs 1999;8(8):1239–54.

Korn T, Reddy J, Gao W, Bettelli E, Awasthi A, Petersen TR, Bäckström BT, Sobel RA, Wucherpfennig KW, Strom TB, Oukka M, Kuchroo VK. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. Nat Med. 2007 Apr;13(4):423-31.

Koyama I, Nadazdin O, Boskovic S, et al. Depletion of CD8 memory T cells for induction of tolerance of a previously transplanted kidney allograft. Am J Transplant 2007;7:1055–1061.

Kreisel D et al. Non-hematopoietic allograft cells directly activate CD8+ T cells and trigger acute rejection: an alternative mechanism of allorecognition. Nat Med 2002; 8(3): pp 233-9.

Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P (2000) Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 288:675–678.

Kwekkeboom J, Kuijpers MA, Bruyneel B, Mancham S, De Baar-Heesakkers E, Ijzermans JN, et al. Expression of CD80 on Kupffer cells is enhanced in cadaveric liver transplants. Clin Exp Immunol 2003;132:345-351.

Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation: how much of the promise has been realized? Nat Med 2005; 11:605–613.

Levitsky J. Operational tolerance: past lessons and future prospects. Liver Transpl. 2011 Mar;17(3):222-32.

Li F, Atz ME, Reed EF. Human leukocyte antigen antibodies in chronic transplant vasculopathy-mechanisms and pathways. Curr Opin Immunol 2009;21:557–562.

Li W, Li B, Fan W, Geng L, Li X, Li L, Huang Z, Li S. CTLA4Ig gene transfer alleviates abortion in mice by expanding CD4+CD25+ regulatory T cells and inducing indoleamine 2,3-dioxygenase. J Reprod Immunol. 2009 Jun;80(1-2):1-11. Epub 2009 Apr 11. PubMed PMID: 19362742.

Liu LU, Schiano TD, Min AD, Kim-Schluger L, Schwartz ME, Emre S, Fishbein TM, Bodenheimer HC Jr, Miller CM. Syngeneic living-donor liver transplantation without the use of immunosuppression. Gastroenterology. 2002 Oct;123(4):1341-5. PubMed PMID: 12360494.

Markus BH, Duquesnoy RJ, Gordon RD, Fung JJ, Vanek M, Klintmalm G, Bryan C, Van Thiel D, StarzlTE. Histocompatibility and liver transplant outcome. Does HLA exert a dualistic effect? Transplantation. 1988 Sep;46(3):372-7.

Mellor AL, Baban B, Chandler P, Marshall B, Jhaver K, Hansen A, Koni PA, Iwashima M, Munn DH. Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. J Immunol. 2003 Aug 15;171(4):1652-5. PubMed PMID: 12902462.42.

Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. J Immunol. 2004 Apr 15;172(8):4676-80. PubMed PMID: 15067041.

Navarro V, Herrine S, Katopes C, Colombe B, Spain CV. The effect of HLA class I (A and B) and class II (DR) compatibility on liver transplantation outcomes: an analysis of the OPTN database. Liver Transpl. 2006 Apr;12(4):652-8.

Neuberger J. Incidence, timing, and risk factors for acute and chronic rejection. Liver Transpl Surg. 1999 Jul;5(4 Suppl 1):S30-6.

Neumann UP, Fischer U, Schmitz V, Lang M, Langrehr JM, Neuhaus P. Long-term graft acceptance after rat liver allograft transplantation induced by application of CTLA4-Ig and donor specific spleen cell administration. Transplant Proc 2002;34: 1400-1401.

Ogura Y, Martinez OM, Villanueva JC, Tait JF, Strauss HW, Higgins JPT, et al. Apoptosis and allograft rejection in the absence of CD8 T cells. Transplantation 2001;71:1827-1834.

Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young AW, et al. Chronic renal failure after transplantation of a non-renal organ. N Engl J Med 2003;349(10):931–40.

Olthoff K, Judge TA, Gelman AE, Xhen XD, Hancock WW, Turka LA, Shaked A. Adenovirus-mediated gene transfer into cold-preserved liver allografts: survival

pattern and unresponsiveness following transduction with CTLA4Ig. Nat Med 1998;4: 194-200.

Pearl JP, Parris J, Hale DA, et al. Immunocompetent T cells with a memory-like phenotype are the dominant cell type following antibody-mediated T cell depletion. Am J Transplant 2005;5: 465–474.

Pilat N, Baranyi U, Klaus C, Jaeckel E, Mpofu N, Wrba F, Golshayan D, Muehlbacher F, Wekerle T. Treg-therapy allows mixed chimerism and transplantation tolerance without cytoreductive conditioning. Transplant Proc. 2010 Apr;10(4):751-62.

Rebellato LM, Gross U, Verbanac KM, Thomas JM. A comprehensive definition of the major antibody specificities in poyclonal rabbit anti-thymocyte globulin. Transplantation 1994;57(5):685–94.

Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004;22:745–763.

Sallusto F, Langenkamp A, Geginat J, et al. Functional subsets of memory T cells identified by CCR7 expression. Curr Top Microbiol Immunol 2000;251:167–171.

Sayegh MH, Carpenter CB. Transplantation 50 years later: progress, challenges, and promises. N Engl J Med 2004; 351:2761–2766.

Schenk AD, Nozaki T, Rabant M, et al. Donor-reactive CD8 memory T cells infiltrate cardiac allografts within 24-h post-transplant in naïve recipients. Am J Transplant 2008;8:1652–1661.

Schramm C, Bubenheim M, Adam R, Karam V, Buckels J, O'Grady JG, Jamieson N, Pollard S, Neuhaus P, Manns MM, Porte R, Castaing D, Paul A, Traynor O, Garden J, Friman S, Ericzon BG, Fischer L, Vitko S, Krawczyk M, Metselaar HJ, Foss A, Kilic M, Rolles K, Burra P, Rogiers X, Lohse AW; European Liver Intestine Transplant Association. Primary liver transplantation for autoimmune hepatitis: a comparative analysis of the European Liver Transplant Registry. Liver Transpl. 2010 Apr; 16(4):461-9.

Seetharam A, Tiriveedhi V, Mohanakumar T. Alloimmunity and autoimmunity in chronic rejection. Curr Opin Organ Transplant 2010;15:531–536.

Serra P, Amrani A, Yamanouchi J, Han B, Thiessen S, Utsugi T, Verdaguer J, Santamaria P. CD40 ligation releases immature dendritic cells from the control of regulatory CD4+CD25+ T cells. Immunity. 2003 Dec;19(6):877-89. PubMed PMID: 14670304.

Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M, Kusne S, Rudert W. A, Trucco M. Baboon-to-human liver transplantation. Lancet. 1993 Jan 9;341(8837):65-71.

Stefanova I, Dorfman JR, Tsukamoto M, et al. On the role of self-recognition in T cell responses to foreign antigen. Immunol Rev 2003;191:97–106.

Strom TB, Tilney NL, Carpenter CB, et al. Identity and cytotoxic capacity of cells infiltrating renal allografts. N Engl J Med 1975; 292:1257–1263.

Tanabe M, Kawachi S, Obara H, Shinoda M, Hibi T, Kitagawa Y, Wakabayashi G, Shimazu M, Kitajima M. Current progress in ABO-incompatible liver transplantation. Eur J Clin Invest. 2010 Oct;40(10):943-9.

Tannapel A, Kohlhaw K, Ebelt J, Hauss J, Liebert U, Berr F, Wittekind C. Apoptosis and the expression of Fas and Fas ligand (FasL) antigen in rejection and re-infection in liver allograft specimens. Transplantation 1999;15:1079-1083.

Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of co-stimulatory blockade on prolonged cardiac allograft survival in mice. Am J Transplant 2002;2:501–509.

Vivarelli M, Dazzi A, Cucchetti A, Gasbarrini A, Zanello M, Di Gioia P, Bianchi G, Tamè MR, Gaudio MD, Ravaioli M, Cescon M, Grazi GL, Pinna AD. Sirolimus in liver transplant recipients: a large single-center experience. Transplant Proc. 2010 Sep;42(7):2579-84.

Vu MD, Clarkson MR, Yagita H, et al. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. J Immunol 2006;176:1394–1401.

Watschinger B. How T cells recognize alloantigen: evidence for two pathways of allorecognition. Nephrol Dial Transplant. 1995;10(9):1556-8.

Webster KE, Walters S, Kohler RE, Mrkvan T, Boyman O, Surh CD, Grey ST, Sprent J. In vivo expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. J Exp Med. 2009 Apr 13;206(4):751-60.

Wu Z, Bensinger SJ, Zhang J, et al. Homeostatic proliferation is a barrier to transplantation tolerance. Nat Med 2004;10: 87–92.

Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008 Jul 18;29(1):44-56.

Yang YJ, Chen DZ, Li LX, Kou JT, Lang R, Jin ZK, Han DD, He Q. Sirolimus-based immunosuppressive therapy in liver transplant recipient with tacrolimus-related chronic renal insufficiency. 2008 Jun;40(5):1541-4.

Yoshizawa A, Takada Y, Fujimoto Y, Koshiba T, Haga H, Nabeshima S, Uemoto S. Liver transplantation from an identical twin without immunosuppression, with early recurrence of hepatitis C. Am J Transplant. 2006 Nov;6(11):2812-6. Epub 2006 Aug 25. PubMed PMID: 16939511.

Zhai Y, Meng L, Gao F, Busuttil RW, Kupiec-Weglinski JW (2002) Allograft rejection by primed/memory CD8+ T cells is CD154 blockade resistant: therapeutic implications for sensitized transplant recipients. J Immunol 169:4667–4673.

Zheng XX, Markees TG, Hancock WW, et al. CTLA4 signals are required to optimally induce allograft tolerance with combined donor-specific transfusion and anti-CD154 monoclonal antibody treatment. J Immunol 1999;162:4983–4990.

5

Clinical Immunosuppression

Chunbao Guo
Department of Hepatobiliary Surgery, Children's Hospital, Chongqing Medical University, Chongqing, P.R. China

1. Introduction

Organ transplantation is the act of transferring organs from donors to recipients. Thus, the malfunction of an organ system can be corrected with the transplantation of an organ; organs that have successfully been transplanted include the kidney, liver, heart, lung, and pancreas. Over the past 50 years, the medical community has witnessed great advances in the care of liver transplant recipients, including new immunosuppression modalities, therapies for chronic rejection, and improved operative and preservation techniques. However, the immune system remains the most formidable barrier for clinicians to perform transplantations as a routine form of medical treatment[1].

The immune system has developed elaborate and effective mechanisms to combat foreign agents, including antigens derived transplantation; thus, in transplant recipients, transplantation elicits a complex series of immunological processes. Rejection results when a pathologic and intense inflammatory response develops, or when repair and remodeling of tissues fails. Knowledge of these mechanisms is important to understand the clinical features of rejection, which enables early diagnosis and the delivery of appropriate treatments[1]. These mechanisms are generally categorized as inflammation, immunity, tissue repair, and structural reinforcement of damaged tissues. Comprehension of these mechanisms is also critical for the development of novel drugs, treatments, and strategies to minimize rejection and inhibit the effects of the immune system on transplanted organs, helping to extend the survival and functionality of transplanted organs[2].

The introduction of routine pretransplant screening of graft recipients for anti-donor antibodies has made hyperacute rejection rare. However, no accepted therapeutic strategy to treat chronic rejection is currently recognized. The control of acute rejection has been the primary aim of immunosuppression, thereby allowing tissue repair to progress[3].

The use of combination immunosuppressive therapy has evolved over time. With all the successes of immunosuppressive therapies comes the obligation to tailor treatments to meet the individual patient's characteristics and to balance the risks and benefits of these medications.

Transplantation tolerance could eliminate many of the adverse events associated with immunosuppressive agents. Safe and reliable strategies for the induction of full tolerance have not yet been developed. However, methods to induce states of "partial tolerance" have

been discovered, where lower-than-conventional amounts of ongoing pharmacologic immunosuppression are needed[3, 4]. Although the induction of immunologic tolerance has been achieved and studied in numerous laboratory animal models, immune tolerance remains an elusive goal of transplantation immunology and clinical organ transplantation.

2. History of immunosuppression

Early efforts at transplantation were unsuccessful because of inadequacies in surgical techniques and a fundamental lack of knowledge regarding the immune system. The development of immunosuppressive drug treatments enabled organ transplantation and improved the survival of transplanted organs since the first liver transplant by Dr. Thomas E. Starzl in 1963. Since then, many new and progressively more selective immunosuppressive agents and treatment strategies have been developed. As knowledge of the immune system evolved, therapies that targeted specific immunoregulatory organs enabled the ability to prolong life through organ transplantation. Initial attempts at immunosuppression were with total body radiation, but all of the patients died. In 1949, corticosteroids were used to treat autoimmune disorders and to prevent allograft rejection. Since then, many new and progressively more selective immunosuppressive agents have been developed. These therapies have enabled organ transplantation and improved the survival of transplanted organs. In 1959, cyclophosphamide was demonstrated to suppress antibody production and was used for bone marrow transplantation. In the 1960s, azathioprine (AZA) was found to delay organ graft rejection and was used to suppress the rejection of transplanted kidneys. The first polyclonal anti-lymphocyte globulin was used in 1967, and it spawned the development of other polyclonal and monoclonal antibodies for immunosuppressive therapy. After the first initially successful series of transplantations were performed between 1962 and 1964 in Denver, Colorado, the combination of azathioprine and steroids came into widespread use, becoming part of the primary immunosuppressive regimen for the next 20 years. The T cell-inhibiting properties of cyclosporine, a calcineurin inhibitor, were discovered in 1976. Subsequently, cyclosporine (Sandimmune and later, Neoral) was introduced in the 1980s, when it was used in combination with azathioprine and steroids to prevent rejection in allograft transplants. Its use was credited with a dramatic improvement in graft survival. In 1969, methotrexate was shown to inhibit antibody production and the development of delayed hypersensitivity in guinea pigs[5].

The development of mycophenolate mofetil (MMF), an inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitor, began in 1982, and research continues on other IMPDH inhibitors. Mycophenolate mofetil rapidly replaced azathioprine almost universally. The next advance occurred in 1987, with the introduction of tacrolimus (FK506) to inhibit interleukin (IL)-2 production and lymphocyte proliferation. Tacrolimus has gradually supplanted cyclosporine in many transplant centers. Interest in the antibiotic sirolimus (SRL), which is also known as rapamycin, was renewed in the 1980s when it was shown to prevent allograft rejection.

Other immunosuppressive agents and their dates of discovery include mizoribine in 1981, leflunomide in 1978, deoxyspergualin in 1981, muromab-CD3 (OKT3) in 1985, brequinar in 1986, azodicarbonamide in 1989, vitamin D analogs such as MC1288 in 1991, and bisindolylmaleimide VIII in 1999. Other agents that were developed include Minnesota anti-lymphocyte globulin and anti-thymocyte globulin (ATG)[6].

3. Clinical stages of rejection

Rejection is the consequence of the recipient's alloimmune response to the non-self antigens expressed in donor tissues[7]. Clinically, it can be divided into the following three stages:

3.1 Hyperacute rejection

In hyperacute rejection, the transplanted tissue is rejected within minutes to hours after graft implantation because transplant patients are serologically presensitized to nonself graft antigens, which are known as alloantigens. Histologically, numerous polymorphonuclear leukocytes (PMNs) exist within the graft vasculature and are associated with widespread microthrombi formation and platelet accumulation. Little or no leukocyte infiltration occurs. Hyperacute rejection is humorally mediated and occurs because the recipient has pre-existing antibodies against graft-derived antigens, which can be induced by prior blood transfusions, multiple pregnancies, prior transplantation, or xenografts. The antigen-antibody complexes activate the complement system, causing massive thrombosis in the capillaries, which prevents graft vascularization. The liver is relatively resistant to hyperacute rejection. Although this may be due to its dual blood supply, it is more likely because of incompletely understood immunologic properties. Hyperacute rejection has become relatively rare since the introduction of routine pretransplantation screening of graft recipients for anti-donor antibodies.

3.2 Acute rejection

Acute rejection can occur within 24 hours of graft implantation and continue over a period of days to weeks. Acute rejection commonly manifests within the first 6 months after transplantation.

Acute cellular rejection is mediated by lymphocytes that have been activated against donor antigens, primarily in the lymphoid tissues of the recipient. Graft antigens are recognized by T cells and the resulting release of cytokines eventually leads to tissue distortion, vascular insufficiency, and cell destruction. Histologically, leukocytic infiltration that is dominated by equivalent numbers of macrophages and T cells is observed within the interstitium. The donor dendritic cells, which are also called passenger leukocytes, enter the circulation and function as antigen-presenting cells (APCs).

The primary aim of immunosuppression has been to control acute rejection by allowing time for tissue repair to occur. Most combination therapies block T-cell activation by providing intense immunosuppression during the immediate post-transplantation period known as the induction phase. Later, during the immunosuppressive maintenance phase, multiple-drug cocktails are administered to maintain a state of low- or nonresponsiveness to the allograft.

3.3 Chronic rejection

Chronic rejection develops months to years after acute rejection episodes have subsided. Chronic rejections are both antibody- and cell-mediated. The use of immunosuppressive drugs and tissue-typing methods has increased the survival of allografts in the first year, but chronic rejection remains unpreventable in most cases[5].

Although chronic rejection appears as fibrosis and scarring in all transplanted organs, the specific histopathological picture depends on the organ transplanted. In liver transplants, chronic rejection is characterized by vanishing bile duct syndrome. Histologically, progressive neointimal formation occurs within large and medium arteries and, to a lesser extent, within the veins of the graft. Leukocyte infiltration usually is mild or absent. These processes result in reduced blood flow, with subsequent regional tissue ischemia, fibrosis, and cell death. In chronic rejection, pathologic tissue remodeling results from peritransplant and posttransplant trauma. Cytokines and tissue growth factors induce smooth muscle cells to proliferate, migrate, and produce new matrix material. Interstitial fibroblasts are also induced to produce collagen. The factors that can increase the risk of chronic rejection include previous episodes of acute rejection, inadequate immunosuppression, extended periods of cold ischemia, the development of posttransplant infections such as cytomegalovirus (CMV), initial delayed graft function, and organ reperfusion injury.

Currently, unless inadequate immunosuppression is the cause of rejection, no accepted therapeutic strategies exist for the reversal of chronic rejection. The CD40 and CD28 pathways have been proposed as being important in initiating T-cell responses and lowering T-cell activation thresholds, respectively. Therefore, blocking T-cell costimulation has been proposed to improve long-term outcomes.

4. Mechanisms of rejection

The immune response to a transplanted organ consists of both cellular (lymphocyte-mediated) and humoral (antibody-mediated) mechanisms. The evolving understanding of liver allograft rejection was reviewed by Eksteen[23].

4.1 Acute humoral rejection

Humoral rejection is a form of allograft injury and subsequent dysfunction that is primarily mediated by antibodies and complement. The antibodies involved are either preformed antibodies or anti-donor antibodies that develop following transplantation. Proteinuria is associated with donor-specific antibody detection; it is likely an important factor in the rapid decline in glomerular filtration rates and early graft failure in patients that develop de novo anti-HLA antibodies. The presence of even low levels of donor-specific antibodies, which may not be detected by complement-dependent cytotoxic and flow cytometry crossmatches, have been shown to be associated with inferior allograft outcomes. These patients may require augmented immunosuppression.

Following transplantation, the inactive product C4d from the classical complement activation pathway is deposited in peritubular capillaries (PTC); immune detection of this product in allograft biopsies is used for the diagnosis of antibody-mediated rejection. However, one study reported substantial fluctuations in C4d Banff scores in the first year following transplantation[8]; these authors suggested that these results may reflect the dynamic and unpredictable nature of the humoral process. Thus, C4d by itself may not be a sufficiently sensitive indicator, and detection of microvascular inflammation utilizing donor-specific antibodies may be more useful for diagnosing antibody-mediated rejection.

4.2 T cell-mediated rejection

Although other cell types are also involved, T cells are central in graft rejection. The rejection reaction consists of the sensitization stage and the effector stage.

Sensitization stage

In this stage, CD4 and CD8 T cells, via their T-cell receptors, recognize the alloantigens expressed on the cells of the foreign graft. Two signals are needed for antigen recognition; the first is provided by the interaction of the T cell receptor with the antigen presented by an MHC molecule, while the second signal is provided by a costimulatory receptor/ligand interaction. Of the numerous costimulatory pathways, the interaction of CD28 on the T cell surface with its APC surface ligands, B7-1 or B7-2 (commonly known as CD80 or CD86, respectively), has been most studied. In addition, cytotoxic T lymphocyte–associated antigen-4 (CTLA4) on T cells also binds to these ligands, providing an inhibitory signal. Other costimulatory molecules include CD40 and its ligand, CD40L (CD154)[9, 10].

Typically, the helices of the MHC molecules form the peptide-binding groove and are occupied by peptides derived from normal cellular proteins. Thymic or central tolerance mechanisms (clonal deletion) and peripheral tolerance mechanisms, such as anergy, ensure that these self-peptide MHC complexes are not recognized by T cells, thereby preventing autoimmune responses.

At least 2 distinct, but not necessarily mutually exclusive, pathways of allorecognition exist: the direct and indirect pathways. Each leads to the generation of different sets of allospecific T cell clones.

4.3 Direct pathway

In the direct pathway, host T cells recognize intact allo-MHC molecules on the surface of the donor cells or APCs. Mechanistically, host T cells see allo-MHC molecule + allo-peptide as being equivalent in shape to self-MHC + foreign peptide; therefore, host T cells recognize the donor tissue as foreign. This pathway is presumably the dominant pathway involved in early alloimmune responses.

The transplanted organ carries a variable number of passenger APCs in the form of interstitial dendritic cells. Such APCs have a high density of allo-MHC molecules and are capable of directly stimulating recipient T cells. The relative number of T cells that proliferate on contact with allogeneic or donor cells is extraordinarily high compared to the number of clones that target antigens presented by self-APCs. Thus, this pathway is important in acute allorejection.

4.4 Indirect pathway

In the indirect pathway, T cells recognize processed alloantigens that are presented as peptides by self-APCs. Secondary responses such as those that occur in chronic or late acute rejection are associated with T cell proliferative responses to a more variable repertoire, including peptides that were previously immunologically silent. Such a change in the pattern of T cell responses has been termed epitope switching or spreading.

A link between self-MHC + allopeptide-primed T cells and the development of acute vascular rejection has been demonstrated to be mediated in part by accelerated alloantibody production. In addition, chronic allograft vasculopathy may be mediated by T cells primed by the indirect pathway[11].

4.5 Molecular mechanisms of T cell activation

During T cell activation, membrane-bound inositol phospholipid is hydrolyzed into diacylglycerol (DAG) and IP3. This increases the concentration of cytoplasmic calcium. The elevation in calcium promotes the formation of calcium-calmodulin complexes that activate a number of kinases as well as protein phosphatase IIB or calcineurin. Calcineurin dephosphorylates cytoplasmic nuclear factor of activated T cells (NFAT), permitting its translocation to the nucleus, where it binds to the IL-2 promoter sequence and stimulates transcription of IL-2 mRNA. Numerous other intracellular events, including protein kinase C (PKC) activation by DAG and the activation of nuclear factor kappa B (NF-κB) also occur at the molecular level[10].

Effector stage

Alloantigen-dependent and -independent factors contribute to the effector mechanisms. Initially, nonimmunologic "injury responses" (ischemia) induce a nonspecific inflammatory response. Because of this, antigen presentation to T cells is increased because of the upregulated expression of adhesion molecules, MHC class II, chemokines, and cytokines. It also promotes the shedding of intact, soluble MHC molecules that may activate the indirect allorecognition pathway. After activation, CD4 T cells initiate macrophage-mediated delayed type hypersensitivity (DTH) responses and provide help to B cells to initiate antibody production.

Various T cells and T cell-derived cytokines such as IL-2 and IFN-γ are upregulated early after transplantation. Later, ß-chemokines, including regulated upon activation, normal T cell expressed and secreted (RANTES), IP-10, and MCP-1 are expressed, promoting intense macrophage infiltration of the allograft. IL-6, TNF-α, inducible nitric oxide synthase (iNOS) and growth factors also play a role in this process. Growth factors, including TGF-ß and endothelin, cause smooth muscle proliferation, intimal thickening, interstitial fibrosis, and, in the case of the kidney, glomerulosclerosis.

Endothelial cells activated by T cell–derived cytokines and macrophages express MHC class II, adhesion molecules, and costimulatory molecules. Therefore, these cells can present antigen and recruit more T cells, amplifying the rejection process. CD8 T cells mediate cell-mediated cytotoxicity reactions by inducing cell lysis or apoptosis.

4.6 Apoptosis

The final common pathway for cytolytic processes is the triggering of apoptosis in the target cell. After CTL activation, CTLs produce cytotoxic granules that contain perforin and granzymes. At the time of target cell identification and engagement, these granules fuse with the effector cell membrane and extrude their content into the immunological synapse. By a yet unknown mechanism, the granzymes are inserted into the target cell cytoplasm where granzyme B triggers apoptosis through several different mechanisms, including the

direct cleavage of procaspase-3 and the indirect activation of procaspase-9. This has been shown to play the dominant role in apoptosis induction in allograft rejection.

Alternatively, CTLs can also use the Fas-dependent pathway to induce cytolysis and apoptosis. The Fas pathway is also important in limiting T cell proliferation in response to antigenic stimulation; this is known as fratricide between activated CTLs. Cell-mediated cytotoxicity has been shown to play an important role in acute, but not chronic, allograft rejection[12].

4.7 Role of natural killer cells

Natural killer (NK) cells are important in transplantation because of their ability to distinguish allogenic cells from self and their potent cytolytic effector mechanisms. These cells can mount maximal effector responses without any prior immune sensitization. Unlike T and B cells, NK cells are activated by the absence of MHC molecules on the surface of target cells, which is commonly referred to as the "missing self" hypothesis. Recognition is mediated by various NK inhibitory receptors triggered by specific alleles of MHC class I antigens on cell surfaces.

In addition, NK cells also possess stimulatory receptors, which are triggered by antigens on nonself cells. NK cell effector responses include both cytokine release and direct toxicity mediated through perforin, granzymes, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL). Through this "double negative" mode of activation, they are thought to play a role in the rejection of both bone marrow transplants and transplanted lymphomas in animal models.

NK cells also provide help to CD28-positive host T cells, thereby promoting allograft rejection. Their importance in bone marrow transplants has been recognized for years. In humans, the NK cell-mediated graft-versus-host alloresponse has been used for its potent graft-versus-leukemia effect, contributing to an increase in the rate of sustained remission in patients with acute myelogenous leukemia[11].

NK cells are now being recognized as active participants in acute and chronic rejection of solid tissue grafts. Recent studies have indicated that NK cells are present and activated following infiltration into solid organ allografts. They may regulate cardiac allograft outcomes. Studies have also shown that humans with killer cell immunoglobulin-like receptors that are inhibited by donor MHC have a decreased risk of liver transplant rejection. In cases of renal transplantation, NK cells are not suppressed by the current immunosuppressive regimens[13].

4.8 Role of innate immunity

Although T cells play a critical role in acute rejection, the up-regulation of proinflammatory mediators in the allograft is now recognized to occur prior to T cell-mediated responses. This early inflammation following engraftment is due to the innate response to tissue injury that is independent of the adaptive immune system. Several recent studies have examined the role of Toll-like receptor (TLR) agonists and TLR signals in allorecognition and rejection.

The activation of innate mechanisms alone does not appear to be sufficient to lead to graft rejection itself. However, they are important for optimal adaptive immune responses to the

graft and may play a major role in resistance to tolerance induction. The development of methods to blunt innate immune responses, which has potential implications for a wide variety of diseases, is likely to have a significant impact on transplantation as well.

5. Minimizing rejection

Rejection is the consequence of the recipient's alloimmune response to the nonself antigens expressed in donor tissues. Although rejection cannot be completely prevented, a degree of immune tolerance to the transplant can develop. Several concepts have been postulated to explain the development of partial tolerance. They include clonal deletion, the development of anergy in donor-specific lymphocytes, development of suppressor lymphocytes, and the production of factors that down-regulate the immune response against the graft. Other hypotheses include the persistence of donor-derived dendritic cells in the recipient that promote an immunologically mediated chimeric state between the recipient and the transplanted organ.

Tissue typing or crossmatching is performed prior to transplantation to assess donor-recipient compatibility for human leukocyte antigen (HLA) and ABO blood group.

The ABO blood group compatibility is tested first because incompatibility between the blood groups leads to rapid rejection. In the lymphocytotoxicity assay, patient sera are tested for reactivity with donor lymphocytes. A positive crossmatch is a contraindication for transplantation because of the risk of hyperacute rejection. The lymphocytotoxicity assay is used mainly for kidney transplantation.

The panel-reactive antibody (PRA) test screens the serum of a patient for lymphocytic antibodies against a random cell panel. Patients with prior transfusions, transplants, or pregnancies may have a high degree of sensitization and are less likely to have a negative crossmatch with a donor. A reduced risk of sensitization at the time of a second transplant has been observed when a more potent immunosuppression therapy comprised of rabbit anti-thymocyte globulin, tacrolimus, and mycophenolate mofetil/sodium is utilized for nonsensitized primary kidney or kidney/pancreas transplant patients. Mixed lymphocyte reactions (MLR) can be used to assess the degree of major histocompatibility complex (MHC) class I and class II compatibility. However, this test is not rapid and can be used only in cases involving living related donors. Therefore, this test is rarely used at present.

The primary aim of immunosuppression has been to control acute rejection by allowing tissue repair to develop. Immunosuppressive drugs are used in 2 phases: the initial induction phase, which requires much higher doses of these drugs, and the later maintenance phase.

Immunosuppression can be achieved by several mechanisms that affect lymphocytes including lymphocyte depletion, diversion of lymphocyte traffic, or blocking lymphocyte response pathways[14, 15].

Most combination therapies block T-cell activation by providing intense immunosuppression during the immediate posttransplantation period (induction phase).
Immunosuppressive agents exert their effects through the following mechanisms.

- Regulators of gene expression: The classic examples are glucocorticoids; others include vitamin D analogs and deoxyspergualin. However, recent studies have shown that glucocorticoids affect inflammation by other nongenomic mechanisms.

- Alkylating agents: Cyclophosphamide and other deoxyribonucleic acid (DNA) alkylating agents are mutagenic and can increase the risk of developing cancer.
- Kinase and phosphatase inhibitors: These include cyclosporin A (CsA), tacrolimus (FK506), and sirolimus (SRL), which inhibit kinase cascades.
- Inhibitors of de novo purine synthesis: The first-generation inhibitors are 6-mercaptopurine and azathioprine; the second-generation inhibitors are mizoribine and MMF. Potential third-generation enzyme targets include inosine monophosphate dehydrogenase and T lymphocyte-specific purine nucleoside phosphorylase. The polygentamate derivatives of methotrexate are antifolate compounds that inhibit de novo purine synthesis.
- Inhibitors of de novo pyrimidine synthesis: These inhibitors include brequinar, leflunomide, and the structurally related malononitrilamides that inhibit dihydroorotate dehydrogenase.

6. Clinically relevant immunosuppressive agents

Immunosuppressive agents are drugs that inhibit or block the activity of the immune system to prevent the rejection of transplanted organs and tissues.

These drugs are not without side effects and risks. Because the majority of immunosuppressive agents act non-selectively, the immune system is less able to resist infections and the spread of malignant cells. There are also other side effects, which include hypertension, dyslipidemia, hyperglycemia, peptic ulcers, and liver or kidney injury. Immunosuppressive drugs also interact with other medicines, affecting their metabolism and action. Actual or suspected immunosuppressive agents can be evaluated in terms of their effects on lymphocyte subpopulations in tissues using immunohistochemistry [16, 17].

Immunosuppressive drugs can be classified into the following groups:

6.1 Corticosteroids

Steroids have been the cornerstone of immunosuppression and remain important in treating episodes of acute rejection. However, newer treatment regimens are trying to minimize the use of steroids to avoid or minimize their adverse effects.

In pharmacologic or supraphysiologic doses, glucocorticoids are used to suppress various allergic, inflammatory, and autoimmune disorders. They are also administered as posttransplant immunosuppressants to prevent acute transplant rejection and graft-versus-host disease. Nevertheless, they do not prevent infection and also inhibit later reparative processes.

Glucocorticoids suppress cell-mediated immunity; they act by inhibiting the genes that encode the cytokines interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, and TNF-α. The inhibition of IL-2 is particularly important because it reduces T cell proliferation.

Glucocorticoids also suppress humoral immunity, causing B cells to express reduced amounts of IL-2 and IL-2 receptors. This diminishes both B cell clonal expansion and antibody synthesis.

Glucocorticoids also stimulate the release of lipocortin-1 into the extracellular space, where it binds to leukocyte membrane receptors and inhibits the following inflammatory events:

epithelial adhesion; emigration; chemotaxis; phagocytosis; respiratory burst; and the release of various inflammatory mediators including, but not limited to, lysosomal enzymes, cytokines, tissue plasminogen activator, and chemokines from neutrophils, macrophages, and mast cells.

6.2 Cytostatics

Cytostatics inhibit cell division. In immunotherapy, they are used in smaller doses than when used to treatment malignant diseases. They affect both T and B cell proliferation. As they are the most effective, purine analogs are most frequently administered.

Alkylating agents

The alkylating agents used in immunotherapy include cyclophosphamide (nitrogen mustards), nitrosoureas, platinum compounds, and others. Cyclophosphamide is probably the most potent immunosuppressive compound. In small doses, it is very efficient for the treatment of systemic lupus erythematosus, autoimmune hemolytic anemias, Wegener's granulomatosis, and other immune diseases. However, high doses cause pancytopenia and hemorrhagic cystitis.

Antimetabolites

Methotrexate is a folic acid analogue. It binds dihydrofolate reductase and prevents the synthesis of tetrahydrofolate. In addition to its use in transplant patients, it is used for the treatment of autoimmune diseases such as rheumatoid arthritis and Behcet's disease.

It is extensively used to control transplant rejection reactions. Azathioprine is nonenzymatically cleaved to mercaptopurine, which acts as a purine analogue and an inhibitor of DNA synthesis. Additionally, mercaptopurine itself can also be administered directly.

By preventing the clonal expansion of lymphocytes in the induction phase of the immune response, it affects both the cell and the humoral immunity. It is also efficient in the treatment of autoimmune diseases.

6.3 Antibodies

Antibodies are sometimes used as a quick and potent immunosuppressive therapy to prevent acute rejection episodes; additionally, antibodies such as monoclonal anti-CD20 antibodies are used in targeted treatment of lymphoproliferative or autoimmune disorders.

Antibodies interact with lymphocyte surface antigens, resulting in the depletion of circulating thymus-derived lymphocytes and interference with cell-mediated and humoral immune responses. Lymphocyte depletion occurs either by complement-dependent lysis in the intravascular spaces or by opsonization and subsequent macrophage phagocytosis. Adverse effects such as fever, chills, thrombocytopenia, leukopenia, and headache typically occur with the first few doses.

Heterologous polyclonal antibodies are obtained from the serum of animals (e.g., rabbit, horse) that were injected with the patient's thymocytes or lymphocytes. Currently, anti-lymphocyte (ALG) and anti-thymocyte antigens (ATG) are used. They are part of the

treatment strategy for cases of steroid-resistant acute rejection reaction and grave aplastic anemia. However, they are added primarily to other immunosuppressives to diminish their dosage and toxicity. They also enable transition to cyclosporine therapy.

Polyclonal antibodies inhibit T cells and cause their depletion through either complement-mediated cytolysis or cell-mediated opsonization that is followed by macrophage phagocytosis and degradation. In this way, polyclonal antibodies inhibit cell-mediated immune reactions, including graft rejection, delayed hypersensitivity, and graft-versus-host disease (GVHD); additionally they also influence thymus-dependent antibody production.

In 2005, two preparations became available: Atgam, obtained from horse serum, and Thymoglobulin, obtained from rabbit serum. Polyclonal antibodies affect all lymphocytes and cause general immunosuppression, possibly leading to post-transplant lymphoproliferative disorders (PTLD) or serious infections, such as those caused by cytomegalovirus. To reduce these risks, treatment is provided in a hospital, where adequate isolation from infection is available. Atgam and Thymoglobulin are usually administered for five days intravenously in the appropriate quantity. Patients stay in the hospital as long as three weeks to give the immune system time to recover to a point where there is no longer a risk of serum sickness.

Monoclonal antibodies are directed towards precisely defined antigens. Therefore, they cause fewer side effects. Especially significant are the antibodies directed against IL-2 receptor- (CD25-) and CD3. They are used to prevent organ transplant rejection, but also to track changes in the lymphocyte subpopulations. It is reasonable to expect the development of similar new drugs in the future.

T-cell receptor directed antibodies

Muromonab-CD3 is a murine anti-CD3 IgG2a monoclonal antibody that prevents T-cell activation and proliferation by binding the T-cell receptor complex that is present on all mature T cells. As such, it is one of the most potent immunosuppressive substances; it is commonly administered to control episodes of steroid- and/or polyclonal antibody-resistant acute rejection. Because it acts more specifically than polyclonal antibodies, it is also used prophylactically in transplantations.

However,the mechanism of action of muromonab-CD3 is only partially understood. It is known that the molecule binds the TCR/CD3 receptor complex. In the first few administrations, this binding non-specifically activates T-cells, leading to a serious syndrome 30 to 60 minutes later that is characterized by fever, myalgia, headache, and arthralgia. Sometimes the syndrome develops into a life-threatening reaction of the cardiovascular system and the central nervous system that requires lengthy therapy for recovery. After this period, CD3 blocks TCR-antigen binding and causes either conformational changes or the removal of the entire TCR3/CD3 complex from the T-cell surface. This lowers the number of available T-cells, perhaps by sensitizing them for uptake by tissue-resident macrophages. Also, cross-linking of CD3 molecules activates an intracellular signal that causes T cell anergy or apoptosis unless the cells receive another signal through a co-stimulatory molecule. Additionally, anti-CD3 antibodies shift the balance from Th1 to Th2 cells.

However, patients can develop neutralizing antibodies that reduce the effectiveness of muromonab-CD3. Muromonab-CD3 can also cause excessive immunosuppression. Although

anti-CD3 antibodies act more specifically than polyclonal antibodies, they lower the cell-mediated immunity significantly, predisposing patients to opportunistic infections and malignancies.

IL-2 receptor directed antibodies

Interleukin-2 is an important immune system regulator that is necessary for the clonal expansion and survival of activated T lymphocytes. Its effects are mediated by the trimeric cell surface IL-2 receptor, which is comprised of α, β, and γ chains. IL-2 receptor α (CD25, T-cell activation antigen, TAC) is expressed only by activated T lymphocytes. Therefore, it is of special significance for selective immunosuppressive treatment, and research has been focused on the development of effective and safe anti-IL-2 antibodies. Through the use of recombinant gene technology, murine anti-TAC antibodies have been modified, leading to the introduction of two chimeric mouse/human anti-Tac antibodies in 1998: basiliximab (Simulect) and daclizumab (Zenapax). These drugs act by binding the IL-2 receptor α chain, preventing IL-2-induced clonal expansion of activated lymphocytes and shortening their survival. Basiliximab and daclizumab are currently being used for the prevention of acute organ rejection after bilateral kidney transplantation; they are similarly effective and are associated with minimal side effects.

Other monoclonal antibodies

Efalizumab is a humanized monoclonal antibody that targets the lymphocyte function-associated antigen-1 (LFA-1) receptor through the CD11a side chain. Efalizumab (Raptiva), a drug indicated for psoriasis, was withdrawn from the US market on June 8, 2009 because of potential risks for progressive multifocal leukoencephalopathy (PML). PML is a rapidly progressive infection of the central nervous system caused by the JC virus that leads to death or severe disability. Demyelination associated with PML results from the JC virus infection. JC virus belongs to the genus *Polyomavirus* of the *Papovaviridae* family. PML should be considered in any patient exhibiting new-onset neurologic manifestations who has taken efalizumab. For more information, please see the Food and Drug Administration MedWatch Safety Alert.

Monoclonal antibodies against B7-1 (CD80) and B7-2 (CD86) have been developed to block T-cell CD28 activation and proliferation. In a recent trial, one of these antibodies, belatacept, was not inferior to cyclosporine as a means of preventing acute rejection after renal transplantation. Cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4Ig) can simultaneously inhibit B7-1 and B7-2 interactions with CD28 and has been used successfully in animal models, demonstrating a beneficial effect on chronic allograft rejection. Monoclonal anti-CD45-RB, leflunomide, FK778, FTY720, alemtuzumab (anti-CD52 antibody), and rituximab are some of the other agents that are currently in different phases of evaluation. Other antibodies targeting CD28 are also in development.

6.4 Immunophilin-binding agents

The available immunophilin-binding agents are cyclosporine and tacrolimus. These agents are calcineurin inhibitors that primarily suppress T lymphocyte activation by inhibiting IL-2 production. They are associated with numerous toxicities that are often dose-dependent. Nephrotoxicity occurs with both the drugs. Hirsutism, gingival hypertrophy, hypertension, and hyperlipidemia develop more often with cyclosporine than tacrolimus.

Calcineurin inhibitors and azathioprine have been associated with post-transplant malignancies and skin cancers in organ transplant recipients. The results of several studies suggest that calcineurin inhibitors have oncogenic properties that are predominantly linked to the production of cytokines that promote tumor growth, metastasis, and angiogenesis. This drug has been reported to reduce the frequency of regulatory T cells; additionally, after converting from CNI monotherapy to a mycophenolate monotherapy, patients were found to have increased graft success and T-Reg frequencies.

Cyclosporine

Since its introduction in 1983, Cyclosporine has become one of the most widely used immunosuppressive drugs. It is a cyclic fungal peptide that is comprised of 11 amino acids. Cyclosporine is thought to bind to the cytosolic protein cyclophilin (an immunophilin) of immunocompetent T lymphocytes. The complex of Cyclosporine and cyclophilin inhibits the phosphatase calcineurin. The drug also inhibits lymphokine production and cytokine release, leading to the reduced functionality of effector T-cells. The adverse events of cyclosporine would be hair growth as well as trembling and shaking of hands. For cyclosporine, the target conventional trough levels (C0) levels were adapted as 300–400 ng/mL during the first postoperative month, 100–200 ng/mL for up to 1 year, and approximately 100 ng/mL or less thereafter. Cyclosporine is used to treat acute rejection reactions, but has been increasingly been replaced by newer, less nephrotoxic immunosuppressants.

Tacrolimus

Tacrolimus (trade name Prograf) is a product of the bacterium *Streptomyces tsukubaensis*. It is a macrolide lactone that acts by inhibiting calcineurin. Although tacrolimus is used particularly for liver and kidney transplants, in some clinics it is used for heart, lung and heart/lung transplants. It binds to the immunophilin FKBP1A; the tacrolimus-FKBP1A complex then binds to calcineurin and inhibits its phosphatase activity. In this way, Tacrolimus prevents the cell from transitioning from the G0 into G1 phase of the cell cycle. While tacrolimus binds to a different intracellular protein (FKBP-12) compared to cyclosporine, it has the same mechanism of action of cyclosporine, is more potent than Cyclosporine and is associated with less pronounced side effects compared to cyclosporine. Tacrolimus treatment was started orally to maintain a C0 whole blood level of 10–15 ng/mL initially with reduction of the dose to obtain C0 levels between 3-10 ng/mL at 1 year after liver transplant. However, neurotoxicity, alopecia, and posttransplant diabetes mellitus develop more frequently with tacrolimus compared to cyclosporine.

Advagraf

Advagraf is a new oral formulation of tacrolimus with prolonged-release characteristics compared to the currently authorised product Prograf(t). Advagraf therapy requires careful monitoring by adequately qualified and equipped personnel. Because the later product is nationally authorised, the invented name may vary depending on the country of authorisation. Advagraf is the first calcineurin inhibitor formulated to enable once daily oral dosing and it is expected that it may help to improve compliance with dosing and cause less interference with the daily life activities of the patient. This medicinal product should only be prescribed, and changes in immunosuppressive therapy initiated, by physicians experienced in immunosuppressive therapy and the management of transplant patients.

Voclosporin

Voclosporin is a calcineurin inhibitor that is under development by Isotechnika as of 2010. This company uses calcineurin as a surrogate marker to assess the amount of immunosuppression achieved using drugs in this category. Other companies also have next generation drugs in this class in their pipelines.

6.5 Antiproliferative agents

Antiproliferative agents inhibit DNA replication and suppress B cells and T cells proliferation. Azathioprine and MMF are commonly used antiproliferative agents. MMF is an organic synthetic derivative of the natural fermentation product mycophenolic acid (MPA) that causes the noncompetitive reversible inhibition of inosine monophosphate dehydrogenase, which interferes with purine synthesis. Adverse effects of MMF include nausea, diarrhea, leukopenia, and thrombocytopenia. Invasive CMV infection has also been rarely associated with MMF. The introduction of MMF has been shown to be associated with improvement or stabilization of renal function, even several years after transplantation.

Other antiproliferative agents, such as cyclophosphamide and, more recently, leflunomide, have also been used.

6.6 Mammalian target of rapamycin (mTOR) inhibitors

Sirolimus is a macrocyclic antibiotic produced by *Streptomyces hygroscopicus* fermentation. It is used to prevent rejection reactions. Although it is a structural analogue of tacrolimus, it acts somewhat differently and has different side effects. Sirolimus binds to FKBP-12 and modulates the activity of mTOR, which inhibits IL-2-mediated signal transduction and results in T- and B-cell cycle arrest in the G1-S phase. Sirolimus is associated with numerous adverse effects including leukopenia, thrombocytopenia, anemia, hypercholesterolemia, hypertriglyceridemia, proteinuria, ae well as leg oedema. Contrary to Cyclosporine and tacrolimus, drugs that affect the first phase of T lymphocyte activation, sirolimus affects the second phase of T lymphocyte activation, signal transduction and lymphocyte clonal proliferation. So sirolimus is not used early after transplant because of wound dehiscence. Also sirolimus carries a black box warning that cautions against possible development of early postttransplant hepatic artery thrombosis. Although sirolimus binds to FKBP-12 like tacrolimus, the complex inhibits mTOR, not calcineurin. Therefore, sirolimus acts synergistically with Cyclosporine, and when used in combination with other immunosuppressants, it has few side effects. Also, it indirectly inhibits several T lymphocyte-specific kinases and phosphatases, preventing their transition from G1 to the S phase of the cell cycle. In a similar manner, sirolimus prevents plasma cell differentiation, reducing the production of IgM, IgG, and IgA antibodies. It has also been associated with mucositis, delayed wound healing, lymphocele formation, pneumonitis, and prolonged delayed graft function. It is also active against tumors that are PI3K/AKT/mTOR-dependent.

6.7 Everolimus

Everolimus is the 40-O-(2-hydroxyethyl) derivative of sirolimus and works similarly to sirolimus as an mTOR (mammalian target of rapamycin) inhibitor. It is currently used as an

immunosuppressant to prevent rejection of organ transplants. Everolimus may have a role in transplantation as it has been shown to reduce chronic allograft vasculopathy in such transplants. Because hypercholesterolemia and hypertriglyceridemia have been reported, monitoring of blood lipid level is recommended.

6.8 Other drugs

Many other agents are used to interfere with secondary signaling, and may therefore aid in tolerance induction[18].

Interferons

IFN-β suppresses the production of Th1 cytokines and monocyte activation; it is used to slow down the progression of multiple sclerosis. IFN-γ can trigger lymphocyte apoptosis.

Opioids

Prolonged use of opioids can cause immunosuppression of both innate and adaptive immune responses. Decreased proliferation and function have been observed in macrophages and lymphocytes. It is hypothesized that these effects are mediated by opioid receptors expressed on the surface of these immune cell populations.

Small biological agents

Fingolimod is a new synthetic immunosuppressant that is currently in phase 3 of clinical trials. It increases the expression or changes the function of certain lymphocyte adhesion molecules, such as α4/β7 integrin, causing their accumulation in the lymphatic tissues and their subsequent removal from circulation. In this respect, it differs from all other known immunosuppressants.

The use of any immunosuppressive drug requires a balance between the risk of loss of transplanted organ and the toxicity of the agent. The goal is to balance an appropriate level of immunosuppression with the long-term risks, which include the development of infections, cancer, and metabolic complications.

7. Therapeutic management

7.1 Phases

Immunosuppressive treatment of the transplanted patient begins with the induction phase, which begins perioperatively and continues immediately after transplantation. Maintenance therapy then continues for the life of the allograft. Induction and maintenance strategies use different medicines at specific doses or at doses adjusted to achieve target therapeutic levels to give the transplanted patient the best hope for long-term graft survival[19].

Induction strategy

The induction strategies include antibody-based therapy and aggressive early immunosuppression to avoid early acute rejection.

Antibody-based therapy:
This therapy uses monoclonal or polyclonal antibodies and is administered in the early posttransplant period (up to 8 wk). Antibody-based therapy allows for avoidance of or

dose reduction of calcineurin inhibitors, possibly reducing the risk of nephrotoxicity. All agents are effective for preventing acute rejections, although the anti-CD25 antibodies may require concurrent administration with calcineurin inhibitors. The adverse effect profiles of polyclonal and monoclonal antibody therapies limit their use in some patients. Patients at a high risk of rejection may receive rabbit anti-thymocyte globulin (Thymoglobulin).

Aggressive early immunosuppression:
This therapy uses maintenance drugs at higher doses to achieve the strongest immunosuppressive effect directly following transplantation. Approximately 50% of patients do not receive antibody therapy at the time of transplantation. The highest doses of calcineurin inhibitors place patients at increased risk of nephrotoxicity and may not be the best strategy for patients at the highest risk for rejection.

Maintenance strategy

Maintenance of immunosuppression is the key for the prevention of acute and chronic rejections throughout the life of the graft.

After induction therapy, whether this involves high-dose steroids that are then tapered off or an anti-thymocyte globulin preparation, maintenance therapy involves maintaining the program of conventional immunosuppression in order to prevent graft rejection[21]. Conventional maintenance therapy has evolved over the years and now includes multiple immunosuppressive agents that are given in non-toxic doses. Historically, corticosteroids and azathioprine were used to maintain grafts after induction therapy. Cyclosporine was then added to the armamentarium for maintenance therapy. Triple-drug therapy using cyclosporine, azathioprine, and prednisone is the most common maintenance regimen for many transplant recipients. Triple-drug therapy permits lower doses of cyclosporine and azathioprine to be given, as well as enabling the use of low-dose steroids or every-other-day steroid therapy.

In stable liver transplant patients, cyclosporine can be discontinued and the recipient maintained on azathioprine or MMF and every-other-day steroids. Alternatively, patients have been maintained on cyclosporine monotherapy with complete withdrawal of MMF and steroids.

Following the clinical introduction of tacrolimus, this potent agent increased rapidly in popularity for maintenance therapy in liver transplant recipients. Tacrolimus-based therapies usually include very-low-dose prednisone, but may also utilize azathioprine or MMF. The potent activity of tacrolimus enables more rapid steroid withdrawal, and many patients can be maintained off steroids altogether with the use of tacrolimus.

7.2 Anti-rejection strategies

Acute rejection

A number of strategies are available for patients who experience an acute rejection episode. For typical patients, alteration in clinical graft function prompts a liver biopsy and pathological evaluation of the graft for rejection. The 3 agents used to treat acute rejection are (1) steroids, (2) anti-thymocyte globulin, and (3) muromonab-CD3.

Steroids are the first-line treatment for rejection. These agents are the mainstay of therapy for acute rejection episodes, preventing macrophage IL-1 release and blocking T cell synthesis of IL-2. Steroids also have anti-inflammatory properties. The typical dosage is 10 mg/kg/d for 3-5 days, which is then tapered down to a maintenance dose. Steroids reverse 60-75% of rejection episodes.

Anti-thymocyte globulin: This agent binds all circulating T and B lymphocytes, which are then lysed or phagocytosed by macrophages and neutrophils. The efficacy of anti-thymocyte globulin is similar to muromonab-CD3. Anti-thymocyte globulin treatment is generally reserved for steroid-resistant acute rejection because of its cost, toxicity, and the development of anti-drug antibodies.

Muromonab-CD3: This agent displaces the T3 molecule from antigen receptors, binds all mature T cells, and prevents alloantigen recognition. The reversal rate of first acute rejection episodes is 94% for patients treated with muromonab-CD3. Muromonab-CD3 is sometimes used as the first-line agent for severe vascular rejections. The development of human anti-murine antibodies allows for the reappearance of CD3 T cells, which may decrease muromonab-CD3 efficacy and necessitate higher doses, possibly increasing the risk of infection. A second course of muromonab-CD3 treatment may be given for recurrent rejection, although repeated treatments can be associated with complications from the development of anti-murine antibodies. The success rate in recurrent episodes is approximately 40-50%.

Chronic rejection

For patients with chronic rejection, newer agents may be on the horizon to slow or reverse the rejection process. Unless inadequate immunosuppression is the cause of rejection, changes in immunosuppressive therapy are generally not effective in reversing chronic rejection. In liver transplant recipients whose organs have significant regenerative abilities, the use of high-dose tacrolimus appears to have some effect in reversing chronic rejection; however patients must be treated early in the course of chronic rejection. The addition of sirolimus to MMF is currently being studied to determine efficacy. Long-term data on transplanted patients treated with sirolimus demonstrated that the chronic rejection rates are much lower compared to rates traditionally reported for cyclosporine-based regimens. Blood pressure management, treatment of hyperlipidemia, and diabetes management are the current mainstays of treatment for graft preservation[20].

7.3 Primary immunosuppressive agents

Calcineurin inhibitors combine with binding proteins to inhibit calcineurin activity. This works to inhibit IL-2, which is critical for T helper cell proliferation. Calcineurin normally exerts phosphatase activity on the nuclear factor of activated T cells. This factor then migrates to the nucleus to initiate IL-2 transcription. Although studies have shown that cyclosporine and tacrolimus were associated with similar rates of graft survival, several studies have shown lower rates of rejection episodes with tacrolimus.

Levels of both cyclosporine and tacrolimus must be carefully monitored. Trough levels appear to correlate well with drug exposure in patients receiving tacrolimus. Initially, levels can be kept in the range of 10-20 ng/mL; however, after 3 months, levels are kept lower

(5-10 ng/mL) to reduce the risk of nephrotoxicity. Controversy continues regarding the best method to monitor cyclosporine levels.

7.4 Adjuvant agents

These agents are usually combined with a calcineurin inhibitor and include steroids, azathioprine, MMF, and sirolimus. Currently, most protocols use a calcineurin inhibitor and steroids with or without MMF. The use of adjuvant agents allows clinicians to achieve adequate immunosuppression while decreasing the dose and toxicity of individual agents.

In kidney transplant recipients, mycophenolate mofetil has assumed an important role in immunosuppression after several clinical trials reported a marked decrease in the prevalence of acute cellular rejection compared to azathioprine; furthermore, a reduction in 1-year treatment failures was also reported for MMF. Ongoing long-term studies suggest MMF also reduces the prevalence of chronic rejection.

Sirolimus has shown great promise for its potential to combat acute cellular rejection and to provide rescue immunosuppression. Current work shows that sirolimus causes a significant decrease in acute rejection and improvement in patient and graft survival compared to azathioprine[21, 22].

8. Complications of immunosuppression

Clinical immunosuppression strategies involve striking a balance between freedom from rejection episodes and freedom from the toxicity and complications of immunosuppressive treatment regimens. As the number of rejection episodes decreases, the likelihood of opportunistic infections, late malignancy and drug toxicity increases.

The specific toxicities of the immunosuppressive drugs are described in the sections pertaining to each drug. In general, the goal of multidrug therapy is to decrease the toxicities that are seen with higher doses of individual drugs.

8.1 Infection and malignancy issues

Opportunistic infections remain an important risk for immunocompromised patient despite the use of prophylactic measures[24]. Exposure to viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus, and human papillomavirus place the recipient at risk for infection and, potentially, later malignancy.

The incidence of CMV has been reduced with the use of antiviral prophylaxis in the first 3 months posttransplant. However, preemptive monitoring and initiation of treatment in the case of significant viremia after discontinuation of prophylaxis remains to be proven as a strategy for reducing the risk of late-onset CMV disease. Approximately 27% of patients who die with a functioning graft die from infectious or malignant complications. This highlights issues regarding the appropriate amount of immunosuppression required to balance aspects of graft function with complications related to therapy.
An increasingly recognized problem associated with immunosuppression is BK virus nephropathy. This virus, a member of the human papovavirus family, lives in the human genitourinary tract and replicates in some immunosuppressed patients, causing allograft

dysfunction. While antiviral agents such as cidofovir and leflunomide are active against the BK virus, the mainstay of therapy is a reduction in immunosuppression. The risk of acute allograft rejection with dose reduction is currently under investigation.

The most serious long-term effects of immunosuppression are the late malignancies that can develop in transplanted patients[25]. In addition to the post-transplantation lymphoproliferative diseases that are specific to chronically immunosuppressed patients, patients may also develop Kaposi's sarcoma. In addition, patients are at higher risk for malignancies that are common in non-immunosuppressed patients. The most common cancer observed in immunosuppressed patients is skin cancer, which mimics its frequency in the general population. A slightly higher incidence of Hodgkin's disease, non-Hodgkin's lymphoma, and breast, colon, lung, uterine and ovarian cancers has been observed in transplant recipients. For this reason, patients should undergo yearly cancer surveillance, including chest radiography and a general physical examination to look for new skin lesions; Pap smears and pelvic examinations are also suggested for women.

8.2 PTLD

Posttransplant lymphoproliferative disease (PTLD) is a growing concern in transplanted patients. Most cases of PTLD are of B-cell origin and are linked to EBV infections. Patients present with constitutional symptoms such as night sweats, fever, and weight loss. An acute rise in creatinine levels, similar to what occurs during acute allograft rejection, may also be seen. Risk factors for PTLD include primary EBV infection; the use of cyclosporine, tacrolimus, and MMF; and exposure to anti-thymocyte globulin (ATG) or OKT3. Treatment options include reduction or discontinuation of immunosuppression with an increase in prednisone to reduce rejection risk.

The long-term survival of liver transplant recipients requires lifelong treatment with immunosuppressive drugs. Despite the use of multiple agents in smaller doses, significant toxicities that are either directly or indirectly related to the immunosuppressive therapy can occur[27]. The primary long-term effects of corticosteroids include growth inhibition, Cushing's syndrome, osteoporosis, avascular femoral head necrosis, cataracts, glaucoma, cardiovascular disease and gastritis-peptic ulcer disease. The long-term effects of azathioprine include hepatitis, pancreatitis and red-cell aplasia. For cyclosporine, the long-term effects include hypercholesterolemia, arteriosclerosis, hypertension and nephrotoxicity. The long-term effects of tacrolimus may include hypertension and nephrotoxicity; however, it is too early to determine what other side effects may develop over time for this drug.

In summary, significant progress has been made in developing effective immunosuppressive protocols. These protocols rely on combination therapy using multiple drugs at low dosage to prevent rejection, treat established rejection episodes and minimize both the short-term toxicities and the long-term complications associated with immunosuppressive therapy.

9. Induction of tolerance

Transplant tolerance is defined as a state of donor-specific unresponsiveness without a need for ongoing pharmacologic immunosuppression. Safe, reliable strategies for the induction of full tolerance have not yet been developed[28]. However, during the study of achieving

immune tolerance, methods to induce states of "partial tolerance" have been discovered; in these cases, lower-than-conventional amounts of ongoing pharmacologic immunosuppression are required to prevent rejection. Nonetheless, immune tolerance remains the holy grail of transplantation immunology and clinical transplantation[29].

9.1 Induction of tolerance in transplant patients

Clinical allograft transplantation research has been conducted to identify methods to induce full or partial tolerance in transplant patients. These strategies are not ready for general clinical use until further evidence-based studies are available.

Full tolerance

The holy grail of organ transplantation is full immunologic tolerance, a state of indefinite survival of a well-functioning allograft that does not require maintenance immunosuppression. In addition, the host must retain a normal immune response and not suffer from immunosuppression-related infections, neoplasia, or other drug-related adverse effects. Rare cases of operational tolerance after transplantation, with complete cessation of immunosuppressive therapy have been observed and reported; these cases generally are associated with patient noncompliance regarding therapy.

Most studies concerning the intentional induction of immunologic tolerance have involved patients with hematologic malignancies. Full tolerance was achieved with myeloablative therapy prior to organ transplantation in combination with induced donor chimerism by means of bone marrow transplantation and excellent human leukocyte antigen (HLA) matching. Mixed chimerism retains a graft-versus-host T-cell effect that allows for transplant acceptance despite the subsequent disappearance of the donor chimerism.

Myeloablative therapy includes total body irradiation and lymphoablative methods, such as total body irradiation and the use of azathioprine and corticosteroids. However, the complications of full tolerance and the unpredictable timing organ transplantation with regards to the time required for myeloablative therapy prior to transplantation precludes the routine application of these therapies.

Cosimi and Sachs studied mixed chimerism in a small number of patients. They used nonmyeloablative conditioning, such as peritransplantation low-dose total-body irradiation or thymic irradiation plus anti-thymocyte globulin therapy combined with splenectomy. Donor-specific marrow infusion was given at the time of transplantation. Cyclosporine was given for about a month after transplantation and then stopped. Patients had transient chimerism for several weeks, and graft survival was approximately 70% over the long term.

Tregs are responsible for maintaining tolerance by broadening suppression through a mechanism termed linked-suppression, where tolerance to a specific epitope is spread to all epitopes of that protein; this tolerance is also spread to cohorts of naïve T-cells as they develop. Immunologist Herman Waldmann described this phenomenon as a process of infectious tolerance. Tregs from tolerant animals can be transferred to naive animals, in which they subsequently confer antigen-specific tolerance, including tolerance to skin and organ allografts. Although not completely understood, this is referred to as adoptive tolerance, and it has been recognized since the 1990s when Dr. Metcalfe at Cambridge published a landmark paper describing this phenomenon.

Tolerance induction through the expansion and transfer of donor Tregs to an allograft recipient or by means of the ex vivo development of Treg from recipient T cells are intriguing but yet-untested strategies in humans. Currently, in heart transplantation, analysis of whether FOXP3 gene expression in peripheral blood cells reflects anti-donor immune responses is underway. Overall, these possibilities represent exciting ways to broaden the translational approach of tolerance induction through the use of T regulatory cells.

Partial tolerance

At present, partial tolerance that requires minimal immunosuppression is possible. This allows for the minimal use of immunosuppressive drugs, which results in reduced risks for infection, neoplasia, and drug-related adverse effects. Partial, or incomplete, donor-specific tolerance has been termed minimal immunosuppression tolerance or prope tolerance from the Latin word for near.

Professor Sir Roy Calne postulated that prope tolerance preserves some of the transplant recipient's immune responses to infection and other antigens, reducing morbidity and mortality caused by immunosuppressive effects.

Although numerous researchers are investigating assays to monitor the degree of immunosuppression, no assays or tests are currently available to monitor tolerance. Dr. Sarwal at Stanford University is currently using exciting microarray technologies to describe genetic identifiers of allograft recipients that are rendered tolerant. This technology may be what is required to overcome the current barriers to allograft tolerance.

Identifying either prope or complete tolerance depends on the elimination, withdrawal, or reduction of maintenance immunosuppression followed by the observation of a favorable response. Allograft biopsies may or may not be helpful in identifying rejection at an early stage if the strategy is unsuccessful. Indeed, a specific directive of granting agencies, such as the National Institutes of Health and the Immune Tolerance Network, is to fund research to develop tolerance assays.

The demonstration of immune tolerance induction in many rodent models stands in stark contrast to the lack of success in humans and primates, with the exception of myeloablative therapy followed by donor-derived stem cell infusion. The specific pathogen-free environment in which rodents are housed for their lifetime limits the number of memory T cells that they develop. In contrast, humans and primates are exposed to many viruses during their long and less pathogen-free lives. In addition, they generate a considerable pool of self-renewing memory T cells; in fact, nearly half of circulating T cells in adult humans are memory T cells. Therefore, they are less immunologically naïve compared to experimental rodents. Many of these memory T cells can cross-react with foreign MHC. Therefore, the translation of tolerance induction strategies from the rodent laboratory models to large animals and then to humans may need to account for differences in previous specific and net immunologic memory.

9.2 Mechanisms for tolerance

Tolerance is generally accepted to be an active process and, in essence, a learning experience for T cells[30]. Tolerance is said to occur mechanistically at 2 levels: centrally and peripherally.

Central and/or Intrathymic Tolerance

The chief mechanism of T-cell tolerance is the deletion of autoreactive T cells in the thymus, rendering the organism tolerant to "self." Immature T cells migrate from the bone marrow to the thymus, where they encounter peptides derived from endogenous proteins that are bound to major histocompatibility complex (MHC) molecules on thymic epithelial cells.

Double-positive ($CD4^+$ and $CD8^+$) thymocytes initially undergo random generation of different T-cell receptors (TCRs). Positive selection, also called thymic education, ensures that only clones with TCRs that exhibit moderate affinity for self-MHC are allowed to develop. Negative selection by means of apoptosis occurs when T cells do not produce functional TCRs, when TCR rearrangement fails, when T cells have low affinity for MHC-self-peptide complexes, or when T cells have extremely high affinity for such complexes. Negative selection also results in the deletion of some thymocytes that interact with autoantigens presented by interdigitating cells and macrophages at the corticomedullary junction. The remaining cells lose either CD4 or CD8 and leave the thymus to function in the periphery as mature, functional $CD4^+$ and $CD8^+$ T cells[31].

Peripheral tolerance

Many potentially reactive T cells escape intrathymic deletion, reflecting the fact that many antigens are absent intrathymically or are present at insufficient levels to induce tolerance in the thymus. Several peripheral, nonthymic mechanisms that prevent autoimmunity by rendering peripheral T cell repertoires tolerant also exist[32].

9.3 Sequestration of antigens into privileged sites

Some antigens are sequestered into privileged sites away from the immune system because of physical barriers, such as tight junctions, or immunologic barriers, such as the expression of Fas ligand (FasL) or reduced MHC class I expression. Thus, antigen-presenting cells (APCs), and subsequently T lymphocytes, may never encounter these self-antigens. Therefore, immune cells remain ignorant of these antigens. At some of these sites, proinflammatory lymphocytes are controlled by apoptosis due to the expression of FasL or the secretion of cytokines such as transforming growth factor-beta (TGF-β) or interleukin (IL)-10. When T cells enter these sites, their Fas receptors interact with the FasL of these sites, and they undergo apoptosis. Privileged sites include the brain, the testes, and the anterior chamber of the eye. Transplanted tissues are most likely to survive in these privileged sites because of the tight control of proinflammatory lymphocytes.

9.4 Apoptosis of T cells due to persistent activation or neglect

Apoptosis, or programmed cell death, of lymphocytes is an important mechanism of immune control and homeostasis. Apoptosis contributes to the deletion of clones that are persistently activated and of activated lymphocytes when the immune response is no longer needed (e.g., after an infection clears). Cells that are persistently stimulated undergo activation-induced cell death involving Fas-FasL signaling or tumor necrosis factor. Most T cells that remain after antigen clearance are deprived of the stimuli required to survive and undergo passive cell death. Apoptosis of donor-reactive lymphocytes is also known as the "deletional" method to induce tolerance; in theory, this represents the most fail-safe

mechanism of tolerance induction. In the absence of donor-reactive lymphocytes, the response to donor antigens could not be induced no matter what antigens are encountered.

9.5 Clonal anergy

T lymphocytes require 2 signals to become activated, proliferate, and differentiate. The first is the recognition of an appropriate MHC-antigen complex by the TCR of the responsive lymphocyte. The second signal is delivered by costimulatory molecules also expressed by APCs; Costimulatory ligands are only able to engage once the first signal is activated. Lack of costimulation causes anergy, when T cells fail to respond to the MHC-peptide complex and remain unresponsive to subsequent challenges.

CD28 is the main costimulatory ligand expressed by naive T cells encountering antigen. CD28 signaling enhances T-cell proliferation by boosting T cell IL-2 production. It also enhances expression of CD40 ligand, which interacts with CD40 on APCs to induce the upregulation of the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) to further enhance costimulatory signaling.

Recently, Rigby et al. used inhibition of T-cell costimulation as an effective means to prevent autoimmunity and allograft rejection in multiple animal models. They studied the effects of anti-CD28 and CTLA4-Ig on diabetes development and the requirements to induce tolerance in nod/scid mice after the transfer of transgenic beta-cell reactive BDC2.5.NOD T-cells. These authors were successful in this set of experiments and have helped to develop the understanding of natural regulatory mechanisms that may have a unique role in establishing targeted, long-standing immune protection and peripheral tolerance.

T lymphocytes also express CD152 (CTLA-4) after CD28 binds to its ligands B7-1 and B7-2 on APCs. The interaction of CTLA-4 and B7 molecules decreases opportunities for B7-CD28 binding and downregulates T-cell IL-2 production, which subsequently reduces T-cell proliferation. CD28 interacts with B7 molecules, first leading to T-cell activation. However, after this effect peaks, upregulation of CTLA-4 with its relatively high affinity for B7 molecules limits the degree of activation. Verbinnen et al. recently studied the involvement of regulatory T cells (Treg) and deletion of alloreactive cells in the induction and maintenance of tolerance after costimulation blockade (CTLA-4) in a mouse model of graft-vs.-host disease. The study showed that clonal deletion of host-reactive T cells was a major mechanism responsible for tolerance.

9.6 Regulatory T cells

Regulatory T cells (Tregs), also called suppressor T cells, suppress the activation of clone-specific T-cell activity. Tregs account for 10-15% of $CD4^+$ T cells and express the transmembrane protein CD25, which is the alpha chain of the IL-2 receptor. $CD4^+CD25^+$ Tregs are anergic to TCR-mediated activation but potently suppress the activation of other T cells. However, not all $CD25^+$ T cells are regulators. Some naive T cells upregulate CD25 in response to antigen, a change that represents the activation rather than the suppression of an immune response. The thymus produces anergic but suppressive $CD4^+$ 25^+ T cells, which are also identified by the expression of FoxP3, the transcription factor responsible for their development. These T cells suppress the activation and expansion of autoreactive $CD4^+CD25^-$ populations.

Studies in mice have shown that Tregs are antigen-specific and that they regulate peripheral tolerance by producing suppressive cytokines such as IL-10 and TGF-beta. They depend on continuous antigen exposure to stay capable of mediating suppression. Antigen removal reduces the quantity of cells.

In allograft rejection, direct stimulation of T cells in response to donor-derived antigens presented by donor APCs had been the focus of transplantation research for many years. However, indirect antigen presentation, in which self-APCs present donor peptides in an MHC-restricted fashion is responsible for the induction of antigen-specific Tregs that can directly and indirectly suppress other alloreactive T cells. Although positive costimulation with CD28 appears to be necessary for the development of intrathymically derived Tregs, costimulation blockade with CTLA-4 is required for the development of peripherally acquired suppressor Tregs.

10. Immunosuppression withdrawal

Although tolerance induction may allow for the withdrawal of immunosuppression in the future, at this time, immunosuppressive medications appear to be necessary for the life of the transplanted organ[33].

10.1 Steroid versus steroid-free protocols

The known toxicity of long-term steroid exposure has prompted the development of steroid-free immunosuppressive regimens. Benefits of the withdrawal or avoidance of steroids include normal growth in children, improved lipid profiles, improved blood pressure, better glycemic control, and a lower risk of bone disease.

The development of cyclosporine prompted attempts to develop steroid-free protocols. Initially, patients were doing well with cyclosporine monotherapy. Over time, 50% of these patients required steroids, usually for episodes of acute rejection. Strong randomized studies are undoubtedly needed to prove both the efficacy and the safety of these protocols.

Steroid withdrawal has been used as a strategy to avoid adverse steroid effects in transplanted patients. Recent data show that the risk of rejection is higher in patients withdrawn from steroids on a cyclosporine-based protocol. After tacrolimus became available, protocols with this drug showed that withdrawal of steroids after 6 months was successful 80% of the time. More recently, studies involving rapid steroid withdrawal (over 1-2 wk) in patients taking tacrolimus show similar graft survival rates compared with patients withdrawn after 3-6 months. Although the roles of sirolimus and MMF in steroid-free protocols have yet to be definitively determined, the future looks promising for greater use of steroid-free protocols.

10.2 Calcineurin inhibitor-free protocols

Because of the risk of both acute and chronic nephrotoxicity attributed to calcineurin inhibitors, the development of protocols free of these agents is desirable. The use of sirolimus, MMF, and anti-CD25 antibodies has been studied to determine whether graft survival and acute rejection rates can be maintained at the present rates in the absence of calcineurin inhibitors.

The withdrawal of cyclosporine has been investigated in several trials. While the long-term graft survival rates were similar in patients withdrawing from cyclosporine compared to those maintained on it, the incidence of acute rejection in the withdrawal group was higher. The addition of sirolimus has been used in these withdrawal protocols. Higher rates of acute rejection were again noted in the withdrawal group.

Many other protocols that minimize exposure to calcineurin inhibitors have been studied. Promising protocols include sirolimus, MMF, and steroids or the combination of anti-CD25 antibodies, sirolimus, MMF, and steroids. These protocols have shown acceptable graft survival rates and acute rejection rates; however, these studies were small in size and further research is warranted[34]. In short, multiple regimens have been shown to be effective.

10.3 Pregnancy

Current data suggest that protocols involving cyclosporine, azathioprine, and steroids are associated with low rates of birth defects. However, patients are treated with high-risk pregnancy strategies. However, children born to parents with previous transplants are often small for their gestational age. Preliminary data suggest the safety of tacrolimus. MMF animal data and some early human studies show adverse effects on fetal development. Presently, few data exist regarding sirolimus and pregnancy.

10.4 Length of treatment

Episodes of acute cellular rejection have occurred after the cessation of medication even 20 years after transplantation. For patients with stable graft function, individual components of the treatment regimen may be gradually diminished or completely discontinued; however, in most patients, some degree of immunosuppression must be continued. Some patients with severe resistant infections or malignancy related to immunosuppressants require the discontinuation of these medicines.

11. Future perspective

Immunity, regulation, graft rejection versus acceptance, and tolerance have proven to be extraordinarily complex. Indeed, currently used drugs and treatment protocols that are largely directed at inhibiting alloreactive T cells, have not optimally improved allograft survival or function. The tremendous progress made in understanding the molecular and cellular basis of allograft rejection has not yet been translated into durable modalities that have advanced clinical care and outcomes. Despite the many advances in both immunological knowledge and the practical application of clinical immunosuppression, the holy grail of indefinite graft survival with immune tolerance in clinical solid organ transplantation remains a distant dream. The current challenge is to integrate molecular, cellular, and anatomic concepts to achieve the equivalent of a unified field theory of the immune response to organ transplantation. A shift in emphasis, focusing on underappreciated immune pathways must now be considered to make further improvement. We highlight 3 areas of recent interest, complement, NK cells and lymphatics[35, 36], which reinforce the concept that the transplant community must direct attention on how the immune system as a whole responds to a transplant. Discoveries of

new molecules, cell populations, functions or pathways have each led to the hope that the field has finally reached the point that reliable immune manipulation can now be achieved. Once that perspective is gained, we may finally be poised to make the major leaps forward in clinical care and outcomes.

12. References

[1] Roitt IM, Delves PJ. Roitt's Essential Immunology. 10th ed. Ames, IA: Blackwell Publishing; 2001.
[2] Rocha PN, Plumb TJ, Crowley SD, Coffman TM. Effector mechanisms in transplant rejection. Immunol Rev. 2003;196:51-64.
[3] Starzl TE, Zinkernagel RM. Transplantation tolerance from a historical perspective. Nat Rev Immunol. 2001;1(3):233-9.
[4] Brent L. The 50th anniversary of the discovery of immunologic tolerance. N Engl J Med. 2003;349(14):1381-3.
[5] Denton MD, Magee CC, Sayegh MH. Immunosuppressive strategies in transplantation. Lancet. 1999; 353(9158):1083-91.
[6] Rocha PN, Plumb TJ, Crowley SD, Coffman TM. Effector mechanisms in transplant rejection. Immunol Rev. 2003;196:51-64.
[7] Kuby J. Immunology. 3rd ed. New York, NY: WH Freeman & Co; 1997.
[8] Loupy A, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). Am J Transplant. 2011;11(1):56-65.
[9] Clarkson MR, Sayegh MH. T-cell costimulatory pathways in allograft rejection and tolerance. Transplantation. 2005;80(5):555-63.
[10] Schwartz JC, Zhang X, Fedorov AA, et al. Structural basis for co-stimulation by the human CTLA-4/B7-2 complex. Nature. 2001;410(6828):604-8.
[11] Sayegh MH, Turka LA. The role of T-cell costimulatory activation pathways in transplant rejection. N Engl J Med. 1998;338(25):1813-21.
[12] Krupnick AS, Kreisel D, Popma SH, et al. Mechanism of T cell-mediated endothelial apoptosis. *Transplantation*. 2002;74(6):871-6.
[13] Jukes JP, Wood KJ, Jones ND. Natural killer T cells: a bridge to tolerance or a pathway to rejection?. Transplantation. 2007; 84(6):679-81.
[14] McDiarmid SV. Management of the pediatric liver transplant patient. Liver Transpl. 2001; 7(11 Suppl 1):S77-86.
[15] Willicombe M, Brookes P, Santos-Nunez E, et al. Outcome of patients with preformed donor-specific antibodies following alemtuzumab induction and tacrolimus monotherapy. Am J Transplant. 2011;11(3):470-7.
[16] Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. J Allergy Clin Immunol. 2010 Feb;125(2 Suppl 2):S324-35. Review.
[17] Food and Drug Administration (FDA). FDA MedWatch - 2009 Safety Alerts for Human Medical Products - Raptiva. FDA; April 8, 2009.
[18] Gummert JF, Ikonen T, Morris RE. Newer immunosuppressive drugs: a review. J Am Soc Nephrol. 1999 Jun;10(6):1366-80.
[19] Hardinger KL, Koch MJ, Brennan DC. Current and future immunosuppressive strategies in renal transplantation. Pharmacotherapy. 2004;24(9):1159-76.

[20] Evrard V, Otte JB, Sokal E, et al. Impact of surgical and immunological parameters in pediatric liver transplantation: a multivariate analysis in 500 consecutive recipients of primary grafts. Ann Surg. 2004; 239(2):272-80.
[21] Harper SJ, Gelson W, Harper IG, Alexander GJ, Gibbs P. Switching to sirolimus-based immune suppression after liver transplantation is safe and effective: a single-center experience. Transplantation. 2011;91(1):128-32.
[22] Geissler EK, Schlitt HJ. Immunosuppression for liver transplantation. Gut. 2009; 58(3):452-63.
[23] Eksteen B, Neuberger JM. Mechanisms of disease: the evolving understanding of liver allograft rejection. Nat Clin Pract Gastroenterol Hepatol. 2008; 5(4):209-19.
[24] Aberg F, Mäkisalo H, Höckerstedt K, Isoniemi H. Infectious complications more than 1 year after liver transplantation: a 3-decade nationwide experience. Am J Transplant. 2011;11(2):287-95.
[25] Jain A, Singhal A, Fontes P, Mazariegos G, DeVera ME, Cacciarelli T, Lopez RC, Sindhi R, Humar A, Marsh JW. One thousand consecutive primary liver transplants under tacrolimus immunosuppression: a 17- to 20-year longitudinal follow-up. Transplantation. 2011;91(9):1025-30.
[26] Avolio AW, Agnes S, Barbarino R, Magalini SC, Frongillo F, Pagano L, Larocca LM, Pompili M, Caira M, Sollazzi L, Castagneto M. Posttransplant lymphoproliferative disorders after liver transplantation: analysis of early and late cases in a 255 patient series. Transplant Proc. 2007;39(6):1956-60.
[27] Sánchez-Fueyo A, Strom TB. Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. Gastroenterology. 2011;140(1):51-64.
[28] Bishop GA, Ierino FL, Sharland AF, Hall BM, Alexander SI, Sandrin MS, Coates PT, McCaughan GW. Approaching the promise of operational tolerance in clinical transplantation. Transplantation. 2011;91(10):1065-74.
[29] Levitsky J. Operational tolerance: past lessons and future prospects. Liver Transpl. 2011;17(3):222-32.
[30] Selzner N, Grant DR, Shalev I, Levy GA. he immunosuppressive pipeline: meeting unmet needs in liver transplantation. Liver Transpl. 2010;16(12):1359-72.
[31] Pepper M, Jenkins MK. Origins of CD4(+) effector and central memory T cells. Nat Immunol. 2011;12(6):467-71.
[32] Thomson AW, Mazariegos GV, Reyes J, Donnenberg VS, Donnenberg AD, Bentlejewski C, Zahorchak AF, O'Connell PJ, Fung JJ, Jankowska-Gan E, Burlingham WJ, Heeger PS, Zeevi A. Monitoring the patient off immunosuppression. Conceptual framework for a proposed tolerance assay study in liver transplant recipients. Transplantation. 2001;72(8 Suppl):S13-22.
[33] Mazariegos GV. Immunosuppression withdrawal after liver transplantation: what are the next steps? Transplantation. 2011;91(7):697-9.
[34] Masetti M, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, Begliomini B, Di Benedetto F, Gerunda GE. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in de novo liver transplant recipients preserves renal function. Am J Transplant. 2010;10(10):2252-62.

[35] Kitchens WH, Uehara S, Chase CM, Colvin RB, Russell PS, Madsen JC. The changing role of natural killer cells in solid organ rejection and tolerance. Transplantation. 2006;81(6):811-7.
[36] Dell-Olio D, Kelly DA. Immunosuppressants: what's new? Curr Opin Organ Transplant. 2010;15(5):594-600.

Part 4

Prognostic Factors in Liver Transplantation

6

Prognostic Factors for Survival in Patients with Liver Cirrhosis

Marcia Samada and Julio C. Hernández
Medical Surgical Research Center
Cuba

1. Introduction

Liver cirrhosis is the final stage of various chronic liver diseases. The concept is essentially morphological, defined as a diffuse alteration of hepatic architecture by the presence of necrosis, fibrosis and regenerative nodules. These disorders conduct to intrahepatic vascular changes and to the reduction of functional mass. Finally, the consequences are the development of portal hypertension and the occurrence of liver failure. (Ampurdanés S, 2002)

For many decades, alcohol was considered the leading cause of cirrhosis. But actually, viral hepatitis by viruses B (HBV) and C (HCV) are recognized as the most important sources. (Mandayam S) Another common cause is Nonalcoholic Fatty Liver Disease (NAFLD), followed by autoimmune diseases with or without cholestasis, among others.

1.1 Natural history of liver cirrhosis and its complications

The studies that provide more data on the natural history of cirrhosis are related to the evolution of chronic hepatitis by HBV and HCV. These are based on prospective, retrospective and cross studies, but are conditioned by factors that make difficult to establish absolute evidence on the natural history of the disease. (Serra MA., 2006)

Of those patients with HCV, 50% usually develop chronic liver disease including cirrhosis and liver cancer. It is estimated that 15% of chronically infected persons develop liver cirrhosis within 20 years. (Wiese M, 2005) However, there are individual differences. Currently it is known that 33% of patients develop cirrhosis in less than 20 years, while another 31% will need many more years in order to develop the same damage. (Serra MA 2006)

Usually it is a silent disease. Most patients are asymptomatic or have nonspecific symptoms until decompensation occurs. They can start with symptoms related to complications of liver failure or portal hypertension.

Ascites is the most common complication and of earlier onset. Once patients with cirrhosis develop ascites the prognosis worsens. It is estimated that approximately 50% of them could die within two years if they do not have a transplant. So, this is a major criterion for liver transplant evaluation in the United States and Europe. (Settle 2004, Sagnelli 2005)

Along with ascites, there may be other serious complication such as spontaneous bacterial peritonitis. In these cases, the probability of survival one year after this complication appears is only 40%. This is a strong reason for evaluating these patients as candidates for transplantation. (Corrao 1997) Similarly, other complications may appear such as hepatic encephalopathy and hepatic-renal syndrome. Both also worsen the prognosis. (Mandaya 2004)

Variceal hemorrhage occurs in 30 to 40% of patients with liver cirrhosis. In the past two decades, even with the improvement achieved in the treatment and in the prognosis after bleeding, mortality at six weeks is still high. It is estimated between 15 and 30% in patients with stage C of Child-Pugh. (Hands 2008)

After a first episode of hepatic encephalopathy the survival of cirrhotic patients is 42% in the first year, and 23% in the following three years. (Mendez-Sanchez 2005)

Hepatocellular carcinoma is another major complication and can occur at any stage of cirrhosis. It is recognized as the leading cause of death in the compensated phase, especially in patients with HCV. (Capocaccia R, 2007; Perz, J.F, 2006)

1.2 Indication of liver transplantation and survival models

Liver transplantation is the treatment of choice in acute and chronic irreversible liver failure of different etiologies, in primary liver tumors and when an impaired quality of life appears by manifestations of liver disease, as intractable pruritus and hepatic osteodystrophy.

The most commonly used survival models to assess the degree of liver failure and to prioritize patients on the waiting list for liver transplantation are: the Child-Pugh score (Oellerich M, 1991) and Model for End Stage Liver Disease (MELD) score (Malinchoc M, 2000). Although currently used primarily MELD, both have been included among the criteria for liver allocation in the United States and Europe (Freeman RB Jr, 2004; Adler M, 2005).

The Child-Pugh score has been widely used both in research and in clinical practice. For these reason, candidates for liver transplantation were prioritized mainly by these score, which included subjective measures of encephalopathy and ascites, and time waiting on the list. However, the need for a more accurate system in which the urgency of assignment was a relevant criterion, determined the introduction of the MELD score that is also very valuable as a predictor of mortality and allocation of organs for patients on the waiting list. The MELD score had been previously validated as a predictor of 3-month mortality for patients with chronic liver disease. (D'Amico G, 2006; Botta F, 2003)

The MELD system appears superior for comparing populations and has had a positive impact on allocation and survival in liver transplantation; however, it is still far from perfect. One of the disadvantage of the MELD formula is the loss of prognostic accuracy in periods longer than 3 months. The Child-Pugh classification provides superior results for periods exceeding one year. For these reasons, some authors recommend implementing both systems. (Forman LM, 2001; D'Amico G, 2006; Prieto M, 2007; Durand F, 2005.)

The Child-Pugh score evaluates five parameters: ascites, encephalopathy, bilirubin, prothrombin time and albumin. Although never formally validated, has been the most

widely used for decades. It is easy to apply and has proved useful in estimating the prognostic index of survival. But some limitations are pointed out such as, not all variables have an independent effect, it includes subjective variables like ascites and encephalopathy, the cutoff points for quantitative variables are not optimal, and it does not take into account certain important prognostic factors such as renal function. (Oellerich M, 1991; Prieto M, 2007)

The MELD model uses a mathematical formula with simple and objective variables such as, serum concentrations of bilirubin, creatinine and international normalized ratio (INR) of prothrombin time. From these variables, you get a score that is predictive of survival. Initially it also included the etiology of the disease, but this variable was excluded because a minimal influence was observed. (Vargas V 2003) Nevertheless, its application is less practical because of the need of computer systems. One of its major limitations is its variability due to changes in creatinine and bilirubin. These parameters can be altered by treatment, sepsis or hemolysis. The value of creatinine is often affected by diuretics and other factors such as age, sex and body mass, which may introduce a bias independent of the severity of liver disease. Moreover, the severity of some medical complications, are not well reflected in the MELD score. (Prieto M, 2007; Vargas V, 2003; de la Mata, 2004)

Research is still going in an effort to improve this mathematical model. Many studies have proposed the addition of variables that may be of prognostic significance. Recently it has been suggested that the addition of sodium to the MELD formula could improve its accuracy. Some studies showed that serum sodium lower than 126 mEq/L is an independent predictor of mortality among patients listed for transplantation, and the addition of sodium to the MELD score increases its prognostic value. In patients with portal hypertension and cirrhosis, hyponatremia may be the earliest harbinger of refractory ascites and hepatorenal syndrome, and possibly a more sensitive marker than rising creatinine. (Taddei TH, 2007) However, this new formula is also subject to interassay variations, as well as the potential manipulation can be generated inadvertently by the use of diuretics. It is also unknown if its use can lead to increased mortality by neurological causes. For all this, it seems premature to use as long as no data are available for validation in larger groups and different cohorts. (Cárdenas A, 2008; Jiang M, 2008) Among the new proposals are to include the introduction of the measurement of the Hepatic Venous Pressure Gradient (Taddei TH, 2007), to include ascites, encephalopathy (Ripoll C, 2005; Stewart CA, 2007), sex (S Huo; 2007) or exclude the INR (Heuman DM, 2007). But is too premature to make conclusions.

Recently, a new estimator has been projected: the Cuban model Bioclim. This is a score calculated by a mathematical model that evaluates the creatinine and bilirubin biochemical parameters. It also takes into account: clinical encephalopathy, ascites and variceal upper gastrointestinal bleeding, considering the positive or negative response to treatment. Compared with Child-Pugh and MELD, the authors of this model (Vilar E, 2009) concluded that Bioclim score seems to have a greater discriminatory power in the short-term survival (4 to 12 weeks), intermediate term (24 to 52 weeks) and long-term (104 weeks).

1.3 Studies of survival and prognostic factors

In order to improve prognostic models many factors have been studied in relation to the survival of patients with liver cirrhosis.

In a review of 118 studies conducted by D'Amico (D'Amico G, 2006) the Child-Pugh was reported as the best predictor of mortality in cirrhosis, followed by the five components measured individually.

Said (Said A, 2004) noted that in one year follow up of cirrhotic patients, male gender, MELD score, Child-Pugh and encephalopathy, were associated with increased mortality. Independent predictors were the Child-Pugh and encephalopathy.

Botta (Botta F, 2003) compared the survival of cirrhotic patients at 6 and 12 months using a multivariate analysis including variables of Child-Pugh, MELD and a quantitative test of liver function test monoetilglicinexylidide (MEGX). At six months, MEGX, creatinine and prothrombin time were disposed as predictive factors of lower survival. The ascites was added at 12 months.

Attia (Attia KA, 2008) reported as independent predictors of mortality in 172 African patients with cirrhosis, the Child-Pugh score, MELD index, and creatinine.

London (London MC, 2007), in a study of 308 cirrhotic patients on the waiting list for liver transplantation, described the serum sodium and MELD score as independent predictors of survival at 3 and 12 months.

Samada (Samada M, 2008) conducted a study in 144 patients with liver cirrhosis and transplant candidates. The variables associated with lower survival at 12 months were: prothrombin time, bilirubin, albumin, cholesterol, serum sodium, sex, history of ascites and encephalopathy. Also MELD index and Child-Pugh stages were evaluated. But only the Child-Pugh score and spontaneous bacterial peritonitis were independent predictors of survival.

In conclusion we can state that detailed clinical evaluation of patients with liver cirrhosis and knowledge of prognostic factors associated with survival, could lead to proper management of these patients, the appropriate indication for liver transplantation and increased survival. This has been the principal motivation of the present study, in order to recognize the prognostic factors for survival in patients with cirrhosis within a three year period.

2. Methods

We performed a descriptive, prospective, and longitudinal study in 194 patients with liver cirrhosis. All were referred to the consultation of hepatology and liver transplantation at CIMEQ hospital between January 2004 and April 2011. The sample was composed of 144 patients who met the following inclusion criteria: diagnosis of liver cirrhosis (confirmed by laparoscopy, liver biopsy or ultrasound) and rolling up at least 36 months (three years). Patients who underwent liver transplantation during the study period, those who were lost to follow-up, died of causes unrelated to liver disease, and those who at the time of assessment presented hepatocellular carcinoma, cholangiocarcinoma or other malignancies were excluded. Were also excluded four patients with spontaneous bacterial peritonitis and hepatorenal syndrome. The frequency of evaluations was determined by clinical assessment of patients at least twice a year.

The confirmation of alcoholic and viral etiology was performed. The surface antigen for hepatitis B virus (HBsAg) by UMELISA HBsAg and antibody for HCV by HCV–UMELISA,

both produced by the National Center for Immunoassay in Havana, Cuba were investigated. HCV infection was confirmed by qualitative PCR (UMELOSA) produced by the National Center for Immunoassay in Havana, Cuba. The criterion for toxic alcohol intake was: 60 g daily intake for men and 40 g for women over 10 years. (Cavalry J, 2002) Patients with HBV or HCV and alcohol were included in the viral etiology, because liver damage is increased more by the virus than by alcohol. (Safdar K, 2004)

2.1 Variables studied

The variables studied were:

- Sex and age.
- Laboratory variables: INR, total bilirubin (normal up to 17μmol/L), albumin (normal value 35-48 g/L) and creatinine (normal value up to 123 μmol/L).
- Presence of esophageal varices: established in present or absence depending on the report of the upper digestive tract endoscopy, once these were classified according Paguet. (González M, 2007)
- Variables related to complications of cirrhosis: diagnostic criteria considered for the purposes of the study were selected taking into account the complications of the cirrhotic disease established AEEH guides. (Berenguer J et al, 2001).
- Ascites: fluid in the abdominal cavity detected by physical examination and/or abdominal ultrasound. We determined the presence of this complication if the patient had ascites at the time of assessment or had a history of it.
- Hepatic encephalopathy: presence of neuropsychiatric disorders that occur in patients with severe liver dysfunction. We used this complication if it was present at the time of the evaluation or the patient's history, family and clinical summary of the center of origin.
- Upper gastrointestinal bleeding (UGB): presence of hematemesis or melena associated with esophageal varices and/or portal gastropathy. We evaluated the history of this complication by questioning the patient, the clinical summary sent by the center of origin or having been submitted for evaluation.
- Hepatocellular carcinoma, we sought the presence of hepatocellular carcinoma during follow-up by surveillance strategy for this disease in cirrhotic patients. The strategy was based monitoring by abdominal ultrasound and determination of serum alpha-fetoprotein every 6 months (twice a year).
- Classification of Child-Pugh: used to evaluate the degree of liver failure in patients with cirrhosis. It has three stages according to score: A, B, C (see Table 1). (Pugh RNH, 1973)
- MELD: score used to assess the degree of liver failure and prioritize patients on transplant waiting list. The MELD score was calculated according to the original formula proposed by the Mayo Clinical group as follows: [9.57 × loge creatinine mg/dL + 3.78 × loge bilirubin mg/dL + 11.20 × loge INR + 6.43 (constant for liver disease etiology)]. (Kamath PS, 2001)
- Compensated liver cirrhosis: patients with or without varices but who have not showed any of the complications of cirrhosis.
- Decompensated liver cirrhosis: defined by the presence of ascites, variceal bleeding, encephalopathy and/or jaundice. (Gines P, 1987)
- Survival time: defined from the first assessment in consultation until the date the study was closed.

	Child-Pugh classification		
Parameter	A	B	C
Ascites	none	slight-moderate	tense
Hepatic encephalopathy (grade)	none	I-II	III-IV
Serum bilirubin (μmol/L)	<51	51-102	>102
Serum albumin (g/L)	>34	25-34	<25
Prothrombin time (%)	>60	46-60	<46
Score	5 to 6	7 to 9	10 to 15

The total score classifies patients into grade A, B, or C (ordinal scale) according to the points on continuous 5-15-point scale, which depends on ascites, encephalopathy, jaundice, serum albumin, and prothrombin time prolongation(Pugh RNH, 1973)

Table 1. Child-Pugh classification for the survival prognosis in liver cirrhosis

2.2 Statistical analysis

The data were processed using SPSS 13.0 for Windows. The results are presented as means ± standard deviations and confidence intervals of 95% for quantitative variables and as percentages for categorical variables. For comparison of continuous variables the t test comparison of independent means and chi-square test to compare categorical variables was used. Survival analysis was performed using the Kaplan-Meier curves; we used the cutoff points at 36 months (three years). We performed a Cox regression analysis to estimate the independent effects of potential predictors of survival that had been significant in univariate analysis. For all tests a significant level of 0.05 was set.

3. Results

Of the 144 patients, 94 (65.2%) were male and 50 (34.7%) females. Everyone had a minimal follow up of 36 months with a mean of 43.27 ± 12.97, minimum of three months for those who died and up to 73 months for the rest.

The average age was 47.8 years with a standard deviation of 12.9. Younger age was 18 years old and the oldest 87. The group with the highest number was between 40 and 60 years old with 84 patients (58.35%) as shown in Table 2.

All patients	144
Follow-up time (months)	43.27 ± 12.97 (3-73)
Sex (Male/Female)	94/50
Age (years)	47.8 ± 12.97 (18-87)
<40 years	33 (22.9%)
40–60 years	84 (58.3%)
>60 years	27 (18.8%)

Table 2. Baseline characteristics of all patients

The most common cause of cirrhosis in the study was HCV (42 patients, 29.1%), followed by alcohol (33 patients, 22.9%). The group "others" (46 patients, 31.9%) involved autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, congenital hepatic fibrosis and cryptogenic (Table 3).

Etiology of cirrhosis	Patients
Alcohol	33 (22.92%)
Hepatitis C virus	42 (29.17%)
Hepatitis B virus	23 (15.97%)
Others	46 (31.94%)
Total	144 (100%)

Table 3. Etiology of cirrhosis

Table 4 and 5 show the clinical and laboratory characteristics of all patients. At the beginning of the evaluation 40 (27.7%) had not developed complications of cirrhosis and 104 (72.2%) had one or more of them.

Compensated cirrhosis (yes/no)	40 (27.8%) / 104 (72.2%)
Presence of varices (yes/no)	96 (66.7%) / 48(33.3%)
Compensated cirrhosis(yes/no)	15 (37.5%) / 25 (62.5%)
Decompensated cirrhosis(yes/no)	81(77.8%)/23 (22.1%)
Child Pugh classification	
A	56 (38.8%)
B	46(31.94%)
C	42(29.16%)
Child - Pugh score	8.02 ± 2.6
MELD score	13.36 ± 5.74
Previous encephalopathy (yes/no)	21 (14.65)/123(85.4%)
Previous ascites (yes/no)	92 (63.9%) / 52 (36.15%)
Previous variceal bleeding (yes/no)	35 (24.3%) / 109 (75.7%)
Hepatocellular carcinoma (yes/no)	7 (4.9%) / 137 (95.1%)

Table 4. Clinical characteristics

According to Child-Pugh stages predominated A (56 patients, 38.8%), followed by B (46 patients, 31.9%) and C (42 patients, 29.1%). The average score was 8.02 ± 2.6. The mean MELD score was: 13.36 ± 5.74.

Laboratory test	
Bilirubin (µmol/L)	48.06 ± 59.4
Albumin (g/L)	34.10 ± 8.30
INR	1.45 ± 0.51
Creatinine (µmol/L)	83.53 ± 25.35

Table 5. Laboratory characteristics

96 patients showed esophageal varices (66.7%). Of these, 15 (37.5%) were in compensated stage and 81 (77.8%) in the decompensated one. Of these, 35 (24.3%) had at least one episode of gastrointestinal bleeding.

The most frequent complication was ascites (92 patients, 63.9%), followed by bleeding from esophageal varices (35 patients, 24.3%) and hepatic encephalopathy (21 patients, 14.6%).

Seven patients were diagnosed with hepatocellular carcinoma during follow-up and in two patients who were in Child A stage, it was considered as the cause of decompensation.

3.1 Survival of patients at three years

Of the 144 patients studied, 65 (45.1%) died from complications of liver cirrhosis between January 2004 and April 2011. Overall survival was 62.5% at three years follow-up, with a mean of 48.05 months and 95% between 43.2-52.8 months (see Figure 1).

As shown in Figure 2 and Table 6, survival of patients with compensated liver cirrhosis was 85% at three years with a mean of 60.27 months (CI 53.96-66.58). Patients who had complications had a survival rate of 53.8% with a mean of 42.4 months (CI 36.59-48.20) ($p < 0.001$). Of the patients with compensated liver cirrhosis at a baseline, the seven who died during the follow-up period were due to complications of the disease. Two of them presented HCC.

Cirrhosis	Median (months)	CI 95%	P
Compensated	60.27	53.96 – 66.58	p<0.001
Decompensated	42.40	36.59 – 48.20	

Table 6. Survival of patients with compensated and decompensated liver cirrhosis

As presented in Table 7, univariate analysis showed continuous variables that were associated with significantly poorer survival such as, age of the patients ($p = 0.017$), bilirubin ($p = 0.04$), albumin ($p < 0.001$), the INR ($p < 0.001$), Child-Pugh score ($p < 0.001$) and MELD score ($p < 0.001$). Creatinine did not behave the same way ($p = 0.779$).

Categorical variables that were associated with a significantly lower survival were, male sex ($p = 0.033$), history of ascites ($p = 0,001$), of hepatic encephalopathy ($p = 0.032$), and the development of hepatocellular carcinoma ($p = 0.003$). The bleeding, did not behave the same way. (Table 7).

Value	Surviving	Deceased	p
Patients	79	65	
Sex (male/female)	46/33	48/17	0.033
Age (years, SD)	45.4 ± 13.2	50.66 ± 12.09	0.017
Previous ascites (yes/no)	41/38	51/14	0.001
Previous encephalopathy (yes/no)	7/72	14/51	0.032
Hepatocellular carcinoma (yes/no)	0/79	7/58	<0.001
Previous variceal bleeding (yes/no)	18/61	17/48	0.639
Compensated cirrhosis (yes/no)	32/47	8/57	<0.001
Bilirubin (µmol/L)	35.18 ± 50.18	63.72 ± 66	0.04
Albumin (g/L)	37.56 ± 7.74	29.97 ± 7.03	<0.001
INR	1.31 ± 0.49	1.62 ± 0.49	<0.001
Creatinine (µmol/L)	84.03 ± 24.6	82.94 ± 26.32	0.779
Child- Pugh score	7.0 ± 2.1	9.27 ± 2.63	<0.001
MELD score	11.45 ± 4.99	15.67 ± 5.77	<0.001

Table 7. Clinical and biochemical characteristics of the 144 cirrhotic patients at 3 years follow-up

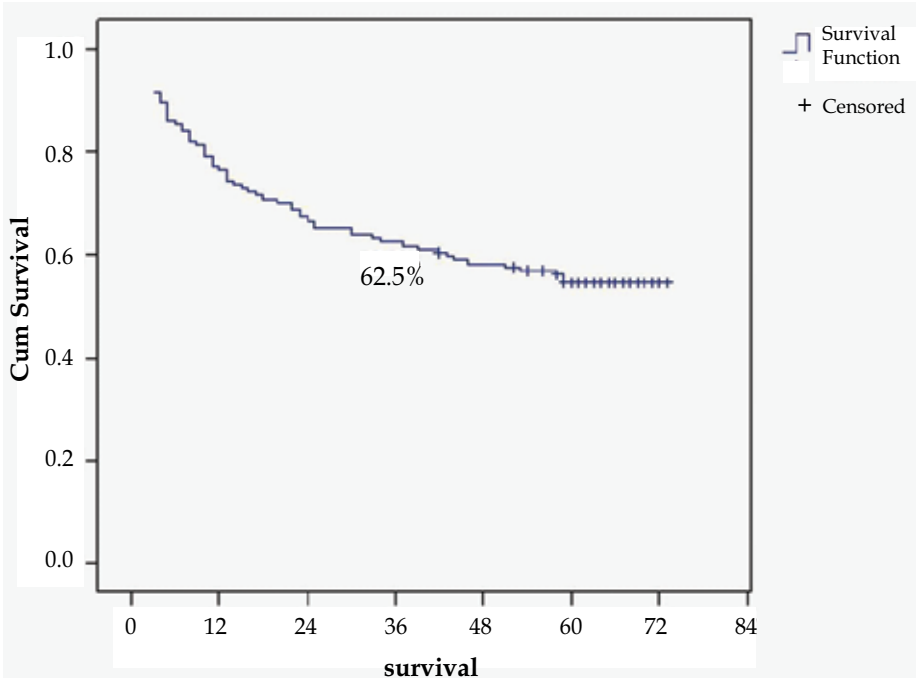


Fig. 1. Overall survival

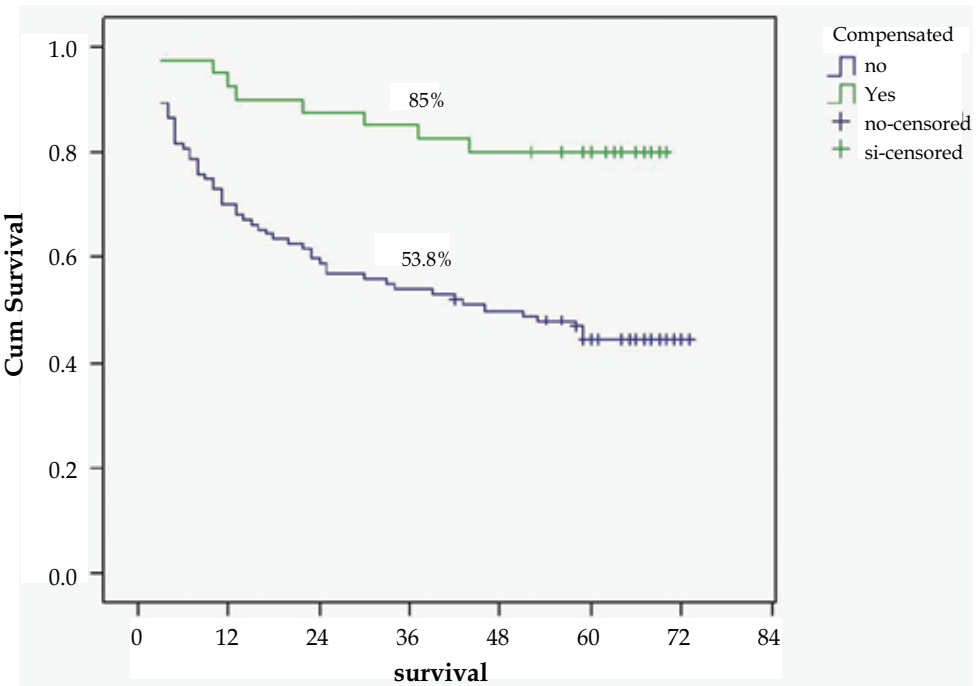


Fig. 2. Survival of patients with compensated and decompensated liver cirrhosis

In the analysis of the survival curves of Kaplan-Meier, categorical variables that had significantly lower survival were male gender (56.4%, average 43.69, 47.56-61.0), the history of ascites (51.1%, average 41.3 months; 35,06-47.53) hepatic encephalopathy (38.1%, mean 30.85 months, 17.67, 44.04), the development of hepatocellular carcinoma (0%, average 11.42, 4.09-18.76) and Child-Pugh stages with 80.8% survival for stage A, 69.9% for B and 31% for C (Table 8). Upper gastrointestinal bleeding for varicose veins was not associated with survival.

Variable	Survival at 36 months (%)	Median (months)	CI 95%	p
Sex: Female	74%	54.28	47.56- 61.0	0.033
Male	56,4	43.69	37.52- 49.87	
Ascites: No	82.7	57.722	51.75- 63.69	0.001
Yes	51.1	41.300	35.06- 47.53	
Encephalopathy: No	66.7	50.95	45.96- 55.92	0.032
Yes	38.1	30.85	17,67- 44.04	
Variceal bleeding: No	61.5	47.85	42.23- 53.46	0.639
Yes	65.7	47.68	38.62- 56.73	
HCC: No	65.7	49.92	45.07-54.77	<0.001
Yes	0	11.42	4.09-18.76	
Child- Pugh: A	80.8	58,04	52.28-63.81	<0.001
B	69.6	51.71	44.30-59.13	
C	31.0	26.42	17.88-34.97	

Table 8. Univariate analysis of categorical variables to three years of survival

	Exp(β)	p
Age	1.024	0.029
HCC	2.377	<0,001
Child-Pugh	1.378	<0,001

Table 9. Predictors of survival of liver cirrhosis at 3 years follow- up Cox regression

4. Discussion

According to different authors, cirrhosis caused by alcohol and HCV are more frequent in the fifth and sixth decades of life, and in males. (Safdar K, 2004; Sagnelli E, 2005; Benvegnù L, 2004) Recent series described that the most common causes of liver cirrhosis are due to HCV, HBV, and alcoholism. These causes can vary between them by geographic area. (Dehesa-Violante M, 2007; Fattovich G, 2008)

These conclusions coincide in this series, since the average age was 47.8 ± 12.95 years. The group more frequent were in patients between 40 to 60 years, male sex and the most frequent causes of cirrhosis were HCV and alcohol.

Cirrhosis is often manifested as a silent disease. In the compensated phase the diagnosis can be made by nonspecific manifestations or laboratory findings, whereas in later stages the disease may debut by its complications. (Heidelbaugh JJ, 2006) At the beginning of the study 27.8% of patients were in compensated phase and 72.2% had developed complications.

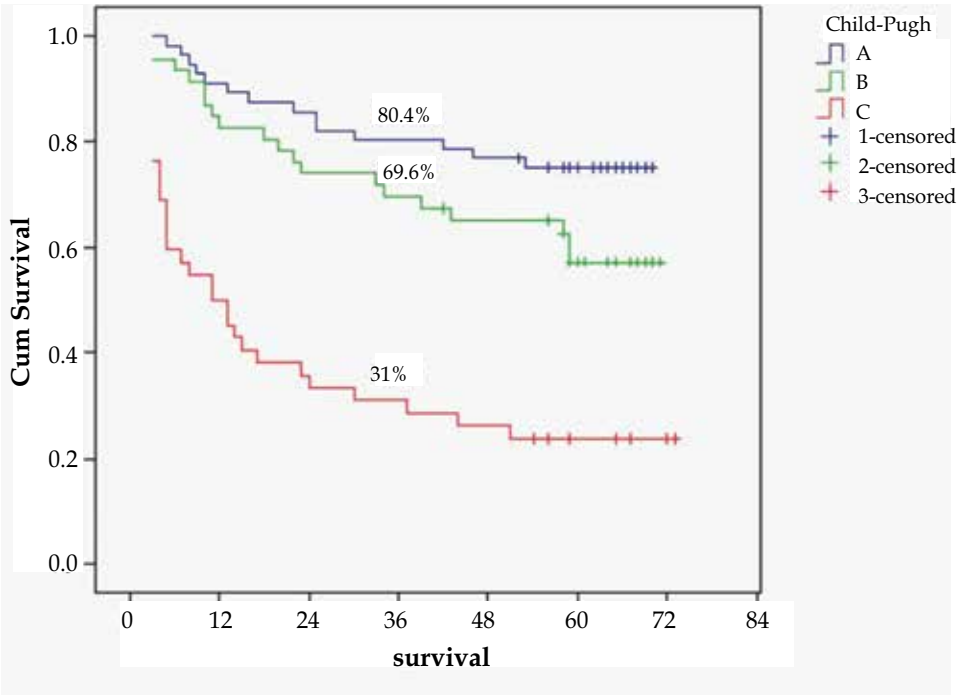


Fig. 3. Survival of patients according the Child-Pugh score

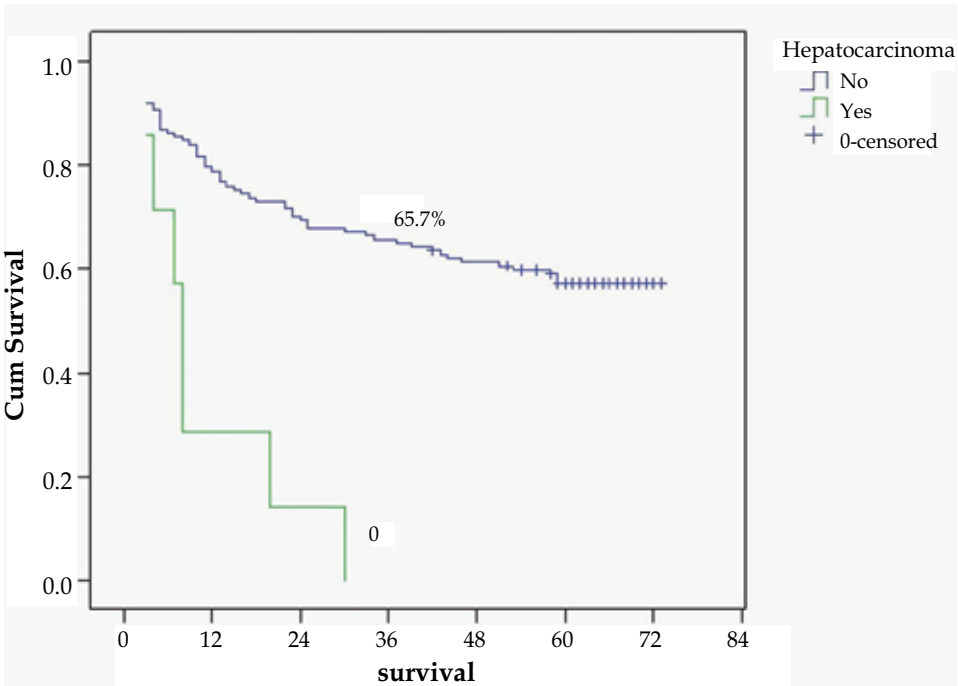


Fig. 4. Survival of patients according the diagnosis of hepatocellular carcinoma

The presence of esophageal varices was more frequent in decompensated liver cirrhosis. Ascites was the most common complication followed by gastrointestinal bleeding, which coincides with reports of other authors (Gines P, 1987). As is known, the higher frequency of esophageal varices is also associated with ascites and with the advanced stage of Child-Pugh. (Samada M, 2008; Sarwar S, 2005; Dib N, 2005)

In this series, overall survival at three years follow-up was 62.5%, which approximates to the average values to the review of natural history and survival in cirrhosis of 118 studies conducted by D'Amico. (D'Amico G, 2006) They reported on 32 studies of survival, with median follow-up of 33 months, cumulative survival of 61%.

Overall survival is less specific because patients are very heterogeneous regarding the presence or absence of complications. In conducting the study it was observed that patients that were at the beginning of the evaluation in compensated and decompensated stage, presented survival rates with significant differences, with 85% and 53.8% respectively. It is reported that the development of hepatocellular carcinoma is a major cause of mortality in patients in compensated phase and the transition to the decompensated stage may be 5 to 7% per year (D'Amico G, 2001). In the present study, HCC was the cause of death in 28.5% of patients who were compensated and the rest died of other cirrhosis complications.

As identified by D'Amico (D'Amico, 2006), compensated cirrhosis by the absence of complications includes two states, patients without varices or ascites (state 1) and patients with varicose veins but without ascites (state 2). Although they have different prognoses, mortality is low (1% per year for state 1 and 3.4% for state 2). On the other hand, decompensated cirrhosis (stages 3 and 4) has a significantly higher mortality (20% per year for the state 3 and up to 57% in 4). This classification was accepted at the Consensus Conference of Portal Hypertension, Baveno IV (De Franchis R, 2000) and modified states Baveno decompensated phase V (D 'Amico G, 2011)

Many factors have been studied in relation to the survival of patients with liver cirrhosis and to improve forecasting models. In the study by D'Amico (D'Amico, 2006), it was reported that the Child-Pugh was the best predictor of mortality in cirrhosis, followed by the five components measured individually. Age was the only variable that was predictive of survival in more than 10 studies, and that was not part of the Child-Pugh score. These data are consistent with those presented by the authors of this paper. The univariate analysis showed that in addition to Child-Pugh, MELD, age, sex and liver cancer were associated with lower survival of the five variables of Child-Pugh, bilirubin, albumin, INR from prothrombin time, ascites and encephalopathy.

In our series, creatinine was not a factor associated with survival, which corresponds with other authors. (Degre D, 2004; Ruf AE, 2005) In relation to sex as a prognostic factor, we must consider that the United States 56 409 deaths related to hepatitis C in the period 1995-2004, there was an increase in mortality from 1.09 to 2.44 per 100 000 inhabitants. This represented an increase of 123% in the period studied, a male predominance with 144% and 81% in females. Alcohol was a cofactor related and could be underestimated. These data confirm the higher male mortality by the two leading causes of cirrhosis, hepatitis C and alcohol (Wise M, 2008)

Ascites is one of the earliest and most frequent complications of liver cirrhosis. Approximately 50% of patients with compensated cirrhosis, can develop ascites within 10 to

15 years after diagnosis, with a mortality of 15% per year and 44% within five years follow-up (Planas R, 2006). In this study, the survival at three years follow-up was 51.1%, indicating that all patients with ascites should be evaluated for liver transplantation, preferably before they develop renal dysfunction, and worsening prognosis.

Encephalopathy is a complication involving low survival. Bustamante et al. (Bustamante J, 1999), followed for 12 ± 17 months 111 patients with cirrhosis who had a first episode of acute encephalopathy, and found that 74% died during follow-up, with a survival rate of 42% per year. In this series the survival at three years was 38.1%.

Hepatocellular carcinoma was the complication that had lower survival; within 11.4 months mean follow-up there was no survivor. This complication can occur at any stage of cirrhosis and is associated with increased frequency in viral causes, so it is very important to increase surveillance programs for early diagnosis and thereby obtain prolonged survivals rates. (Capocaccia R, 2007; Perz JF, 2006) In studies of cirrhosis caused by HBV and HCV has been the leading cause of mortality, especially in patients with HCV. (Perz JF, 2006)

In the survival analysis of MELD and Child-Pugh, it was observed that as they grow, the rate decreases in relation to time tracking. Although the MELD system is the outcome of choice to give priority to patients on the waiting list for liver transplantation in the Consensus Document of the Spanish Society for Liver Transplantation published in 2008, it is argued that currently there are not available data for the Child-Pugh classification no longer used in favor of the MELD system and recommend to apply both models with their advantages and limitations in the future to decide which method is most convenient. Spanish Society for Liver Transplantation. (Spanish Society for Liver Transplantation, 2008)

In the work developed in short and long term, the Child-Pugh score has proven to be a good predictor of mortality. In a review by Cholongitas (Cholongitas E, 2006) on studies that compare the MELD and Child-Pugh, performed with patients on the waiting list for liver transplantation that included 12 532 cirrhotic patients, only 4 of 11 studies showed that the MELD is superior to Child-Pugh in predicting short-term mortality and Gotthardt et al (Gotthardt D, 2009) from the results of their work, consider that for the prediction of long-term mortality (estimated at one year) and removal from the waiting list of patients awaiting transplantation, monitoring should be better by Child-Pugh score than by MELD. This might have implications for the development of new improved scoring systems

In this series, we found on univariate analysis similar significance of Child-Pugh and MELD index as predictors of survival at three years follow-up. But the Child-Pugh acted as an independent predictor of survival.

The prediction of MELD can rise to associate other factors such as clinical or biochemicals. Some studies have shown the prognostic contribution of the addition of sodium to MELD (Ruf AE 2005, Biggins SW 2005), as well as ascites and encephalopathy. (Somsouk M, 2009, Stewart CA, 2007) It has been shown that patients with severe ascites and low sodium, low MELD even have very poor prognosis and suggest incorporating these two elements to the MELD (Heuman DM, 2007).

In another review of prognostic models for priority to liver transplantation, with numerous suggestions for additions to the MELD concluded that the MELD-sodium store is better able to predict survival on the waiting list than the Standard MELD score. (Cholongitas E, 2010)

Biselli M et al (Biselli M 2010) evaluated the survival of patients with advanced liver cirrhosis, liver transplant candidates at 3, 6 and 12 months. Six scoring systems used, included the modified Child Pugh (MCTP) and the standard MELD, and four of its modifications. The modified CTP (Huo TI, 2006) was obtained by assigning an additional point in patients whose serum bilirubin was > 8 mg/dL, prothrombin time prolongation >11 seconds, or albumin <2.3 g/dL, accordingly a mCTP score of 16-18 was defined as class D, which identifies severely decompensated cirrhosis. In this study population, the prognostic power of mCTP did not differ from that of MELD, MELD-sodium and integrated MELD were the best prognostic models.

Although these models are used to assess the short-term survival and give priority for liver transplantation, it would be interesting to determine their behavior in pursuit of longer than one year.

5. Conclusions

Age, Child-Pugh score and the development of hepatocellular carcinoma behaved as independent predictors of survival within three years of monitoring.

Undoubtedly, the Child-Pugh has a good long-term predictive capacity and the development of hepatocellular carcinoma can vary the prognosis of these patients in the short term, so you should keep a surveillance system for early detection.

6. References

Adam, R.; McMaster, P.; O'Grady, JG.; Castaing, D.; Klempnauer, JL.; Jamieson, N.; Neuhaus, P.; Lerut, J.; Salizzoni, M.; Pollard, S.; Muhlbacher, F.; Rogiers, X.; Garcia Valdecasas, JC.; Berenguer, J.; Jaeck, D. & Moreno Gonzalez, E. (2003). Evolution of liver transplantation in Europe: report of the European Liver Transplant Registry. *Liver transplantation*, Vol. 9, No. 12, (Dec), pp. 1231-43, ISSN 1527-6465.

Adler, M.; De Gendt, E.; Vereerstraeten, P.; Degré, D.; Bourgeois, N.; Boon, N.; Gelin, M.; Ickx, B. & Donckier, V. (2005). Value of the MELD score for the assessment of pre- and post-liver transplantation survival. *Transplantation Proceedings*, Vol. 37, No. 6, (Jul-Aug), pp. 2863-4, ISSN 0041-1345.

Ampurdanés, S. & Bruguera, M. (2002). Cirrosis hepática compensada, In: *Gastroenterología y Hepatología*, J. Berenguer, (Ed.), 643-645, Elselvier Science, Barcelona.

Attia, KA.; Ackoundou-N'guessan, KC.; N'dri-Yoman, AT.; Mahassadi, AK.; Messou, E.; Bathaix, YF. & Kissi, YH. (2008). Child-Pugh-Turcott versus Meld score for predicting survival in a retrospective cohort of black African cirrhotic patients. World Journal of Gastroenterology. Vol. 14, No. 2, (Jan 14), pp. 286-91, ISSN 1007-9327

Benvegnú, L.; Gios, M.; Boccato, S. & Alberti, A. (2004). Natural history of compensated viral cirrosis: a prospective study on the incidente and hierarchy of mayor complications. *Gut*, Vol. 53, No. 5, (May), pp. 744-49, ISSN: 0017-5749.

Biggins, S.W.; Rodriguez, H.J.; Bacchetti, P.; Bass, N.M.; Roberts, J.P. & Terrault, N.A. (2005). Serum sodium predicts mortality in patients listed for liver transplantation. *Hepatology*, Vol. 41, No. 1, (Jan), pp. 32-9, ISSN 0270-9139.

Biselli, M.; Gitto, S.; Gramenzi, A.; Di Donato, R.; Brodosi, L.; Ravaioli, M.; Grazi, G.L.; Pinna, A.D.; Andreone, P. & Bernardi, M. (2010). Six score systems to evaluate candidates with advanced cirrhosis for orthotopic liver transplant: Which is the winner? *Liver transplantation*, Vol. 16, No. 8, (Aug), pp. 964-73, ISSN 1527-6473.

Botta, F.; Giannini, E.; Romagnoli, P.; Fasoli, A.; Malfatti, F.; Chiarbonello, B.; Testa, E.; Risso, D.; Colla, G. & Testa, R. (2003). MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study. *Gut*, Vol. 53, No. 1, (Jan), pp. 134–139, ISSN: 0017-5749.

Bustamante, J.; Rimola, A.; Ventura, PJ.; Navasa, M.; Cirera, I.; Reggiardo, V. & Rodés, J. (1999). Prognostic significance of hepatic encephalopathy in patients with cirrhosis. *Journal of Hepatology*, Vol. 30, No. 5, (May), pp. 890-5, ISSN 0168-8278.

Capocaccia, R.; Sant, M.; Berrino, F.; Simonetti, A.; Santi, V. & Trevisani, F. (2007). Hepatocellular Carcinoma: Trends of Incidence and Survival in Europe and the United States at the end of the 20th Century. *The American Journal of Gastroenterology*, Vol. 102, No. 8, (Aug), pp. 1661–70, ISSN 0002-9270.

Carbonell, N.; Pauwels, A.; Fourdan, O.; Lévy, V.G. & Poupon R. (2004). Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology*, Vol. 40, No. 3, (Sep), pp. 652-5, ISSN 0270-9139.

Cárdenas, A. & Ginès, P. (2008). Predicting mortality in cirrhosis--serum sodium helps. The New *England Journal of Medicine*, Vol. 359, No. 10, (Sep 4), pp. 1060-2, ISSN 1533-4406.

Cholongitas, E.; Germani, G. & Burroughs, A.K. (2010). Prioritization for liver transplantation. *Nature reviews. Gastroenterology & hepatology*, Vol. 7, No. 12, (Dec), pp. 659-68, ISSN 1759-5045.

Cholongitas, E.; Marelli, L.; Shusang, V.; Senzolo, M.; Rolles, K.; Patch, D. & Burroughs, AK. (2006). A Systematic Review of the Performance of the Model for End-Stage Liver Disease (MELD) in the Setting of Liver Transplantation. *Liver Transplantation*, Vol. 12, No. 7, (Jul), pp. 1049-61, ISSN 1527-6465.

Corrao, G.; Ferrari, P.; Zambon, A.; Torchio, P. (1997). Are the recent trends in liver cirrhosis mortality affected by the changes in alcohol consumption? Analysis of latency period in European countries. *Journal of Studies on Alcohol*, Vol. 58, No. 5, (Sep), pp. 486–94. ISSN 0096-882X.

D´Amico, G. (2001). Natural history of compensated chirrosis and varices. In: *Complications of cirrhosis: pathogenesis, consequences and therapy*., T.D. Boyer & R.K Groszmann, (Ed.), 118-23, AASLD, Dallas.

D´Amico, G. (2011). Stages Classfication of Cirrhosis: Where Do We Stand? In: *Portal Hypertension V Proceedings of the Fitth Baveno International Concensus Workshop*, R. De Franchis, (Ed.), 132-136, Elselvier Science, Barcelona, ISBN 978-1-4443-3449-4.

D´Amico, G.; Garcia- Tsao, G. & Pagliaro, L. (2006). Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. *Journal of Hepatology*. Vol. 44, No. 1, (Jan), pp. 217-31, ISSN 0168-8278.

De Franchis, R. Updating Consensus in Portal Hypertension: Report of the Baveno III Consensus Workshop on definitions, methodology and therapeutic strategies in portal hypertension. *Journal of Hepatology*, Vol. 33, No. 5, (Nov), pp. 846-52, ISSN 0168-8278.

De la Mata, M.; Barrera, P.; Fraga, E.; Montero, JL.; Pozo, J.C.; Rufián, S.; Padillo, J. & Solórzano, G. (2004). Impacto del sistema MELD en la selección de candidatos para trasplante hepático. *Gastroenterologia y Hepatologia*, Vol. 27, Suppl. 4, pp. 62-5, ISSN 0210-5705.

Degré, D.; Bourgeois, N.; Boon, N.; Le Moine, O.; Louis, H.; Donckier, V.; El Nakadi, I.; Closset, J.; Lingier, P.; Vereerstraeten, P.; Gelin, M. & Adler, M. (2004). Aminopyrine breath test compared to the MELD and Child-Pugh scores for predicting mortality among cirrhotic patients awaiting liver transplantation. *Transplant International*, Vol. 17, No. 1, (Jan), pp. 31-8. ISSN 0934-0874.

Dehesa-Violante, M. & Nuñez-Nateras, R. (2007). Epidemiology of Hepatitis Virus B and C. *Archives of Medical Research*, Vol. 38, No. 6. (Aug), pp. 606-11. ISSN 0188-4409.

Dib, N.; Konate, A.; Oberti, F. & Calès, P. (2005). Non-invasive diagnosis of portal hypertension in cirrhosis. Application to the primary prevention of varices. *Gastroenterologie Clinique et Biologique*, Vol. 29, No. 10, (Oct), pp. 975-87, ISSN 0399-8320.

Durand, F. & Valla, D. (2005). Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. *Journal of Hepatology*, Vol 42, Suppl. 1, pp. S100-7. ISSN 0168-8278.

Fattovich, G.; Bortolotti, F. & Donato, F. (2008). Natural history of chronic hepatitis B: Special emphasis on disease progression and prognostic factors. *Journal of Hepatology*, Vol. 48, No. 2, (Feb), pp. 335–52. ISSN 0168-8278.

Fernández-Esparrach, G.; Sanchez-Fueyo, A.; Ginés, P.; Uriz, J.; Quinto, L.; Ventura, P.J.; Cárdenas, A.; Guevara, M.; Sort, P.; Jiménez, W.; Bataller, R.; Arroyo, V. & Rodés, J. (2001). A prognostic model for predicting survival in cirrhosis with ascites. *Journal of Hepatology*, Vol. 34, No. 1, (Jan), pp. 46-52, ISSN 0168-8278.

Follo, A.; Llovet, J.M.; Navasa, M.; Planas, R.; Forns, X.; Francitorra, A.; Rimola, A.; Gassull, M.A.; Arroyo, V. & Rodés, J. (1994). Renal impairment after spontaneous bacterial peritonitis in cirrhosis: incidence, clinical course, predictive factors and prognosis. *Hepatology*, Vol. 20, No. 6, (Dec), pp. 495-501, ISNN 0270-9139.

Forman, L.M. & Lucey, M.R. (2001). Predicting the prognosis of chronic liver disease: an evolution from child to MELD. Mayo End-stage Liver Disease, *Hepatology*, Vol. 33, No. 2, (Feb), pp. 473-5, ISSN 0270-9139.

Freeman, R.B. Jr. MELD and liver allocation: continuous quality improvement. (2004). *Hepatology*, Vol. 40, No. 4, (Oct), pp. 787-9, ISSN 0270-9139.

Garcia-Tsao, G. (2004). Spontaneous bacterial peritonitis: a historical perspective. *Journal of Hepatology*, Vol. 41, No. 4, (Oct), pp. 522-527, ISSN 0168-8278.

Ginés, P.; Quintero, E.; Arroyo, V.; Terés, J.; Bruguera, M.; Rimola, A.; Caballería, J.; Rodés, J. & Rozman, C. (1987). Compensated cirrhosis: natural history and prognosis. *Hepatology*, Vol. 7, No. 1, (Jan-Feb), pp. 122-8, ISNN 0270-9139.

González M y Albillos A. Hemorragia digestiva por várices esofagogástricas. Gastropatía de la hipertensión portal. En: Gastroenterología. Endoscopia diagnóstica y terapéutica. Editor Abreu L, Editorial Médica Panamericana: Segunda edición; Madrid. p. 2007:173-83.

Gotthardt, D.; Heinz Weiss, K.; Baumgärtner, M.; Zahn, A.; Stremmel, W.; Schmidt, J.; Thomas Bruckner, T. & Saber, P. (2009). Limitations of the MELD score in

predicting mortality or need for removal from waiting list in patients awaiting liver transplantation. *BMC Gastroenterology*, Vol 9, (Sep 25), pp. 72, ISSN 1471-230X.

Heidelbaugh, J.J. & Bruderly, M. (2006). Cirrhosis and Chronic Liver Failure: Part I. Diagnosis and Evaluation. *American Family Physician*, Vol. 74, No. 5, (Sep):556-62. ISSN 0002-838X.

Heuman, D.M.; Abou-Assi, S.G.; Habib, A.; Williams, L.M.; Stravitz, R.T.; Sanyal, A.J.; Fisher, R.A. & Mihas, A.A. (2004). Persistent ascites and low serum sodium identify patients with cirrhosis and low MELD scores who are at high risk for early death. Hepatology, Vol. 40, No. 4, (Oct), pp. 802-10, ISSN 0270-9139.

Heuman, D.M.; Mihas, A.A.; Habib, A.; Gilles, H.S.; Stravitz, R.T.; Sanyal, A.J. & Fisher, R.A. (2007). MELD-XI: a rational approach to "sickest first" liver transplantation in cirrhotic patients requiring anticoagulant therapy. *Liver Transplantation*, Vol. 13, No. 1, (Jan), pp. 30–7, ISSN 1527-6465.

Huo, S.C.; Huo, T.I.; Lin, H.C.; Chi, C.W.; Lee, P.C.; Tseng, F.W & Lee, S.D. (2007). Is the corrected-creatinine model for end-stage liver disease a feasible strategy to adjust gender difference in organ allocation for liver transplantation? *Transplantation*, Vol. 84, No. 11, (Dec), pp. 1406–12, ISSN 0041-1337.

Huo, T.I.; Lin, H.C.; Wu, J.C.; Lee, F.Y.; Hou, M.C.; Lee, P.C.; Chang, F.Y. & Lee, S.D. (2006). Proposal of a modified Child-Turcotte-Pugh scoring system and comparison with the model for end-stage liver disease for outcome prediction in patients with cirrhosis. *Liver Transplantation*, Vol. 12, No. 1, (Jan), pp. 65-71, ISSN 1527-6465.

J. Berenguer, M.; Bruguera, M.; García & L. Rodrigo L. (2001) *Tratamiento de las enfermedades hepáticas y biliares*. Elba SA, Madrid.

Jiang, M.; Liu, F.; Xiong, W.J.; Zhong, L. & Chen, X.M. (2008). Comparison of four models for end-stage liver disease in evaluating the prognosis of cirrhosis. *World Journal of Gastroenterology,*Vol. 14, No. 32, (Nov), pp. 6546-50, ISSN 1007-9327.

Kamath, P.S.; Wiesner, R.H.; Malinchoc, M.; Kremers, W.; Therneau, T.M.; Kosberg, C.L.; D'Amico, G.; Dickson, E.R. & Kim, W.R. (2001). A model to predict survival in patients with end-stage liver disease. *Hepatology*, Vol. 33, No. 2, (Feb), pp. 464-70, ISSN 0168-8278.

Londoño, M.C.; Cárdenas, A.; Guevara, M.; Quintó, L.; de Las Heras, D.; Navasa, M.; Rimola, A.; Garcia-Valdecasas, J.C.; Arroyo, V. & Ginès, P. (2007). MELD score and serum sodium in the prediction of survival of patients with cirrhosis awaiting liver transplantation. *Gut*, Vol. 56, No. 9, (Sep), pp. 1283–90, ISSN: 0017-5749.

Malinchoc, M.; Kamath, P.S.; Gordon, F.D.; Peine, C.J.; Rank, J. & ter Borg, P.C. (2000). A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology*, Vol. 31, No. 4, (Apr), pp. 864-71, ISSN 0168-8278.Mandayam, S.; Jamal, M.M. & Morgan, T.R. (2004). Epidemiology of Alcoholic Liver Disease. *Seminars in Liver Disease*, Vol. 24, No. 3, pp. 217-28, ISSN 0272-8087.

Manos, M.M.; Leyden, W.A.; Murphy, R.C.; Terrault, N.A. & Bel, B.P. (2008). Limitations of Conventionally Derived Chronic Liver Disease Mortality Rates: Results of a Comprehensive Assessment. *Hepatology*, Vol. 47, No. 4, (Apr), pp. 1150-57, ISSN 0270-9139.

Méndez-Sánchez, N.; Almeda-Valdés, P. & Uribe, M. (2005). Alcoholic liver disease. An update. *Annals of Hepatology*, Vol. 4, No. 1, (Jan-Mar), pp. 32-42, ISSN 1665-2681.

Oellerich, M.; Burdelski, M.; Lautz, H.U.; Binder, L. & Pichlmayr, R. (1991). Predictors of one-year pretransplant survival in patients with cirrhosis. *Hepatology*, Vol. 14, No. 6, (Dec), pp. 1029-34, ISSN 0270-9139.

Perz, J.F.; Armstrong, G.L.; Farrington, L.A.; Hutin, Y.J. & Bell, B.P. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of Hepatology*, Vol. 45, No. 4, (Oct), pp. 529-38, ISSN 0168-8278.

Planas, R.; Montoliu, S.; Ballesté, B.; Rivera, M.; Miquel, M.; Masnou, H.; Galeras, J.A.; Giménez, M.D.; Santos, J.; Cirera, I.; Morillas, R.M.; Coll, S. & Solà, R. (2006). Natural history of patients hospitalized for management of cirrhotic ascites. *Clin Gastroenterol Hepatol*, Vol. 4, No. 11, (Nov), pp. 1385-94, ISSN 1542-3565.

Prieto, M.; Aguilera, V.; Berenguer, M.; Pina, R. & Benlloch, S. (2007). Candidate selection for liver transplantation. *Gastroenterologia y Hepatologia*, Vol 30, No. 1, (Jan), pp. 42-53, ISSN 0210-5705.

Prieto, M.; Clemente, G.; Casafont, F.; Cuende, N.; Cuervas-Mons, V.; Figueras, J.; Grande, L.; Herrero, J.I.; Jara, P.; Mas, A.; de la Mata, M.; Navasa, M. & Asociación Española para el Estudio del Hígado. (2003). Consensus document on indications for liver transplantation. 2002. *Gastroenterologia y Hepatologia*, Vol. 26, No. 6, (Jun-Jul), pp. 355-75, ISSN 0210-5705.

Pugh, R.N.; Murray-Lyon, I.M.; Dawson, J.L.; Pietroni, M.C. & Williams. R. (1973). Transection of the oesophagus for bleeding oesophageal varices. *The British journal of surgery*, Vol. 60, No. 8, (Aug), pp. 646-9, ISNN 0007-1323.

Ripoll, C.; Bañares, R.; Rincón, D.; Catalina, M.V.; Lo Iacono, O.; Salcedo, M.; Clemente, G.; Núñez, O.; Matilla, A. & Molinero, L.M. (2005). Influence of hepatic venous pressure gradient on the prediction of survival of patients with cirrhosis in the MELD Era. *Hepatology*, Vol. 42, No. 4, (Oct), pp. 793-801, ISSN 0270-9139.

Ruf, A.E.; Kremers, W.K.; Chavez, L.L.; Descalzi, V.I.; Podesta, L.G. & Villamil, F.G. (2005). Addition of serum sodium into the MELD score predicts waiting list mortality better than MELD alone. *Liver Transplantation*, Vol. 11, No. 3, (Mar), pp.336-43, ISSN 1527-6465

Safdar, K. & Schiff, E.R. (2004). Alcohol and Hepatitis C. *Seminars in Liver Disease*, Vol. 24, No. 3, (Aug), pp. 305-10, ISSN 0272-8087.

Safdar, K. & Schiff, E.R. (2004). Alcohol and Hepatitis C. *Seminars in Liver Disease,* Vol. 24, No. 3, (Aug), pp. 305-10, ISSN 0272-8087.

Sagnelli, E.; Stroffolini, T.; Mele, A.; Almasio, P.; Coppola, N.; Ferrigno L, Scolastico, C.; Onofrio, M.; Imparato, M. & Filippini, P. (2005). The Importance of HCV on the Burden of Chronic Liver Disease in Italy: A Multicenter Prevalence Study of 9,997 Cases. *Journal of Medical Virology*, Vol. 75, No. 4, (Apr), pp. 522–7, ISSN 0146-6615.

Said, A.; Williams, J.; Holden, J.; Remington, P.; Gangnon, R.; Musat, A. & Lucey, M.R. (2004). Model for end stage liver disease score predicts mortality across a broad spectrum of liver disease. *Journal of Hepatology*, Vol. 40, No. 6, (Jun), pp. 897–903, ISSN 0168-8278.

Samada, M., Hernández, J.C.; Ramos, L.; Barroso, L.; González, L.; Cepero Valdés, M.; Hernández Rivero, H.; Abdo Cuza, A.; Valdés, A.R.; Pérez Bernal, J. & Bernardos, A. (2008). Factors that predict survival in patients with cirrhosis considered for

Liver Transplantation. *Transplantation Proceedings*, Vol. 40, No. 9, (Nov), pp. 2965-67, ISSN 0041-1345.

Samada, M.; Hernández, J.C.; Barroso, L.; Chao, L.; González, M. & Fernández, I. (2008). Identificación de factores de riesgo de presencia de várices esofágicas en pacientes con cirrosis hepática. *Revista Cubana de Medicina Militar*, Vol. 37, No. 1, (Ene-Mar), ISSN 0138-6557.

Sarwar, S.; Khan, A.A.; Alam, A.; Butt, A.K.; Shafqat, F.; Malik, K.; Ahmad, I. & Niazi, A.K. (2005). Non-endoscopic prediction of presence of oesophageal varices in cirrhosis. *Journal of the College of Physicians and Surgeons--Pakistan*, Vol. 15, No. 9, (Sep), pp. 528-31, ISSN 1022-386X.

Serra, M.A. (2006) Consenso para el tratamiento de las hepatitis B y C. Virus de la hepatitis C. Historia natural de la infección por virus C. (2006). *Gastroenterología y Hepatología*, Vol. 29, Sup. 2, pp. 101-06, ISSN 0210-5705.

Sociedad Española de Trasplante Hepático. (2008). Consensus document of the Spanish Society of Liver Transplantation. *Gastroenterologia y Hepatologia*. Vol. 31, No. 2, (Feb), pp. 82-91, ISSN 0210-5705.

Somsouk ,M.; Guy, J.; Biggins, S.W.; Vittinghoff, E.; Kohn, M.A. & Inadomi, J.M. Ascites improves upon [corrected] serum sodium plus [corrected] model for end-stage liver disease (MELD) for predicting mortality in patients with advanced liver disease. *Alimentary pharmacology & therapeutics*, Vol. 30, No. 7, (Oct), pp. 741-8, ISSN 0269-2813.

Stewart, C.A.; Malinchoc, M.; Kim, W.R. & Kamath, P.S. (2007). Hepatic encephalopathy as a predictor of survival in patients with end-stage liver disease. (2007). *Liver Transplantation*, Vol. 13, No. 10, (Oct), pp. 1366-71, ISSN 1527-6465.

Taddei, T.H. & Strazzabosco, M. (2007). Hepatic venous pressure gradient (HVPG), serum sodium (SNa), and model of end-stage liver disease score (MELD): prognostic significance and correlations. *Journal of Clinical Gastroenterology*, Vol. 41, No. 7, (Aug), pp. 641-3, ISSN 0192-0790.

Thabut, D. & Bernard-Chabert, B. (2007). Management of acute bleeding from portal hypertension. *Best Practice & Research Clinical Gastroenterology*, Vol. 21, No. 1, pp. 19-29, ISSN 1521-6918.

Titó, L.; Rimola, A.; Ginés, P.; Llach, J.; Arroyo, V. & Rodes, J. (1988). Recurrence of spontaneous bacterial peritonitis in cirrhosis: frequency and predictive factors. *Hepatology*, Vol. 8, No. 1, (Jan-Feb), pp. 27-31, ISSN 0270-9139.

Torre, A.; Martín-Llahí, M. & Ginès, P. (2004). Dilutional hyponatremia, refractory ascites and hepatorenal syndrome: current treatment. *Gastroenterología y Hepatología*, Vol. 27, Sup. 4, pp. 26-39, ISSN 0210-5705.

Vargas, V. & Ortiz, M. (2003). Prognosis of liver cirrhosis. The Model of End-Stage Liver Disease (MELD). *Gastroenterolía y Hepatología*, Vol. 26, No. 4, pp. 257-9, ISSN 0210-5705.

Vilar, E.; Calzadilla, L.; Gra, B.; Arus, E.; Llanio, R.; Diaz, J.; Villa, O. & Abreu, M.R. (2009). Application of a biochemical and clinical model to predict individual survival in patients with end-stage liver disease. *World Journal of Gastroenterol,*Vol. 15, No. 22, (Jun), pp. 2768-77, ISSN 1007-9327..

Wiese, M.; Grungreiff, K.; Guthoff, W.; Lafrenz, M.; Oesen, U.; Porst, H. & East German Hepatitis C Study Group. (2005). Outcome in a hepatitis C (genotype 1b) single source outbreak in a Germany - a 25- year multicenter study. *Journal of Hepatology*, Vol. 43, No. 4, (Oct), pp.590-8, ISSN 0168-8278.

Wise, M.; Bialek, S.; Finelli, L.; Bell, B.P. & Sorvillo, F. (2008). Changing trends in hepatitis C-related mortality in the United States, 1995-2004. *Hepatology*, Vol 47, No. 4, (Apr), pp. 1128-35, ISSN 0270-9139.

7

The Rise of Glutaminase in End-Stage Liver Diseases

Maria Jover-Cobos[1], Nathan Davies[1],
Rajiv Jalan[1] and Manuel Romero-Gómez[2]
[1]*University College London*
[2]*University of Seville*
[1]*United Kingdom*
[2]*Spain*

1. Introduction

Ammonia plays a major role in the pathogenesis of hepatic encephalopathy (HE). Systemic hyperammonemia has been largely found in patients with HE with underlying cirrhosis and acute liver failure. HE is associated with a poor prognosis of both acute and chronic liver disease (Bustamante, Rimola et al. 1999; Hui, Chan et al. 2002). Cirrhosis is the major cause of chronic liver dysfunction and affects 6.5 million people worldwide (Eurostat. 2010, as cited in Albrecht, 2010). HE is the hallmark for acute liver failure (ALF), a syndrome with very high mortality in which liver transplant is the often the only effective treatment.

The HE pathophysiological mechanisms include alterations of blood brain barrier function cytokine production, ammonia-induced changes in neurotransmitter synthesis and release, neuronal oxidative stress, impaired mitochondrial function and osmotic disturbances resulting from astrocytic metabolism. However, none of the metabolic discoveries have yet lead to a therapy which improves prognosis. In the clinic, liver transplantation remains the only curative therapeutic option. Cytotoxic brain edema and intracranial hypertension occurring in encephalopathic ALF patients account for a large number of deaths owing to cerebral herniation. It has been shown that in chronic liver failure there is a low grade brain edema (Andrade, Lucena et al. 2005) that is resolved after transplantation. In comatose patients, moderate hypothermia using cooling blankets to depress energy consumption in the brain seems to be the only relatively effective palliative therapy, but it is expensive, difficult to implement, and not routinely available (Stravitz, Lee et al. 2008; Albrecht 2010).

Studies focusing on inter-organ ammonia metabolism in patients with cirrhosis indicate that the liver, muscles, kidney and the small bowel are important in regulating the circulating levels of ammonia. Historically it was thought that the majority of ammonia was produced by gut bacteria, and treatment regimens including non-absorbable antibiotics and enemas have been extensively used. Contrary to popular belief, it has now been shown that at least 50-60% of total gut ammonia is derived from uptake of glutamine, which is metabolized to glutamate and ammonia by the enzyme glutaminase (GA) (Olde Damink, Jalan et al. 2002; Romero-Gomez, Ramos-Guerrero et al. 2004). Ammonia that would normally be converted

to urea by a healthy liver increases to toxic levels. In this situation, the enzyme glutamine synthetase (GS) plays a pivotal role in ammonia detoxification, effectively removing ammonia during the conversion of glutamate to glutamine (Rose, Michalak et al. 1999). Glutamine deamidation by intestinal GA seems to be the main source of ammonia in patients with cirrhosis (Olde Damink, Jalan et al. 2002), and hyperammonaemia and hepatic encephalopathy can appear without the participation of gut bacteria (Weber and Veach 1979).

The following data support the hypothesis that a genetic factor is implicated in the development of overt hepatic encephalopathy: Glutaminase activity has been linked to hepatic encephalopathy and ammonia production; 40% of persons with cirrhosis and minimal hepatic encephalopathy do not develop overt hepatic encephalopathy in long-term follow-up (Romero-Gomez, Boza et al. 2001); patients with cirrhosis who have the same degree of liver dysfunction and the same precipitating factor (for example, variceal bleeding) may or may not develop overt hepatic encephalopathy; and at least 2 different polymorphisms in the promoter region of the glutaminase gene influence protein activity by increasing or decreasing glutaminase activity (Taylor L 2001).

In this chapter we analyze the studies supporting the inhibition of glutaminase based in the identification of mutations in the glutaminase gene to facilitate selection of patients for close monitoring and evaluation for expedited transplantation. Firstly, we have identified a variant in the promoter region of the glutaminase gene that increases glutaminase activity and is associated with the development of HE. Following a simple blood test to identify these patients with the variant, it would be possible to offer a treatment with glutaminase inhibitors. Based on these and another studies we have developed a new molecule, THDP17 that inhibits glutaminase in CACO-2 cell cultures (intestinal cells).

An alternative treatment to HE is currently being investigated: Ornithine phenylacetate (OP). OP is a novel drug that is targeted at reducing ammonia concentration in patients with liver disease and therefore a potential treatment for HE (Jalan, Wright et al. 2007). The mechanism by which OP directly reduces ammonia levels in cirrhosis is by normalization of gut glutaminase activity and concomitant increasing muscle glutamine synthesis activity, subsequently trapping the increased glutamine with phenylacetate,and increasing ammonia excretion as phenylacetylglutamine in the urine.

These studies support glutaminase such as focus for new treatments of HE and the identification of a genetic variation greatly facilitates the selection of patients for close monitoring and evaluation for expedited transplantation.

2. Glutaminase genetic study

It has been described that there are two forms of mitochondrial glutaminase in the body, liver type glutaminase (L-GA) and kidney type glutaminase (K-GA) or extrahepatic (located in other organs such as the intestine). In humans, increased glutaminase activity is localized to the duodenum. There are indirect data suggesting that glutaminase activity in the enterocyte is increased in cirrhotic patients, as demonstrated by the fact that after oral administration of glutamine there is a rapid increase in blood ammonia level in cirrhotic individuals but not in healthy controls (Romero-Gomez, Grande et al. 2002).

Hyperammonemia is noticably marked in patients with liver cirrhosis with poor liver function, but this increase in ammonia production following a glutamine challenge returns to normal after liver transplantation and normalization of liver function. The specific activity of glutaminase in the enterocyte is a crucial point in the stability of nitrogen metabolism in patients with liver cirrhosis. It has been shown that glutaminase activity is increased in cirrhotic subjects compared to controls and that this activity is related to the presence of encephalopathy and the degree of hepatic dysfunction (Romero-Gomez, Ramos-Guerrero et al. 2004). Thus, also the accumulation of glutamine in the astrocyte is responsible, in large part on the toxicity induced by ammonia (Albrecht and Norenberg 2006).

The cDNA of the human renal-type glutaminase (HK-GA) was cloned in 1998 and subsequently further cDNAs have been isolated encoding for three isoforms of HK-GA, which were designated as K-GA (which is predominantly expressed in kidney, intestine and brain but not liver), M-GA (which is expressed only in cardiac and skeletal muscle), and C-GA (which is expressed primarily in cardiac muscle and pancreas but not in brain or liver). The K-GA isoform is localized to the kidney and has 669 amino acids, C-GA, a protein of 598 amino acids and differs from the K-GA in the carboxyl terminus and the M-GA is a protein of 169 amino acids, which is identical to C-GAP up to amino acid 161 only varying for the c-terminal 8residues. After sequencing the genomic DNA, it was proposed that the three isoforms were the product of alternative splicings of the same gene. Recently described two haplotypes of this gene: TACG and CACG, which place a lower activity of glutaminase, which results in lower intestinal production of ammonia and improved liver function and lower risk of developing hepatic encephalopathy (Romero-Gomez 2005).

We assessed whether alterations in the glutaminase gene could explain, at least in part, the risk for overt hepatic encephalopathy in patients with cirrhosis. We have described a variation in the promoter region of the glutaminase gene that is associated with development of HE in patients with cirrhosis (Romero-Gomez, Jover et al. 2010).

This study included subjects from outpatient clinics from 6 Spanish hospitals: 109 consecutive patients with cirrhosis in the estimation cohort, 177 patients in the validation cohort, and 107 healthy control participants. Patients were followed every 3 or 6 months until the development of hepatic encephalopathy or liver transplantation, death, or the end of the study.

The genetic analyses showed that glutaminase TACC and CACC haplotypes were linked to the risk for overt hepatic encephalopathy. Mutation scanning of the glutaminase gene identified a section in the promoter region where base pairs were repeated (a microsatellite). Over a mean follow-up of 29.6 months, hepatic encephalopathy occurred in 28 patients (25.7%) in the estimation cohort. Multivariable Cox models were used to determine the following independent predictors: Child–Turcotte–Pugh stage (hazard ratio [HR], 1.6 [95% CI, 1.29 to 1.98]; P=0.001), minimal hepatic encephalopathy (HR, 3.17 [CI, 1.42 to 7.09]; P=0.006), and having 2 long alleles of the microsatellite (HR, 3.12 [CI, 1.39 to 7.02]; P=0.006). The association between 2 long alleles of the microsatellite and overt hepatic encephalopathy was confirmed in a validation cohort (HR, 2.1 [CI, 1.17 to 3.79]; P=0.012). Functional studies showed higher luciferase activity in cells transfected with the long form of the microsatellite, which suggests that the long microsatellite enhances glutaminase

transcriptional activity. In Figure 1 is shown that patients with long microsatellite showed higher risk of overt hepatic encephalopathy.

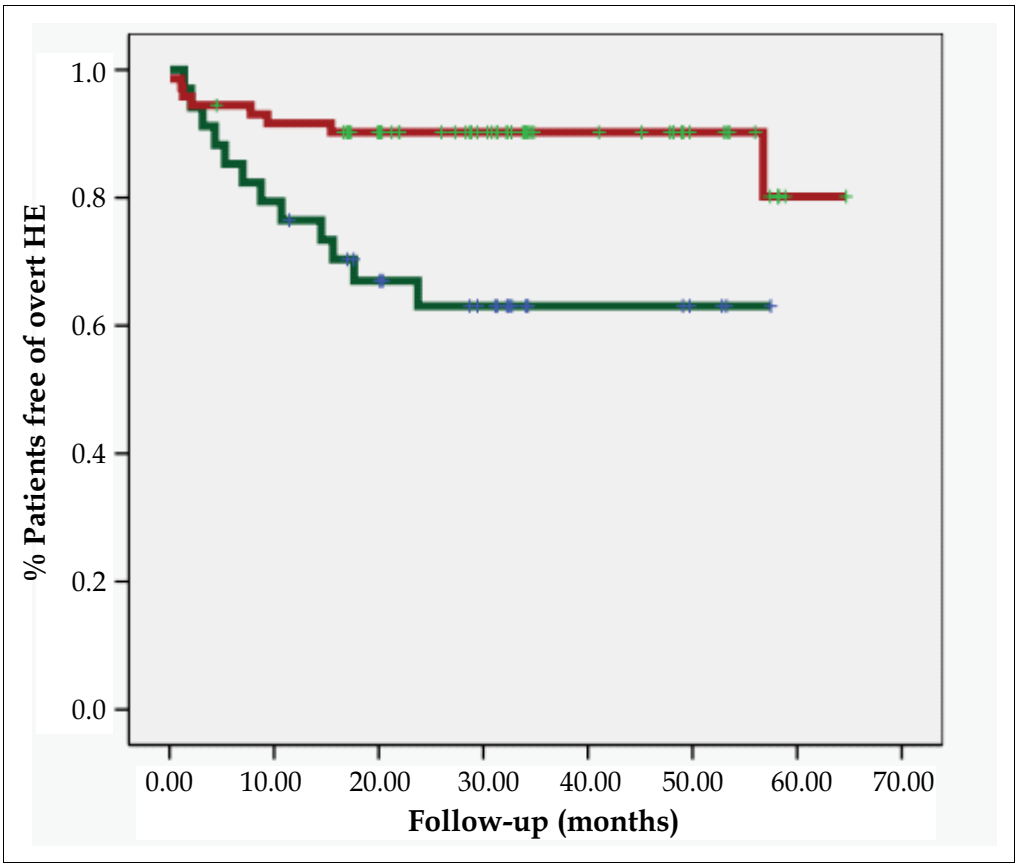


Fig. 1. Actuarial curve showing patients free of bouts of overt hepatic encephalopathy according to the microsatellite in the promoter region. Patients with long microsatellite showed higher risk of overt hepatic encephalopathy (log-rank: 7.74; $p<0.01$)

3. Effects of Ornithinephenylacetate on glutaminase activity in bile duct rats: Inflammation and ammonia

In cirrhotic patients it has been shown that the effects of hyperammonemia are synergistic with inflammation (Shawcross, Davies et al. 2004). The effects on cell swelling by cytokines in ammonia-sensitized cultured astrocytes has also been shown (Rama Rao, Jayakumar et al. 2010). However, the mechanisms by which ammonia produces brain swelling are still subject of much investigation. Although the effects on inflammatory processes have been found to contribute to the formation of cerebral edema, it is not clear whether ammonia promotes inflammation or both are independent factors. Inflammatory pathways identified as contributing to the edema include cyclo-oxygenase, nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling and cytokine release (Andrade, Lucena et al. 2005; Montoliu, Piedrafita et al. 2009; Montoliu, Rodrigo et al. 2010).

Hyperammonemia could increase blood-brain-barrier permeability to systemic cytokines. It is also possible that several factors associated with the systemic inflammatory response syndrome could modulate brain dysfunction induced by hyperammonaemia. These processes, together with a genetic factor, may help to explain the differences that sometimes exist between lower ammonia levels and observed brain impairment in some patients. It has been shown that the presence of HE grade 3/4 correlates better with inflammation than with ammonia plasma levels (Shawcross, Sharifi et al. 2010), though extracellular brain ammonia levels may be significantly higher. One recent study showed that in a cirrhosis animal model in which plasma and brain cytokines were markedly elevated following administration of lipopolysaccharide (LPS), pre-treatment with OP prevented increased levels of TNFα and IL-6 (trend) in plasma and in brain observed in the control group (Wright G 2010). Moreover, OP reduced LPS induced development of pre-coma/coma and worsening of brain edema. It is well-known that the transcription of NFkB directly increases pro-inflammatory cytokines and leads to induction of nitric oxide synthase. (Li and Verma 2002). OP reduced iNOS and NFkB expressions in the cortical brain region of cirrhotic animals, indicating that ammonia reduction may modulate neuroinflammation. In cirrhosis a paradox exists between reduced intrahepatic NO generation and excess NO in the splanchnic circulation. Splanchnic vasodilatation leads to vasoconstriction of numerous vascular beds, including the liver, kidneys, and has significant effects on the brain. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of eNOS (endothelial nitric oxide synthase), the levels of which are increased in liver failure (Leiper, Nandi et al. 2007; Mookerjee, Malaki et al. 2007). It has been shown that treatment of cirrhotic rats with OP resulted in restoration of the NOS pathways which may have a direct effect on cerebral perfusion (Balasubramaniyan, Wright et al. 2011).

Physical symptoms of HE and minimal HE have been detected by motor-evoked potentials (MEP) which examines the function of signal transmission along the nerve, which is perturbed by low grade brain edema. Similar disturbances have been found in patients with cirrhosis using magnetic resonance (MR), with signs compatible with low-grade edema along the corticospinal tract. These abnormalities were related to functional impairment detected by transcranial magnetic stimulation and were found to be reversed after liver transplantation. Recently the assessment of MEP in awake rats has been validated to monitor HE in animal models of liver failure (induced by portocaval anastomosis-PCA) and precipitated HE (simulated gastrointestinal bleed-GB). These models have been utilized to test the efficacy of OP, demonstrating that OP treatment prevents the neurophysiological abnormalities induced by a GB insult in PCA animals. Administration of OP over differing time periods (between 3 hours and 3 days) as a pretreatment prevents the decrease in the amplitude and increase in MEP latency at 6 hours post GB (Oria M, Romero-Gimenez J et al. 2011).

In preliminary studies, it has been shown that the combination of ornithine with phenylacetate to treat hyperammonaemia in cirrhosis is effective in animal models. Administration of OP results in increased conversion of glutamate to glutamine by stimulation of GS activity in the muscle with the subsequent excretion of phenylacetylglutamine in the urine. GA has been found to contribute to hyperammonaemia in cirrhosis and in hyperammonaemia animal models (Romero-Gomez, Grande et al. 2004; Romero-Gomez, Jover et al. 2006).

In this novel approach to target the altered interorgan ammonia metabolism in liver failure, OP utilizes the activity of GS to trap ammonia as glutamine, then phenylacetate facilitates its excretion as phenylacetylglutamine (Davies, Wright et al. 2009; Ytrebo, Kristiansen et al. 2009). The effectiveness of this approach with OP has been confirmed in animal models of cirrhosis and ALF. The reduction (≈50%) of plasma ammonia was associated with (a) an improvement in grade of HE in cirrhotic patients and (b) a reduction in ICP in acute liver failure. OP treatment significantly reduced ammonia concentrations, which was associated with a reduction in brain water and an increase in myoinositol levels, indicating an improvement in brain metabolism (Jalan, Wright et al. 2007; Davies 2009; Ytrebo LM 2009).

In a devascularised pig model of ALF, the rise in arterial ammonia was attenuated with OP which was accompanied by a significant decrease in extracellular brain ammonia and prevention of intracranial hypertension (Ytrebo, Sen et al. 2006).

In our studies we included twenty-five male Sprague-Dawley rats: 4 sham operated, and 21 BDL. 5 BDL's received OP (5 days, IP 0.6 g/kg), 5 BDL's received ornithine (5 days, IP 0.6 g/kg), 5 BDL's received phenylacetate (5 days, IP 0.6 g/kg) and 6 received saline (IP). We measured plasma levels for: ammonia and standard biochemical markers. Expressions of GS, GA and ornithine amino transferase (OAT) were determined by Western-blot (expressed as a % of sham values) and enzyme activity was determined by end-point methods in liver, kidney, gut, muscle and lung. We found that plasma ammonia was decreased in BDL-OP rats vs. BDL-saline (58.97±6.02 vs. 106.2±20.56 μmol/L;$P<0.05$). BDL-OP rats showed increased GS expression in liver (66% BDL-OP vs. 55% BDL-saline; $P < 0.01$) and showed further increased levels in the muscle (153% BDL-OP vs.142% BDL-saline). OP ameliorates the BDL related increases in glutaminase expression (124%vs.163%; $P<0.05$) and activity (0.45±0.16mIU/mg protein BDL-OP vs. 1.14±0.046mIU/mg protein BDL-saline; $P<0.01$) in gut. We demonstrated that this prevention is due to effect of ornithine in glutaminase activity (0.46±0.17mIU/mg protein BDL-O vs. BDL-saline; $P<0.05$) and not to phenylacetate. OP treatment in BDL rats increased the conversion of glutamate to glutamine by stimulation of GS in the muscle and also resulted in normalization of glutaminase expression and activity in the gut, indicating that OP effectively restricts the production of *in vivo* ammonia in a cirrhotic. Mechanism of action of OP on the metabolism of ammonia is shown in the Figure 2.

4. THDP-17: Glutaminase inhibitor in CACO-2 cultures

It has been described as a glutaminase inhibitors Mersalyl, *N*-ethylmaleimide and 6-diazo-5-oxo-L-norleucine (DON). DON has been used in the inhibition of glutaminase in cell cultures of astrocytes in studies demonstrating the importance of GA activity in cell damage induced by ammonia. Also, in rats subjected to portocaval shunt, neomycin inhibits intestinal glutaminase activity (Hawkins, Jessy et al. 1994), although the mechanisms by which neomycin may inhibit glutaminase activity are not described. GA activity is increased in patients with high levels of nitric oxide, glucagon, or tumor necrosis factor, and in a wide number of cancer types as in liver, breast and leukemia as glutamine is implied as a factor in in cell proliferation (Perez-Gomez C 2005; Gao P 2009).

So based on the above and in the previous studies of assosiation of the presence of long-long microsatellite in the glutaminase gene and the mechanism of action of OP in gut

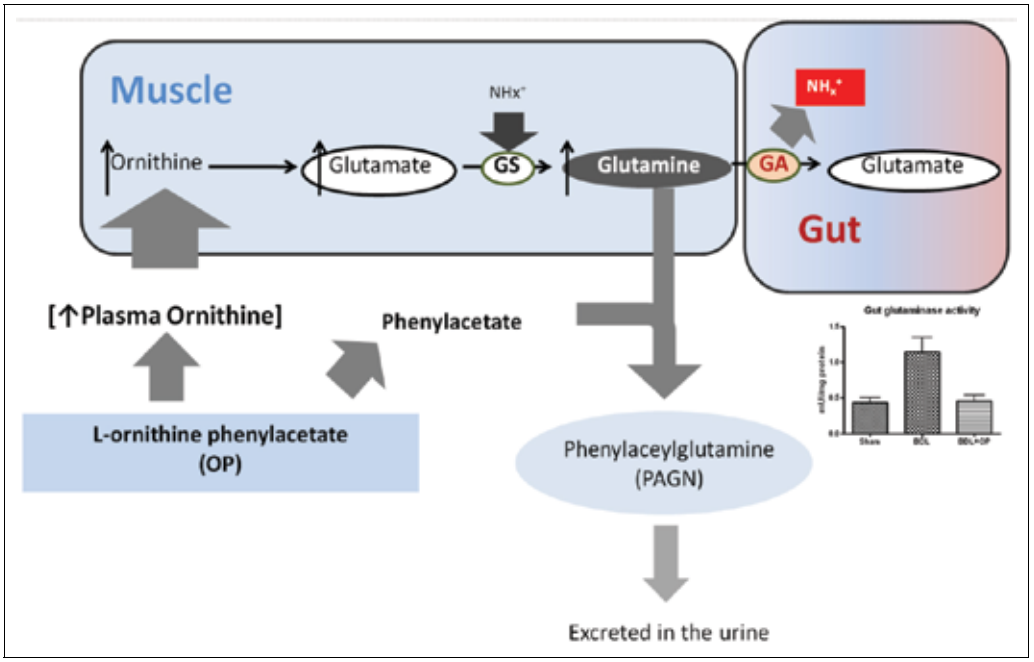


Fig. 2. Mechanism of action of OP in glutamine synthetase (GS) and glutaminase (GA) enzymes. GS is stimulated in muscle by glutamate increased levels. At the same time PAGN is formed and excreted in the urine. In adition, GA is restored to normal levels in the gut

glutaminase, the inhibitition of glutaminase activity is a therapeutic target in the management of hepatic encephalopathy. It is very important to investigate new molecules for the purpose of decreasing the intestinal production of ammonia, since it determines a high risk of encephalopathy and decreased survival. However, when developing these inhibitory molecules is necessary to note that complete inhibition of the enzymatic activity of glutaminase could severely damage the normal function of the enterocytes, so that molecules inhibiting the activity of glutaminase should cause a reduction in activity ie, a partial inhibition of enzyme activity, without significantly affecting the functionality of the enterocyte.

Colon carcinoma cell cultures (CACO2) have been used to test this compound. 50,000 cells per well were used in a 12-well plate with 1.2ml DMEM medium supplemented with 2mM L-Glutamine, 15% FBS, 1X antibiotic/antifungal solution, 1X non-essential amino acids (PAA Laboratories GmbH, Linz, Germany). Plates were incubated at 37°C and 5% CO_2 for 24 and 48h. THDP-17 and 6-diazo-5-oxo-norleucina (DON) (Sigma, St. Louis, EE.UU.) were assayed in duplicates at 0, 5, 20 and 100µM. The glutaminase activity was assayed using the protocol described by Heini (Heini Hans G. 1987).

Using both THDP-17 and DON (positive control) 100µM, glutaminase activity was inhibited after 48h. The product THDP-17 reduces 42% of the initial glutaminase activity, while DON reduces 46% of the initial activity. Therefore THDP-17 is considered to have potential to act as a therapeutic option for the hepatic encephalopathy as a glutaminase partial inhibitor that is able to cross the cellular and mitochondrial membranes. Further studies to evaluate toxicity and *in vivo* experiments to as certain efficacy need to be done in the future. In Figure

3 it can be seen that glutaminase activity is inhibited at 48 hours with differing concentrations of THDP-17 and DON (0,5,20 and 100μM).

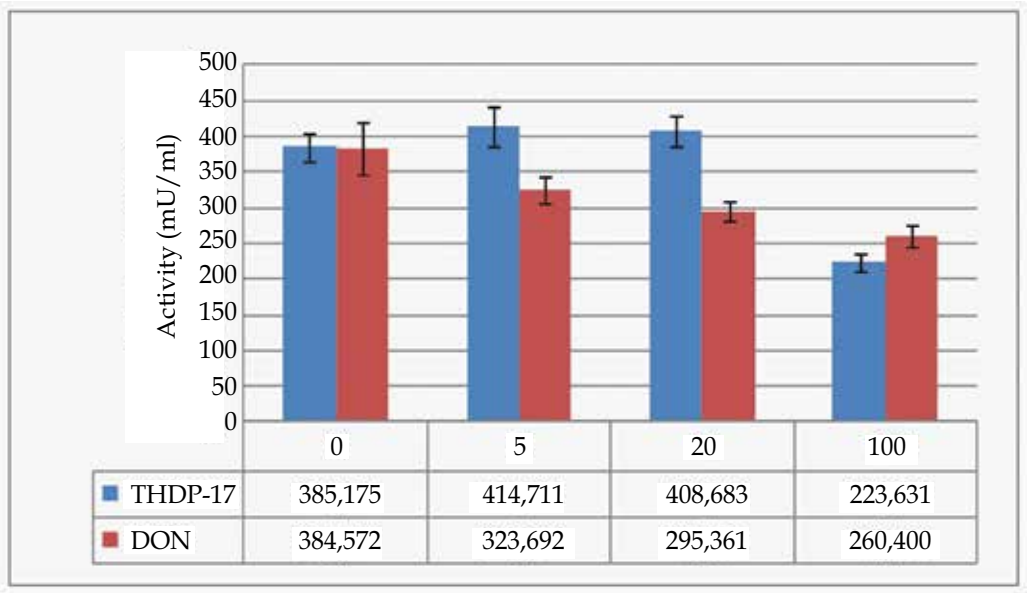


Fig. 3. Inhibition of GA activity following 48 hrs incubation with either THDP 17 or DON in Caco-2 cells

5. Future research

Glutaminase plays a major role in the cause of hepatic encephalopathy. However, a prospective study is required to evaluate the clinical utility of the genetic marker we have identified for predicting overt hepatic encephalopathy before it can be recommended for clinical practice.

In the gut, intestinal glutaminase activity is increased in patients with cirrhosis and correlates with minimal hepatic encephalopathy (Romero-Gomez, Ramos-Guerrero et al. 2004). Glutaminase is also a key factor in the brain. Glutamine synthesis detoxifies ammonia in the brain, but glutaminase transforms glutamine into ammonia, glutamate, and free radicals in the mitochondria, and these byproducts are implicated in mitochondria dysfunction and further neurotransmission impairment (Trojan horse hypothesis) (Albrecht and Norenberg 2006).

Studies to date have indicated that OP is safe and patient studies in minimal HE and HE are needed to establish whether OP or glutaminase inhibitors such TPHD-17 (a promising GA inhibitor) could be used as a treatment for this significant complication of liver disease.

6. Conclusion

We identified a microsatellite in the promoter region of the glutaminase gene that is linked to the development of overt hepatic encephalopathy in patients with cirrhosis. This

promoter is associated with an increase in enzyme activity when the long allele is present. This genetic marker might help identify patients at risk for overt hepatic encephalopathy so that they could be more carefully monitored and could receive intensive treatment and/or define priority in liver transplant waiting list. However, additional studies are needed before this biomarker can be recommended for use in clinical practice.

Our studies support the role of glutaminase in HE and the use of OP, a novel treatment in developing for HE in reducing plasma ammonia. The mechanism by which OP directly reduces ammonia levels in cirrhosis is by increasing muscle GS activity, subsequently trapping and increasing ammonia excretion as phenylacetylglutamine, with the concomitant normalization of gut GA activity. The reduction on ammonia (by OP) leads to a reduction in ICP in ALF and is associated with an improvement in inflammation in the context of chronic liver disease. Moreover, OP modulates iNOS and NFkB mechanisms and prevents LPS-induced brain edema in cirrhotic rats.

And finally, we present a new molecule, THDP-17 as a new therapeutic option for the treatment of hepatic encephalopathy as a GA partial inhibitor that is able to cross the cellular and mitochondrial membranes.

In summary, we believe that further investigation of GA is warranted, because increased knowledge of the pathways involved might lead to the uncovering of new drug targets (such as OP and THDP-17) and other treatments for hepatic encephalopathy. These findings suggest developing approaches to target GA to prevent ammonia release and subsequent HE as a valid therapeutic strategy in the management of patients with liver disease.

7. Acknowledgment

The experiments performed in the authors' laboratories were or are supported by grants Intra-European Fellowship Marie Curie in Seventh Framework Programme (FP7-PEOPLE-2010-IEF number PIEF-GA-2010-272485), Excellency Project P06-CTS-01887 from Junta de Andalucia and PI07425 del Ministerio de Sanidad y Consumo from Spain, and from Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas del Instituto de salud Carlos III of the Ministry of Spanish Health. Ornithinephenlacetate has been provided by Ocera Therapeutics, San Diego, CA.

8. References

Albrecht, J. (2010). "Hepatic encephalopathy in our genes?" *Ann Intern Med* 153(5): 335-6.

Albrecht, J. and M. D. Norenberg (2006). "Glutamine: a Trojan horse in ammonia neurotoxicity." *Hepatology* 44(4): 788-94.

Andrade, R. J., M. I. Lucena, et al. (2005). "Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period." *Gastroenterology* 129(2): 512-21.

Balasubramaniyan, V., G. Wright, et al. (2011). "Ammonia reduction with Ornithine phenylacetate restores brain eNOS activity via the DDAH-ADMA pathway in bile duct ligated cirrhotic rats." *Am J Physiol Gastrointest Liver Physiol* In press.

Bustamante, J., A. Rimola, et al. (1999). "Prognostic significance of hepatic encephalopathy in patients with cirrhosis." *J Hepatol* 30(5): 890-5.

Davies, N., Wright G, Ytrebo LM, Stadlbauer V, Fuskevag O-M, Zwingmann C, Davies DC, Habtesion A, Hodges SJ, Jalan R (2009). "L-ornithine and phenylacetate synergistically produces sustained reduction in amnonia and brain water in cirrhotic rats." *Hepatology* Jul;50(1):155-164..

Eurostat (2010). Accessed at <http://ec.europa.eu/health/ph_information/dissemination /echi/echi_en.htm> on 26 July 2010.

Gao P, T. I., Chang T, Lee Y, Kita K, Ochi T, Zeller K, De Marzo AM, Van Eyk JE, Mendell JT and Dang CV (2009). "c-Myc suppression of miR-23 enhances mitochondrial glutaminase and glutamine metabolism." *Nature* 458(7239): 762–765.

Hawkins, R. A., J. Jessy, et al. (1994). "Neomycin reduces the intestinal production of ammonia from glutamine." *Adv Exp Med Biol* 368: 125-34.

Heini Hans G., G. R., Brecht Andreas and Mecke Dieter (1987). "Purification and characterization of rat liver glutaminase." *Eur. J. Biochem* 162: 541 -546.

Hui, A. Y., H. L. Chan, et al. (2002). "Survival and prognostic indicators in patients with hepatitis B virus-related cirrhosis after onset of hepatic decompensation." J Clin *Gastroenterol* 34(5): 569-72.

Jalan, R., G. Wright, et al. (2007). "L-Ornithine phenylacetate (OP): A novel treatment for hyperammonemia and hepatic encephalopathy." *Med Hypotheses* 69(5): 1064-9.

Leiper, J., M. Nandi, et al. (2007). "Disruption of methylarginine metabolism impairs vascular homeostasis." *Nat Med* 13(2): 198-203.

Li, Q. and I. M. Verma (2002). "NF-kappaB regulation in the immune system." *Nat Rev Immunol 2*(10): 725-34.

Montoliu, C., B. Piedrafita, et al. (2009). "IL-6 and IL-18 in blood may discriminate cirrhotic patients with and without minimal hepatic encephalopathy." *J Clin Gastroenterol* 43(3): 272-9.

Montoliu, C., R. Rodrigo, et al. (2010). "Cyclic GMP pathways in hepatic encephalopathy. Neurological and therapeutic implications." *Metab Brain Dis* 25(1): 39-48.

Mookerjee, R. P., M. Malaki, et al. (2007). "Increasing dimethylarginine levels are associated with adverse clinical outcome in severe alcoholic hepatitis." *Hepatology* 45(1): 62-71.

Olde Damink, S. W., R. Jalan, et al. (2002). "Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS." *Hepatology* 36(5): 1163-71.

Perez-Gomez C, C. S. J., Alonso FJ, Segura J, Manzanares E, Ruiz-Sanchez P, Gonzalez M, Marquez J, Mates JA (2005). "Co-expression of glutaminase K and L isoenzymes in human tumour cells." *Biochem. J.* 386: 535–542.

Rama Rao, K. V., A. R. Jayakumar, et al. (2010). "Marked potentiation of cell swelling by cytokines in ammonia-sensitized cultured astrocytes." *J Neuroinflammation* 7: 66.

Romero-Gomez, M. (2005). "Role of phosphate-activated glutaminase in the pathogenesis of hepatic encephalopathy." *Metab Brain Dis* 20(4): 319-25.

Romero-Gomez, M., F. Boza, et al. (2001). "Subclinical hepatic encephalopathy predicts the development of overt hepatic encephalopathy." *Am J Gastroenterol* 96(9): 2718-23.

Romero-Gomez, M., L. Grande, et al. (2004). "Prognostic value of altered oral glutamine challenge in patients with minimal hepatic encephalopathy." *Hepatology* 39(4): 939-43.

Romero-Gomez, M., L. Grande, et al. (2002). "Altered response to oral glutamine challenge as prognostic factor for overt episodes in patients with minimal hepatic encephalopathy." *J Hepatol* 37(6): 781-7.

Romero-Gomez, M., M. Jover, et al. (2010). "Variations in the promoter region of the glutaminase gene and the development of hepatic encephalopathy in patients with cirrhosis: a cohort study." *Ann Intern Med* 153(5): 281-8.

Romero-Gomez, M., M. Jover, et al. (2006). "Phosphate-activated glutaminase activity is enhanced in brain, intestine and kidneys of rats following portacaval anastomosis." *World J Gastroenterol* 12(15): 2406-11.

Romero-Gomez, M., R. Ramos-Guerrero, et al. (2004). "Intestinal glutaminase activity is increased in liver cirrhosis and correlates with minimal hepatic encephalopathy." *J Hepatol* 41(1): 49-54.

Rose, C., A. Michalak, et al. (1999). "L-ornithine-L-aspartate lowers plasma and cerebrospinal fluid ammonia and prevents brain edema in rats with acute liver failure." *Hepatology* 30(3): 636-40.

Shawcross, D. L., N. A. Davies, et al. (2004). "Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis." *J Hepatol* 40(2): 247-54.

Shawcross, D. L., Y. Sharifi, et al. (2010). "Infection and systemic inflammation, not ammonia, are associated with Grade 3/4 hepatic encephalopathy, but not mortality in cirrhosis." *J Hepatol*.

Stravitz, R. T., W. M. Lee, et al. (2008). "Therapeutic hypothermia for acute liver failure: toward a randomized, controlled trial in patients with advanced hepatic encephalopathy." *Neurocrit Care* 9(1): 90-6.

Taylor L, L. X., Newsome W, Shapiro RA, Srinivasan M, Curthoys NP (2001). "Isolation and characterization of the promoter region of the rat kidney-type glutaminase gene." *Biochim Biophys Acta* 1518: 132-136.

Weber, F. L., Jr. and G. L. Veach (1979). "The importance of the small intestine in gut ammonium production in the fasting dog." *Gastroenterology* 77(2): 235-40.

Wright G, S. R., Brooks HF, Stadlbauer V, Vairappan B, Davies NA, Andreola F, Hodges S, Moss RF, Davies DC, Jalan R. (2010). "Role of aquaporin-4 in the development of brain oedema in liver failure." *J Hepatol*. Jul;53(1): 91-7.

Ytrebo LM, K. G., Maehre H, Fuskevag O-M, Kalstad T, Revhaug A, Cobos MJ, Jalan R, Rose C (2009). "L-ornithine phenylacetate attenuates increased arterial and extracellular brain amnonia and prevents intracranial hypertension in pigs with acute liver failure." *Hepatology* Jul;50(1):165-174.

Ytrebo, L. M., R. G. Kristiansen, et al. (2009). "L-ornithine phenylacetate attenuates increased arterial and extracellular brain ammonia and prevents intracranial hypertension in pigs with acute liver failure." *Hepatology* 50(1): 165-74.

Ytrebo, L. M., S. Sen, et al. (2006). "Interorgan ammonia, glutamate, and glutamine trafficking in pigs with acute liver failure." *Am J Physiol Gastrointest Liver Physiol* 291(3): G373-81.

Part 5

Quality of Life Before and After Liver Transplantation

8

Disease Targeted Measures of Health Related Quality of Life (HRQOL) in Patients with Advanced Liver Disease Before and After Liver Transplantation

Teresa Casanovas Taltavull,
Maria Carmen Peña-Cala and Eva Rodríguez Bruzos
Hospital Universitari de Bellvitge, Institut Català de la Salut, IDIBELL, L'Hospitalet de Llobregat-Barcelona
Spain

1. Introduction

Diseases of the liver are becoming increasingly recognized due to their elevated prevalence and their impact on patients' daily life (Younossi,1998; Armstrong, 2000). Besides, in recent years enormous progress in diagnosis and therapeutics has been made. Currently, liver transplantation is the treatment of choice in selected patients for acute and chronic liver failure (Tomé, 2008; Desai, 2008). Liver transplantation has shown that it improves both survival and perceived changes of quality of life (Tomé, 2008; Duffy, 2010). Survival after liver transplantation is excellent, both in the short and long term. Patient survival rates of approximately 85% after the first year and 75% five years after transplantation have been reported in the European Liver Transplant Registry (www.eltr.org).

Quality of life (QOL) is a broad concept which includes all aspects of life such as where and how one lives and the role he/she plays in society (Bergner, 1989). We have to consider that QOL will also be affected by factors such as interactions with the environment, previous experiences, cultural background, life expectancy, family life, social interactions, present circumstances; financial situation, housing and job satisfaction, which are difficult to incorporate in research (Sanders, 2008; Flamme, 2008).

QOL is a complex concept involving patient's perception of his/her ability to perform functions such as work, but also comprises the physical effects of the illness and concomitant psychological conditions, anxiety, depression, stigma and feeling of hopelessness (Gutteling, 2007; Cordoba, 2003). Other related issues are studied such as sexual problems, relationships with his/her family, friends and the healthcare team (Ware, 1992; Carver, 2005; Day, 2009).

The present review focuses on relevant patient-reported outcomes such as self-perceived symptoms (some are related to immunosuppressor drugs), medication adherence and long term HRQOL (Health Related Quality of Life) after liver transplant (Osterberg, 2005). In our opinion, although these are interrelated issues we will consider here the most recent development of HRQOL in advanced liver disease and transplantation (Unal, 2001).

There is some doubt as to what differentiates HRQOL and QOL. Some concepts that can be encapsulated under the term QOL include social functioning, emotional well-being, role functioning, cognitive functioning, sleep problems, sexual functioning, vitality/energy, pain, life satisfaction, body image and general perceptions of health (Table 1) (Borgaonkar, 2000; van den Plas, 2003). In this chapter we will discuss HRQOL because in clinical practice both concepts HRQOL and QOL are used as equivalents, especially for patients with severe disease.

Due to the multidimensionality of HRQOL, it is not possible to measure every dimension simultaneously, therefore a more limited and focused assessment should be undertaken. In patients with chronic diseases such as advanced liver disease, QOL is based on health parameters, and not on more general factors such as socioeconomic status or housing conditions since these are often considered as not having any relevance to their medical concerns. However, some problems related to finances, corporal image or specific architectural needs are contemplated in relation to some medical conditions as it is done for the study of QOL in oncological patients using specific tools (Roila & Cortesi, 2001; Gangeri, 2007).

Leisure and recreation, **Mobility and self-care,** Travel, Walking, Food/drink, Running, Visit friends' homes, Climbing, Vacation, Eating, Nearnes sto toilet facilities, Grooming, Hobbies and sports, Physical endurance, Relationships, **Emotional,** Intimacy and sexual function, Anger, Body image, Embarrassment, Understanding from others, Anxiety, Coping and support, Irritability, Relations with children and extended family, Happiness, Friendships, Worries or fears, Pain and discomfort, Ability to relax, Chest pain, Frustration, Abdominal pain, Depression/sadness, Abdominal cramps, Satisfaction, Abdominal discomfort, **Job-education,** Rectal pain, Satisfaction, Back pain, Attendance, Headaches, Concentration, Extra intestinal pain, Task completion, Joint pain, Achievement/promotion, **Well being**, Financial reward, Energy, **Treatment,** Fatigue, Efficacy, Sleep, Adverse effects, Self-control

Table 1. Problems, issues and domains of health related quality of ife (HRQOL) studied by questionnaires (Borgaonkar, 2000.)

2. Methods for studying QOL

In practice most research has measured HRQOL as a multi-dimensional construct, instruments used to measure it must have at least three core domains: physical, psychological and social (Unal, 2001; Drent, 2009). Broadly speaking QOL measures can be divided into two categories: generic or condition-specific (Patrick, 1989; van den Plas, 2003):

2.1 Generic questionnaires

Generic questionnaires are comprehensive and they can apply to different patient populations, allowing comparisons between different diseases. These generic measures have the advantage that the obtained scores can be compared with the scores of other patient populations or with a healthy control group, stratified by age and gender. A recognized disadvantage is that generic instruments are not designed to identify disease specific

domains which may be important when we want to establish whether clinical changes have occurred and whether or not they are significant (Jay, 2009).

In the last few years one of the major clinical concerns has been to obtain a good level of QOL after therapeutic interventions. It is therefore crucial to study QOL by asking questions which are relevant to the patients (Hays, 2000). The concept of patient reported outcome is related to an in depth study of the meaningful changes perceived by the patients (Gill, 1994).

A patient-reported outcome (PRO) can be defined as "any outcome based on data provided by patients or by patient proxy as opposed to data provided from other sources". PROs that are of importance to liver transplant patients are: symptom experience, medication adherence and HRQOL. The effectiveness of treatment after organ transplantation depends both on the skills of the health care team and on the life-long, active cooperation of the patient. (Bayliss,1999).

The clinical information provided to the patient and caregivers about long-term side effects of drugs, and of the possibility of developing recurrent or "de novo" disease is essential (Nickel, 2002). This means that transplantation will have an impact on the daily life and the well-being of the patient and will remain as a chronic condition (Stewart, 1989). The three most commonly used generic HRQOL instruments are: the Nottingham Health Profile (NHP), the Medical Outcomes Study Short Form-36 (SF-36) and the Sickness Impact Profile (SIP). (Table 2) (Coons, 2000; Hunt, 1980; de Bruin, 1992; Bergner, 1976). All three instruments have sufficient psychometric properties.

The Medical Outcomes Study 36-item Short Form Health Survey (SF-36), consists of 36 items which measure eight scales: physical functioning (PF), (10 items), role limitations due to physical problems (RP), (4 items), bodily pain (BP), (2 items), general health (GH), (5 items), vitality (VT), (4 items), social functioning (SF), (2 items), role limitations due to emotional problems (RE), (3 items), and mental health (MH) (5 items). On the basis of these separate subscales, component summary scores can be calculated to provide a global measure of physical (Physical Component Summary score, PCS) and mental functioning (Mental Component Summary score, MCS), respectively. The scale scores range from 0 to 100, with higher scores indicating a better health status. The PCS and MCS have been standardized on the basis of a normative general population of different countries, with the mean set at 50 (SD 10) (Guyatt,1993) The SF-36 is currently the most used instrument worldwide, and a shorter version is available (SF-12) (Bruns, 2010).

2.2 Nottingham Health Profile (NHP)

(1) Part I: 38 questions in 6 subareas, with each question assigned a weighted value; the sum of all weighted values in a given subarea adds up to 100, (Hunt, 1985),

- Energy level (EL): 3,
- Pain (P): 8,
- Emotional reaction (ER): 9,
- Sleep (S): 5,
- Social isolation (SI): 5,
- Physical abilities (PA): 8

(2) Part II: Seven daily life areas that can cause problems in your present state of health:

- Work (i.e. paid employment);
- Looking after the home (cleaning & cooking, repairs, odd jobs around the home, etc.);
- Social life (going out, seeing friends, going to the movies, etc.);
- Home life (i.e. relationships with other people in your home);
- Sex life;
- Interests and hobbies (sports, arts and crafts, do-it-yourself, etc.);
- Vacations (summer or winter vacations, weekends away, etc.) (Hunt,1980).

Generic Tests	**Nottingham Health Profile (NHP)**	**Medical Outcomes Study Form (SF-36)**	**Sickness Impact Profile (SIP)**
Authors	Hunt et al. 1980, 1985	Ware et al. 1992 Brazier et al. 1992 (Validation)	Bergner et al. 1981
Number of items	38	36	136
Number of subscales	6	8	12
Total score	No	Yes, -Emotional role** -Physical role**	Yes
Reliability	IC:Cronbach *alpha*: 0.70-0.85 TRT.r: 0.75-0.88	IC: Cronbach *alpha* > 0.84 TRT.r: 0.60-0.81	IC:Cronbach *alpha*: 0.94 TRT.r: 0.87-0.97
*Validity**	CV: Ill vs healthy people DV: Between groups with various health statuses	Conv. V: correlations between four comparable dimensions of SF-36 and NHP were high DV: Correlations between non comparable dimensions of SF-36 and NHP were low	Conv. V: E.g. Activity of daily living. Index:r:0.55-0.61 DV: E.g. explained variance of Speech Pathology Ratings Clinical and Descriptive validity
Subscales/ domains	- Energy - Pain - Emotional reactions - Sleep, - Social isolation - Physical mobility - Seven activity of daily living questions	- Physical functioning - Role limitations due to physical problems - Bodily pain, - General health - Vitality, - Social function - Role limitations due to emotional problem - Mental health	

IC: Internal consistency; TRT: Test Re-test reliability; CV: Construct validity; Conv. V: Convergent validity; DV: Discriminant validity.
*All reported validities have been established
** Mental component score and physical component score

Table 2. Most commonly used generic HRQOL instruments

2.3 Disease-specific questionnaires have been developed to be valid only for one specific condition

A review of QOL instruments used in liver transplantation has been published recently (Jay, 2009). In this article, among others, authors discussed existing QOL instruments with its individual strengths and limitations.

In this chapter we will discuss four specific questionnaires, that have been designed for CLD: (Table 3.) (Gutteling, 2007). In table 3 we present the two more frequently used questionnaires.

Specific HRQOL instruments	**Chronic Liver Disease Questionnaire (CLDQ)**	**Liver Disease Quality of Life Questionnaire (LDQOL)****
Authors	Younossi et al.1999	Gralnek et al.2000
Number of items	29	101
Number of subscales	6	20
Total score	Yes	No
Reliability	TRT: IC: 0.59	IC: Cronbach *alpha*> 0.70 1 subscale *alpha*:0.62
*Validity**	CV: Worse CLDQ scores with increased disease severity	CV: Worse LDQOL scores with increased disease severity for all subscales
Subscales	- Fatigue - Activity - Emotional function - Abdominal symptoms - Systemic symptoms - Worry	8 subscales of the SF-36 adding specific scales: - CLD-related symptoms - CLD-related effects on activities of daily living, - Concentration, - Memory, - Sexual functioning - Sexual problems, - Sleep, - Loneliness, - Hopelessness, - Qual.of social interaction, - Health distress - Self-perceived stigma of CLD

IC: Internal consistency; TRT: Test Re-test reliability; CV: Construct validity; Conv. V: Convergent validity; DV: Discriminant validity; CLD: Chronic Liver Disease.
*All reported validities have been established
**A prospectively validated Short Version of LDQOL has been published (Kanwal, 2008)

Table 3. The two most commonly used specific HRQOL instruments for candidates or recipients of liver transplantation (Jay, 2009)

2.3.1 Hepatitis quality of life questionnaire (HQLQ) (Bayliss, 1998)

In addition to a standard SF-36 generic core, comprised of eight scales, HQLQ contains five generic items consisting of two questions for the social functioning scale and one question each for physical role, emotional role and vitality scales to augment existing SF-36 scales. HQLQ is addressed only to hepatitis C patients. This instrument will be useful in studies of health outcome among patients with chronic hepatitis C, a condition whose health burden appears to have been underestimated in studies to date.

2.3.2 Chronic Liver Disease Questionnaire (CLDQ) (Younossi, 1999)

This instrument introduced in 2000, has two parts: the generic SF-36 and six specific scales, with a number of individual items: abdominal symptoms, fatigue, systemic symptoms, activity, emotional function and worry. These items were selected based on 60 chronic liver disease patients, 20 liver experts and a review of the literature. Younossi et al. established construct validity according to significant differences in CLDQ scores according to Child's classification.

2.3.3 Liver Disease Quality of Life Questionnaire (LDQOL) (Gralnek, 2000)

LDQOL is a targeted instrument which incorporates the generic SF-36 as well as 12 disease-targeted multi-item scales: liver disease-related symptoms, liver disease-related effects on activities of daily living, concentration, memory, sexual functioning, sexual problems, sleep, loneliness, hopelessness, quality of social interaction, health distress, and self-perceived stigma of liver disease. This questionnaire was designed for patients with liver disease, such as transplant candidates or liver transplant recipients. Gralnek et al., established the validity of this instrument for measuring QOL in patients with chronic liver disease in a multi-center study of patients referred for liver transplant evaluation. More recently a prospective validation of a short form version of the LDQOL including 36 targeted items representing nine domains in addition to the SF-36 has been published (Kanwal, 2008).

The LDQOL was published in 2000, but has only been introduced to clinics recently and some studies have been published (Dias Teixeira, 2005; Kim, 2007; Gotardo, 2008; Casanovas 2010;).

2.3.4 National Institutes of Diabetes and Digestive and Kidney (NIDDK)

The Liver Transplant Data Base (NIDDK-LTD), (Belle, 1997) which was developed from standardized instruments it is a QOL questionnaire for adults, which includes both physical and mental domains and specific areas. Unlike the previous specific instruments, it has not been developed in other cultures.

2.4 Complementary QOL indexes

Some complementary QOL indexes and questionnaires may allow implementation research. The opinion of several authors, such as Jay et al. however, is that, the lack of a gold-standard QOL instrument for liver transplant recipients is an impediment to cross-study comparisons. Depending on the objective of the study or the target population, complementary questionnaires should be used, since other areas may be affected in advanced liver disease (Foster, 1998; Dwigt, 2000; Gutteling, 2006).

2.4.1 Fatigue Impact Scale (FIS) (Fisk,1994; Hassoun, 2002; Jones, 2009)

FIS is a validated questionnaire for assessing fatigue. It is used in many chronic conditions. The multidimensional assessment of fatigue is complicated by the interrelation of its multiple causes and effects. A significant proportion of liver patients suffer fatigue, even patients with non advanced disease. Fatigued patients have more sleep problems and higher depression scores than non-fatigued patients (Poynard, 2002). Self rated depression is present in 28% of fatigued patients compared with 4% of non-fatigued patients. Long term fatigue affects 68% of the patients with PBC but it is not related to the severity of their liver disease (Morana, 2009).

2.4.2 Beck's Depression Inventory (BDI) (Beck, 1961)

Beck's Depression Inventory is a 21-item self-report rating inventory measuring characteristic attitudes and symptoms of depression. The total score ranges between 0 and 63; scores below 14 are considered normal, scores from 14 to19 indicate mild to moderate depression, scores from 20 to 28, moderate to severe depression, and scores higher than 28 are indicative of severe depression. This questionnaire is regularly used as a complementary measure.

2.4.3 State-Trait Anxiety Inventory (STAI) (Guillén-Riquelme, 2011)

Since physical symptoms associated with end-stage liver disease, such as poor appetite and fatigue, are also associated with depression, the scores of liver transplant candidates in three separate areas, somatic, cognitive, and affective, have to be computed. Therefore, the State-Trait Anxiety Inventory is one of the most frequently used instruments for measuring anxiety in adults and has been demonstarted to be valid and reliable. It consists of 20 statements that assess how respondents feel "generally." Scores can vary between 20 and 80, with higher scores indicating more anxiety.

2.4.4 Cognitive Operations Preference Enquiry-Easy (COPE-EASY) (Francis, 2009)

Independently of important cultural differences and health systems, coping with chronic diseases is crucial for patients and caregivers (Saab, 2011). The Coping Operations Preference Enquiry-Easy (COPE-Easy) consists of 32 questions, incorporating 15 distinct coping strategies that can be grouped into three subscales: active problem focused coping, avoidant coping and seeking social support (Gutteling 2007).

3. Factors influencing QOL measurements: Age, gender, expectancies, cause of liver disease, differences between men and women and mode of administration

Persons with severe liver disease often have a poor quality of life before liver transplantation (van den Plas, 2003). This poor quality of life is related to chronic disease and a decline in health caused by poor liver function (Marchesini, 2001). Medical treatments may be of some help in limiting symphtoms in cirrhosis and its complications (Hussain, 2001; Younossi, 2001; Girgrah, 2003; Gutteling, 2008). However, a more complete return of quality of life and health must usually wait until after recovery from a successful liver transplantation procedure (Castaldo, 2009). In addition, cross-cultural issues have to take into account (Hunt, 1986).

Currently, liver transplantation is the treatment of choice in selected cases of acute and chronic liver failure and HRQOL reached is in general satisfactory, although below the level of the general population (Cleemput, 2007). Balanced results have been measured one year post- transplant (Takinella, 2010). Results, however, must be interpreted with caution as QOL improvements may have been overstated due to variables such as selection bias, exclusion of severely ill and deceased patients, too many short-term studies and suboptimal methodology (Younossi, 1998).

In the studies published about QOL after liver transplantation in the nineties, only generic instruments were used (Levy, 1995). It is only recently that QOL data have been obtained through specific liver disease questionnaires (Jay, 2009). We will mention some studies that have identified factors known to enhance QOL.

For example, patients with a history of alcoholism or who were regular drug users can be accepted in a transplant program only, after rehabilitation (Lucey, 2002; Gangeri, 2002). However, some studies observed that they have a greater incidence of psychiatric disease or psychological disorders which are responsible for a reduction in their QOL (Dew, 2000; 2001). Recent publications have addressed this problem, as a transplant candidates, alcoholic patients may be considered as a transplant candidates after psychiatric assessment. The detection of urinary ethylglucuronide allows the detection of alcohol consumption in alcoholic liver disease patients awaiting liver transplantation, (Erim, 2007), although fully consensued recommendations have not been achieved, the general recommendation is to dedicate more time assessing patients and increasing communication within the multidisciplinary transplant team (Kotlyar, 2008).

The differences in QOL between male and female patients continue to be a subject of research (Cowling, 2004; Lowry, 2010). QOL assessed gender differences have been detected in chronic illnesses: e.g. women scoring lower levels of QOL (Vazquez, 2004). The same results were obtained when assessing chronic HCV-related liver diseases. Teixeira et al. 2006, using the SF-36 or the specific LDQOL instrument found statistically significant differences. They tested differences in the following domains: liver disease symptoms, concentration, memory, worrying about the disease and sexual problems. Russell et al 2008, with the administration of, SF-36 the Center for Epidemiologic Studies Depression Scale (CES-D), and Beck Anxiety Inventory Scale, observed similar results.

Gifford. et al, in Australia, using the generic instrument SF-12, observed a reduction of QOL for women between 15 and 71 years of age (Gifford, 2003). While the reasons for the lower QOL in women have not yet been clearly defined, the findings indicate that social and cultural problems associated to the disease may be implicated. Interestingly, these observations have been repeated for different chronic conditions, backgrounds or geographic origins. In Spain this has been corroborated by Ferrer et al using the CLDQ (Ferrer, 2006).

The main concern among men is related to their professional activities; they are worried about not being able to provide for their families. Although there are different circumstances implicated in symptoms or in alteration in the QOL, the way that patients react to the diagnosis or treatments and its consequences are different in both genres (Gifford, 2005).

In our experience in the Liver Transplant Unit, we observed, using the generic NHP that three months after liver transplant women were scoring higher than men, but after six

months men were improving progressively whereas women were not, not correlating with clinical results (data not published). These facts may corroborate the usual role of women in family life.

The weight of the stigma felt by liver disease sufferers in the past is still present nowadays (Scambler, 1998; 2009; Zickmund, 2004). Stigma is defined as the opinion of a dominant group with a preformed judgement about attitudes or situations considered socially unacceptable. Stigma is found in all levels of society (Zickmund, 2003).

One explanation is that drug use, a risk factor for AIDS and chronic HCV-related liver disease, projects a negative image of these diseases (Kanwal, 2005). In the past, before the discovery of the hepatitis C virus in 1989, this situation was observed in cirrhosis patients who, despite not being drinkers, were always asked about their drinking habits and were sometimes labelled as alcoholics. Another reason is that people living with a carrier are afraid of being infected (Marcellin, 2007).

Women have reported experiencing greater stigma than men. The presence of this stigma can affect self-esteem and cause alteration in the QOL (Strauss, 2006).

The pre-transplant physician/patient relationship and the coordination with other members of the transplantation team are vital. At this stage, the medical information that patients receive and the attitudes of the medical team is highly significant (Cordoba, 2003; Zickmund, 2004; Flamme, 2008). Knowing about experiences of other patients with the same health problems is also positive. Information supplied to the patient and his/her family members as well as psychological and social support induces behavioural changes, which may be reflected in an improved physiological process (van der Plas, 2003).

Realizing that social and/or psychological factors play a significant role in patients' HRQOL, transplant teams could take advantage of information collected so far and implement new programs (Rodger, 1999; Pieber, 2006; van den Berg, 2006; DiMartini, 2011.)

Mode of administration	**Strengths**	**Weaknesses**
Self	Minimal resources required	Greater likelihood of low response rate
Interviewer	Maximizes response rate. Few, if any, missing items	Requires many resources training of interviewers May diminish willingness to acknowledge problems Limits format of instrument
Surrogate Responder	Reduces stress for target group (very sick or elderly)	Perceptions of surrogate may differ from target group
Telephone	Few, if any, missing items Minimizes errors of misunderstanding Less resource intensive than Interviewer administered mode	Requires resources training of interviewers

Table 4. Modes of administration of questionnaires of HRQOL (Hays, 2009)

Effects on the properties of questionnaires of HRQOL related to the mode of administration have been studied (Table 4) (Hays, 2009; Gundy, 2010). Significant differences were detected in measurements –after adjustment- researchers found that, for the Emotional Functioning (EF) scale, patients who had completed the written questionnaire at home had significantly lower levels of (EF) compared to those interviewed over the telephone.

Child-Turcotte-Pugh (CTP), Prognosis of liver disease. (CTP A = 5-6 p, CTP B = 7-9 p, CTP C = 10-15 p)	1 point each	2 points each	3 points each
Ascites	None	Controlled	Poor control
Encephalopathy	None	Grade I-II	Grade III-IV
Total bilirubin, μmol/L (normal = 17.1 μmol/L)	< 34	34 – 50	> 50
Albumin, g/L	>3.5 g/dL)	2.5-3.5 g/dL	< 2.5 g/dL
INR	< 1.7	1.7–2.2	>2.2

Table 5. Scoring severity of liver disease MELD (Malinchoc, 2000; Kamath, 2001) and Child-Pugh (Pugh, 1973). MELD score was developed to determine the severity of liver disease based on the patient's serum bilirubin, serum creatinine, and the international normalized ration (INR).It has been proposed to replace the Child-Turcotte-Pugh (CTP) score as a "more objective" measure of chronic liver disease severity

4. HRQOL in patients with chronic liver disease is not associated with disease severity as measured by MELD (Model for End-stage Liver Disease) score

Liver transplant is indicated in selected patients with advanced liver disease in which other therapeutic measures have failed or are not possible, and with no absolute contraindications for this procedure. (Consensus Document of the Spanish Society of Liver Transplant, 2009).

Some type of balance between need and utility has to be considered, meaning that we should be cautious with patients with a very severe clinical prognosis when taking advantage of a scarce resource, a liver graft. In our opinion, ideally, there should be a balance between the subjective perception of health by the patient, friends and close family and the clinical severity of the disease based on medical data when deciding on when to do the transplant.

However, some difficulties have to be taken into account. Clinicians base their decisions on objective measures, such as analysis and image tests for clinical diagnosis and treatment but the moment of transplant also depends on the waiting list and the feasibility of a suitable donor. Ideally, it would be desirable to consider both biological objective measures and more subjective measures, such as HRQOL (Kanwal, 2004).

HRQOL in chronic liver patients has been shown to be impaired in numerous studies. Gutteling et al. 2006, studied the impact of physical and psychosocial determinants on a weighted score of HRQOL in patients with chronic liver disease. They showed that HRQOL was related to disease severity and joint pain. Also depression, decreased appetite and fatigue were strongly related to HRQOL. In hepatitis C patients, fatigue and depression were powerful determinants of HRQOL (Strauss, 2006). Patients with cirrhosis who had a

higher Child-Pugh score (measuring disease severity) presented symptoms such as muscle cramps, pruritus, and fatigue (Marchesini, 2001), significant factors relating to QOL. Co-morbid conditions and the duration of disease have not shown in the majority of studies a significant relationship with QOL in these patients. However, the relationship between psychological distress, symptoms and QOL is less known.

Some authors, studying the association between HRQOL and survival in patients with cirrhosis, showed that the relation between HRQOL and survival was MELD (Model of En-Stage Liver Disease) independent (Kanwal, 2004; Saab, 2005). Kanwal et al., found that higher baseline HRQOL predicted lower mortality (Kanwal, 2009). Specifically, for each 1-point increase in HRQOL, there was a 4% decrease in mortality. Both social relations and support have proved to be favourable predictors.

Considering that HRQOL has been recognized as an important outcome in chronic liver diseases, and clearly determined by disease severity, some changes might be applied in the clinical practice. For example, it could be useful to develop a form of intervention aimed at improving adaptation to the more frequently identified symptoms and to implement the use of a comprehensive assessment of QOL in the evolution of chronic liver disease patients with the aim of better clinical management.

However, in everyday practice, the instruments evaluating QOL in liver disease are rarely used due to lack of time and resource constraints (Sanders, 1998; Gutteling, 2007). Some doctors, having different priorities, are hesitant to implement this issue. MELD is being used to prioritize patients for liver transplantation, with the purpose of limiting mortality in patients on the waiting list (Wiesner, 2003). There are paucity of data evaluating associations between MELD score and patient-centered outcomes (HRQOL). Kanwal et al. in 2004 publication "Does Model for End-stage Liver Disease (MELD) predict HRQOL in patients with advanced chronic liver disease?" explore these associations. Their research on correlations between MELD/CTP and patient-centered outcomes evidenced that in persons with advanced chronic liver disease, MELD score predicts patient self-rated severity of liver disease symptoms, but fails to predict disability days and the more global outcome of HRQOL (Kanwal, 2004).

CTP score may be a better proxy measure than MELD, due to being based on clinical data and is more patient centered disease in persons with decompensated chronic liver disease (Saab, 2005). Both hepatic encephalopathy and ascites, which can affect quality of life, are not part of the MELD score. Furthermore, the MELD score has not been correlated with the severity of ascites and hepatic encephalopathy. Thus, liver disease severity assessed by the MELD score may no longer correlate with quality of life. (Saab, 2005) (Table 5).

MELD score allows prioritizing patients on the waiting list, putting the "sickest first" (Schaffer, 2003). However, we have to take into account that the MELD score does not always adequately reflect disease severity and prognosis (Frost, 2002). In patients with fulminant hepatic failure, metabolic disease, hepatocellular carcinoma, refractory ascites, hepato pulmonary syndrome etc. MELD does not apply (Schaffer, 2003).

Nowadays, although one of the most used disease severity indices is MELD score, it is not associated with HRQOL. Several patients with decompensated liver disease do not have a high enough MELD score, so we should examine possible causes of the present situation and justify and validate other specific instruments or formulas.

5. Does etiology of the liver disease appear to influence individual's HRQOL? Issues concerning Acute Liver Disease vs. Chronic Liver Disease

Pretransplantation HRQOL scores are affected by the etiology of liver cirrhosis, therefore it is important the knowledge of the impact of a particular disease. Patients with hepatocellular and cholestatic etiologies have higher HRQOL scores than alcohol or viral hepatitis patients (Bianchi, 2003; Steel, 2007). Although the individual's perception of his/her state of health is crucial, different etiologies may identify some of the characteristics of the components of QOL in liver diseases (Krasnoff,2005). In general terms, younger and male people perceive a better QOL compared to older and women patients (Painter, 2001).

In early stages, liver disease patients show few or non-specific symptoms, therefore reporting insignificant effects on HRQOL. As the disease progresses to cirrhosis and complications arise (ascites, muscle cramps, fatigue etc.) individuals report noteworthy effects on HRQOL (Gutteling, 2006; Björnsson, 2009). In patients with advanced disease the etiology of the liver disease does not seem to influence patients' ratings. Although in earlier cases of chronic cholestatic liver disease significant pruritus and fatigue may have a significant impairment of HRQOL (Poupon, 2004). These symptoms that may also be observed in chronic hepatitis C may not be present in those with other forms of chronic liver disease such as chronic hepatitis B or iron overload (Lam, 2009).

We will explore the most important issues that have been developed in the last few years (Jay, 2009). Some determinants of HRQOL are derived from specific symptoms and concerns of liver diseases, but a generic HRQOL test is unable to measure them (Gutteling, 2007; Kotlyar, 2008). Examples of some specific problems observed in advanced liver disease are: sleep related problems, sleep pattern changes, stigma of liver disease, symptoms and effects of liver disease and their treatments (Holzner, 2001).

5.1 Issues related to HRQOL in hepatitis C and hepatitis B

Patients with chronic hepatitis C assessed by the SF-36 show diverse and non specific symptoms (fatigue, anorexia, weight loss, abdominal distress…) and usually have significant reductions in their SF-36 scores for all of the scales (both mental and physical components) (Dwigt, 1998; Foster, 2000; Strauss, 2006) .

Patients with chronic hepatitis B virus (HBV) infection show a reduction in the SF-36 scores that assessed mental functions, but they have no reduction in the scores that measured physical symptoms, indicating that the symptoms associated with chronic HCV infection are qualitatively different to those associated with chronic HBV infection (Curry, 2004; Spiegel, 2005; Lam, 2009).

Patients with chronic HCV infection who had used intravenous drugs in the past had the greatest impairment in QOL scores, but the reduction in QOL scores was still found in patients who had never used drugs (Weissman, 1980; Fowler 1980). The reduction in QOL could not be attributed to the degree of liver inflammation or to the mode of acquisition of the infection. Recent studies have demonstrated through cerebral magnetic resonance images of abnormal cerebral metabolism and cognitive impairments in patients with chronic hepatitis C (Hilsabeck, 2002; Forton, 2008; Bokemeyer, 2011)

5.2 Hepatocellular Carcinoma (HCC)

Development of a malignant tumour might occur in a cirrhotic liver that can have a preserved liver function. For this reason, clinical guidelines recommend screening surveillance programs in every cirrhotic patient in order to detect possible tumours in early stages to assure treatment efficacy (Ruppert, 2010). Owing to recent advances in early stage liver tumour diagnosis, presumably, these patients do not have specific symptoms and their perceived HRQOL is perhaps stable, but due to the little research available in this area the result for the moment remains uncertain (Kotlyar, 2006; Steel, 2007).

Indications for orthotopic liver transplantation according to the European Liver Transplant Registry (2008), are the following. Virus related indication in 38%; Alcoholic liver disease (ALD) related indication in 33%, and 4% had combined aetiology of ALD and hepatitis C and B. (Varma, 2010)

We could assume that at the time of diagnosis of the HCC, patients may suffer distress and be disturbed by the prospect of a therapeutical intervention such as a possible liver transplant. If the HCC is diagnosed at an early stage the prognosis is good but the patient is not adapted to the disease as is the case in a chronic patient (Gangeri, 2007; Castaldo, 2009; Crone, 2010). After transplant they might have recurrent viral hepatitis which worsens the clinical situation and even the prognosis (Bownik, 2010). New approaches for the prophylaxis of recurrent hepatitis C are under evaluation but whether this treatment will influence the severity of liver disease or the outcome of recurrence is still unknown.

5.3 Alcoholic Liver Disease (ALD)

Patient selection for liver transplantation has always been a demanding responsibility for transplantation teams and professionals. In alcoholic liver disease patients, issues related to liver transplantation have remained unresolved despite the convincing reports of similar survival post transplant in selected ALD patients, compared to those who received transplant for other indications (Roberts, 2004).

A period of abstinence is recommended for alcoholics before being considered possible candidates for transplant and before being accepted to undergo the procedure (Kotlyaar, 2008). Also a reasonable familiar and social support is required. DiMartini et al. have proposed a selection method to identify alcoholic patients suitable for transplantation. Lucey et al have reported on a multidisciplinary collaboration of transplant hepatologists, surgeons and psychiatrists that identifies psychosocial predictors of long term sobriety and compliance after liver transplant in alcoholics (Weinrieb and Lucey, 2007; Dew, 2008). Pretransplant abstinence has two purposes; it allows a window of opportunity for the liver to stabilize (it is not exceptional that some cases have been withdrawn from the waiting list due to improvement), and it allows the opportunity to examine the patient's commitment.

5.3.1 Alcoholic Acute Hepatitis

No systematic evaluation has been performed on patients transplanted for alcoholic liver disease (ALD). Data are limited on the impact of structured management of the alcohol problem on the risk of recidivism following transplantation in ALD. The question of a possible transplant during the acute episode remains unanswered. In these patients short

term survival is good after transplant. However, due to the relative scarcity of donors, the majority of transplant teams do not accept to list patients who are actively consuming alcohol. (Cowling, 2004), but it is controversial (Mathurin, 2011).

5.4 Autoimmune and Cholostatic Liver Diseases

Liver transplant is a well established therapy for patients with autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). The perceived QOL in patients with cholestatic liver disease before and after LT was measured using some aspects of QOL, including symptoms (pruritus, fatigue); physical, social, and emotional functioning; health perceptions (stigma); and overall QOL (Gross, 1999). Changes in these QOL parameters before and after LT were studied and also the relationships between clinical and QOL factors and was demonstrated that cholestatic liver disease displayed the best cost-effectiveness ratio after LT (Longworth, 2003). Following transplantation QOL was substantially better than before transplantation and there were no differences in QOL parameters between patients with AIH, PBC and PSC. Some authors have pointed out that a patient's QOL 1 year after transplant could not be predicted by pre-transplantation QOL variables (Krasnoff, 2005; Bownik, 2009).

5.5 Acute Liver Disease

Acute liver failure continues to be associated with a high mortality rate, and emergency liver transplantation is often the only life-saving treatment (Riordan& Williams, 2003). Although, the short-term outcomes are worse in comparison with those for non-urgent cases, due to the initial recovery process, the majority of patients transplanted with acute liver failure, reported that they have a good quality of life (Sargent, 2007; Dobbels, 2010). The keys to long-term success and continued progress in urgent liver transplantation are the use of good-quality whole grafts and a short waiting list time, both of which depend on access to a sufficient pool of organ donors. In this group the pre-transplantation HRQOL data could not be assessed due to patients' clinical situation. However, there have been studies published reporting an acceptable survival and QOL., in the short or medium term (Chan, 2009).

5.6 Issues related to HRQOL in receptors HIV+

More relevant problems at present are: - Primary disease recurrence, especially Hepatitis C virus, -Scarcity of donors, -Complications related to the chronic administration of immunodepressors (Tomé, 2008) The Spanish Liver Transplant Program for selected HIV+ carriers was initiated in 2002 due to the increasing burden of liver disease in patients with HIV (Joshi, 2011). Some new developments, both in the treatment of HIV+ and in the long-term management of liver transplant recipients have enabled further improvement of the results. (Consensus conference, 2009).

Studies are fragmented. Until recently different constructs and researches covered 1-2 years after transplantation and it is only now that there is a focus on the long term results. In acute liver failure a "better HRQOL related to shorter duration and lesser severity of liver disease" was observed. Disease recurrence has little impact on graft survival rates within 7-10 years of transplantation, in contrast, hepatitis C recurrent disease is an important concern in relation to survival and QOL (Holzner, 2001; Karam, 2003; Sainz-Barriga, 2005).

Survival of liver transplanted patients at five years depends on when the patients received liver transplant (K. Bjøro, 1999). In recent years, there has been considerable improvement in long-term clinical management and an increase in knowledge about risks, such as developing chronic renal disease, "de novo" tumours, primary disease recurrence etc. Present challenges are related to studies with a longer follow up, 10 to 20 years, some of which have been recently published (de Kroon, 2007; Ruppert, 2010).

6. Indications of liver transplant and prioritization criteria on the waiting list

When should a liver transplant be considered? There are medical and ethical concerns about the appropriate use of scarce resources, and the degree of priority given to patients with ALD has always been a controversial issue (Kotlyar, 2008). In recent years, approximately 1200 liver transplants have been performed in Spain yearly. This means that on average three livers are received every day.

Referrals to transplant centres should be made considering there is enough time to evaluate the candidate. Pretransplant evaluation and follow-up is a combined effort of clinicians, psychiatrists and substance abuse specialists. Assessment from medical, surgical and psychosocial points of view takes time and sometimes it can be like a race against time (Gutteling, 2007). Later referrals leave little scope to explore further medical management options or to allow time to work with the substance misuse or psychiatric team. Abstinence before transplantation evaluation and listing is important to select patients who would benefit the most from transplantation, as some would get better in this period (Drent, 2009). There should be reservations in listing patients with a lack of social support, active smokers, having psychotic or personality disorders, or a pattern of nonadherence.

7. Studying QOL after liver transplant. Results in HCV, liver transplantation and QOL

A review of quality of life instruments used in liver transplantation (Jay, 2009) has been recently published. Factors influencing the QOL of an individual patient include pretransplant disease severity, complications during the perioperative period, long-term adverse effects of immunosuppressive drugs, the etiology and recurrence of the underlying liver disease and the information received from the medical team throughout the process (Paterson, 2000).

Nowadays, liver transplantation is a common therapeutical option. However transplantation teams have to deal with strict selection of candidates, due to the relative shortage of donors. Moreover we have to take into consideration the present available information on the natural history regarding the risks of "de novo" diseases, or malignancy or the primary disease recurrence in the long term (Telles-Correia, 2011; Estraviz, 2007). Other goals may be more widely accepted, for example, describing functional health and HRQOL before and after transplantation; comparing contrast outcomes and exploring whether physiological performance, demographics, and other clinical variables are predictors of post-transplantation QOL (Bravata& Keeffe, 2001; Ho, 2006; Aberg, 2009).

In particular, HCV-infected patients generally report a worse QOL before transplant and a lesser increase in QOL after transplant than patients transplanted for other reasons (Feurer, 2002).

The major predictors of poor adherence to medication gives an idea of how we can intervene early in treatment (Bernstein, 2002). There are few studies addressing QOL in relation with anti-HCV treatment after liver transplant (Alsatie, 2007; Neri, 2010). Patient and treatment factors to be aware of are treatment of asymptomatic disease; the presence of psychological problems, particularly depression; a patient's lack of belief in the benefit of treatment; the complexity of the treatment; and adverse events (Schiano, 2006). Other factors that perhaps are harder to quantify are a poor provider-patient relationship, inadequate follow-up or discharge planning, missed appointments, and the cost of medication, copayment, or both. (Ghobrial, 2001).

Liver transplant recipients do not, however, achieve the same QOL scores as healthy controls, and the prevalence of psychiatric comorbidities including depression is higher than controls (Dew, 1997). Patients experience more acute anxiety and depression, especially ex-alcoholics and hepatitis C patients (Paterson, 2000).

Despite few physical manifestations of disease at the time of HCV recurrence, patients report an impaired quality of life and functional status compared with other recipients without recurrence (Feurer, 2002). This suggests that patient knowledge of the diagnosis of recurrent HCV alone can negatively impact HRQOL (Hauser, 2004). They perceive themselves as unwell and have significant changes in their mental and physical health despite the absence of disease-related complications. However, only a limited number of studies have investigated the influence of gender, HCV genotype, or HCV antiviral treatment on the HRQOL of liver transplanted patients with HCV recurrence (Feurer, 2002; Saab, 2010).

Complexity of the treatment requires an extra effort by the transplant team, for example before patients leave the hospital after transplant. Adverse events of medication have to be taken into account, possibly in the long-term, due to poorer physical functioning, depression, and greater rates of fatigue some patients can miss some doses. More than 50% of liver transplants recipients survive more than 20 years, achieve important self-achievements, and report quality of life superior to patients with liver disease or other chronic conditions (Ruppert, 2010).

8. Clinical relevance of measuring QOL and methodological difficulties

Liver transplant is a surgery that restores both long-term physiology and well-being in patients with end-stage liver disease. (Tomé, 2008) Factors that have to be considered include the stress of waiting for a liver transplant - with its uncertainty in terms of both timing and outcome - as well as the physical and psychological demands of the procedure in the pre- and post-transplant period (Goetzmann, 2006). Other demands on the long term are linked to general quality of life (QOL) and treatment adherence (Drent, 2009).

It is necessary to differentiate the clinical situation of patients with acute liver disease versus those with chronic liver disease, due to the process of adaptation that usually happens in chronic diseases. Several cross-sectional and longitudinal studies show a statistically significant increase in QOL after transplantation in the majority of patients. Longitudinal studies are preferable to cross sectional studies.

Capture of the HRQOL experiences across disease severity and etiology of the liver disease is challenging because of subtle differences in the disease and the background of the person

(Norman, 2003). Post-transplantation HRQOL scores are not affected in general by the etiology of the original liver cirrhosis, but transplant recipient scores continue to remain significantly lower than those of healthy patient controls. Prospective studies, showing the QOL evolution in the long term follow up, are starting to show differences between the cause of transplant and clinical evolution (Ruppert, 2010). Minimal clinical important difference is a concept defined as the minimal change in HRQOL which is important for the patient, allowing patients to report a minimal yet perceptible change in their health (Norman, 2003). Confirmation of the preliminary results in this group of patients is necessary.

Relapse of substance abuse, especially alcohol consumption, often affects not only QOL, but also adherence to immunosuppressive therapy and thus long-term survival after OLT. As relapse of alcohol addiction occurs in 10 to 30% of OLT recipients, continuous psychological support has an important role in post-transplant care (Pfitzmann, 2007).

The risk of recurrent disease in the graft influences the clinical prognosis. In previous alcoholics, or other addicts, disease recurs in a minority of patients. For example, in alcoholic disease histologically proven disease recurrence is not frequent whereas it is the rule in hepatitis C, and is not common in cases of primary biliary cirrhosis, auto-immune hepatitis, or primary sclerosing cholangitis (Kotlyar, 2006).

In addition to being influenced by the psychological and physical condition of the patient, QOL is also affected by social function and occupational activity. Employment rates in liver transplanted recipients depend on several factors, such as age, education, duration of their disability and country. The number of patients returning to work after transplant ranges from 30% in Germany to about 55% in the United States and Canada (Bravata, 2001; Aberg, 2009).

During the first 6 months after liver transplantation, the majority of physical and mental components of health-related quality of life scores improve, but these increases are not sustained in the long term (De Bona, 2000). At 1 year after liver transplantation, emotional and mental health-related quality of life scores are balanced with a tendency to decrease (Paterson, 2000). In the postoperative years 1 to 5, possible episodes of acute cellular rejection, recurrent disease and patient age over 60 years decrease physical function and overall general QOL scores (Levy, 1995). Beyond 5 years after liver transplantation, osteoporosis, and episodes of chronic rejection may decrease QOL scores through decreases in the physical function and bodily pain domains (Karam, 2003).

9. The importance of psychological aspects

With regard to psychopathology, it is important to note that it is not always a contraindication for transplant *per se* (Jowsey, 2001; Gutteling, 2010). Some studies however, show that psychiatric diagnosis is common among transplant candidates specially in patients with previous alcoholic liver disease and hepatitis C carriers who may have worse clinical outcome after transplantation (Sherman, 2004).

Telles-Correia, et al, found that in the pre-transplant period, the prevalence of depression was observed in 33% of patients, anxiety was observed in 34%, and dependency on alcohol or drugs was observed in 59%. After transplant, depression prevalence was observed in 30%, anxiety in 26%, and psychosis in 6.4% (Telles-Correia, 2006).

As we mentioned above, non-adherence before transplantation is predictive of non-adherence after transplantation. It is known that anti hepatitis C virus viral treatment is associated with neuropsychiatric side effects (Quelhas,& Lopes, 2009). Therefore, in these situations, psychopharmacological treatment is required to be initiated as soon as possible, especially in patients with a history of psychiatric disorder, to assure adherence to medication (Gangeri, 2007; Quelhas & Lopes, 2009). Many factors may affect the process of adaptation to the disease (Kendall; 1995; Uchino, 1996; Telles-Correia, 2008). Patients can have different coping strategies, the most common being, stoic acceptance, denial, hopelessness, anxious concern and fighting spirit.

Coping strategies may change over time, depending on specific stressors and the development that follows the disease. Studies show that fighting spirit and denial are ways of coping better than the rest, in the sense that facilitate adjustment to illness (Carver, 2005; Russell, 2008).

9.1 Psychosocial aspects in family and advanced liver disease

Emotions are stressful for the patient and also for the family and can lead to physical and emotional exhaustion (Nickel, 2002). Family members usually have emotional troubles, sometimes overlapping those presented by the patient. The most common are: fear, anxiety, sleep disturbances, difficulty concentrating, loss of appetite, and fatigue (Sanders, 2008). When the patient gets all the attention, a feeling of isolation among other family members may appear (Pérez San Gregorio, 2008; Stilley, 2010). Moreover, other perceptions have been observed, such as guilt, when the patient is left alone, or fear of not doing enough or not doing something correctly, etc..,

It is also important to mention two phenomena that may affect the whole family, and they are: the conspiracy of silence and family claudication (Miyazaki, 2010). The conspiracy of silence attempts to prevent that the parties involved become aware of the diagnosis and prognosis of the disease. The results are the emergence of mistrust, isolation, limited emotional expression and isolation which can create family-physician-patient misleading relationships (Bolkhir, 2007). To avoid the conspiracy of silence we must always take into account the real needs and desires of the patient information that can change with the time (Carr, 2001).

Family claudication: expresses the absence of the family's ability to respond adequately to the demands and needs of the patient due to a state of exhaustion and an overwhelming feeling that results in difficulties in maintaining a positive communication between the patient, family members, and the healthcare team (Szeifert, 2010; Kramer, 2011).

9.2 Psychosocial intervention in patients with advanced liver disease

The role of the psychological assessment and monitoring during the pre-and post-transplant phases, has been recognized. Identifying and reducing psychological risk factors may play a role in the long-term success of transplantation (Crone, 2006).

Previous studies suggest that psychological intervention during the process of hepatitis C disease and transplantation is important and necessary (Quelhas &Lopes, 2009). Emotional support during the illness, before and after transplantation, both individually or in groups, can improve the emotional well being, HRQOL, survival, facilitate the adaptation and adherence of transplant candidates and transplant patients (Knott et al. 2006; Quelhas &

Lopes, 2009; Steel, 2007). The goals of psychological intervention are to improve the QOL in patients with liver disease, facilitating their adaptation to the disease, accepting adaptive coping strategies and ultimately improving the patient's sense of self-control (Goetzmann et al, found in a sample of 69 patients, that almost half (47%) expressed the need for emotional support during the assessment for the procedure of liver transplantation (Goetzmann, 2007).

Van den Plas et al., showed that for transplant patients psychological support in the pre-and post-transplant periods besides the rehabilitation provided by the medical and nursing team and family, were one of the essential aspects of the transplant program (Van den Plas, 2003).

In mood disorders and anxiety disorder, psychopharmacological therapy in conjunction with psychotherapy may ameliorate the disturbance to the point at which patients can reach an emotional, and affective balance. Their equilibrium, allows them to manage the eventual distress related to the transplant (Crone, 2006). Such patients, however, need constant support before, during and after transplantation; during the pre-transplantation phase, specifically for sensitivity to stress, (Table 6) and in the post-transplant phase, most significantly because of immunosuppressant therapy that might precipitate mood swings, irritability, mania and anxiety. Psychotherapy and/or psychotherapy in conjunction with pharmacological treatment might be indicated during all the phases of the transplant process as they are individuals who tend to manifest traits such as depression, anxiety and phobia (Marcellin, 2007). Anxiety reduction techniques, autogenic training, systematic relaxation techniques, guided imagery, and pain management are recommended (Pasquini, 2006; Pelgur, 2009).

Informed consent	Personality profile	Psychopathology
Past/present psychiatric history	Effect of illness on daily life activities	
Use/abuse of alcohol and/or drugs		
Defense mechanism employed and coping skills		
Motivation for surgery		Treatment compliance
Perceived Quality of Life	Support from the family and friends	
Socioeconomic support (together with nurses team and social worker's evaluation)		
Awareness of information regarding the actual surgical event and future treatments		

Table 6. Domains of the pre-transplant psychological evaluation

A mail survey was done to assess the importance of professionals assigned to psychosocial factors in evaluations for liver transplant candidacy and liver transplant surgeons from the highest volume liver transplant centers in the United States. Psychosocial evaluators assigned greater importance to availability of transportation, adaptation to stress and coping skills and were less likely than transplant surgeons to recommend that a patient with a history of poor social support be listed for liver transplant. The results of the study showed that factors identified by psychosocial evaluators are important and transplant outcomes should be studied (Santos Junior, 2007).

10. Experiences in our unit

Our Liver Transplant Unit started the liver transplant program in 1984. In 1987 we began to study QOL using the generic test NHP (Figueras, 1989). Interestingly, this test allowed us to confirm that after one year of having received transplant, alcoholic patients showed a recovery in all questions related to their daily life. Their recuperation was similar to that of female patients transplanted for primary biliary cirrhosis. Our explanation was that ex-alcoholics, recipients of transplantation, not only resolved their medical problems while abstaining from alcohol but also experienced a global improvement and had better self-esteem. In our experience patients are offered a new outlook on life post-transplant. Even patients who are suffering from self-inflicted damage (ex-alcoholics or ex-drug users) perceive the donation as proof of solidarity, which sometimes results in them strengthening their relationships with family and friends and in some cases, renewing past relationships. Congress of Spanish Liver Transplantation Groups (1992 Murcia), lecture's main focus was to discuss the "QOL after liver transplant" and whether the etiology of alcohol could affect in the results. It is interesting that we have detected some ex-alcoholics who are riskier cases. They have to attend regular visits with the psychiatric team. After liver transplant some patients may relapse and return to alcohol use. Rehabilitation in these cases is also possible. In our experience, severe cases are the exception; younger males (under 50 years old) who have shown a strong tendency to relapse and who have presented severe cases of recidivism, with small likelihood of rehabilitation.

After 2000, due to the relevance of the use of a specific disease questionnaire for QOL assessment in clinical liver diseases and liver transplantation settings, we started using the LDQOL questionnaire, which was translated and adapted to the Spanish population by our group (Casanovas, 2003; 2007).

We then made correlations with clinical and analytical data pre and post-transplant, and with validation and outcome studies (Casanovas, 2010a). The administration of this long questionnaire is time consuming. We are therefore currently planning to administer the SF-LDQOL questionnaire, which has already been validated by its authors (Kanwal, 2008). Recently, some research on QOL in patients with chronic liver disease, with or without HCC, awaiting liver transplantation and the sensitivity to change of the LDQOL questionnaire to determination of the quality of life of liver transplanted patients prospectively followed for twoyears, has been presented.

The LDQOL 1.0 has proven to be a useful and valid tool for measuring QOL cross-sectionally in patients with liver disease. However, its sensitivity to change, or capacity to reflect actual changes in QOL after an intervention of assumed effectiveness, has not been studied to date. Studies on sensibility to change assessed using a prospective follow up from baseline, before transplant to 2 years after transplant, were presented at the AASLD meeting (Casanovas, 2010 b).

11. Recommendations for future QOL after liver transplant studies

Areas of future research related to QOL might help to settle long term problems associated with liver transplantation. There are a number of reliable and valid instruments, however none of them can be considered as the gold standard outcome to be used in all situations.

In future studies, more attention should be paid to the QOL outcomes in liver transplant recipients with alcoholic liver disease, hepatocellular carcinoma and those with hepatitis C. Some articles also suggest that female liver transplant recipients should receive special attention (Russell, 2008).

Others point out the importance of focusing on the recovering functions both during the initial period post-transplant and on the long term results. How the new available immunosuppressor drugs have to be adjusted will depend on further research incorporating HRQOL results. (Crone, 2006)

In relation to patients with previous addictions, there is no definitive biochemical test to identify alcohol relapse and the tests available have poor sensitivity and specificity, so emphasis on QOL is also important. More research is required in this field to detect alcohol ingestion before it has an effect on the new liver or when social and familiar behaviour could be difficult to manage. (Stilley, 2010)

More attention for caregivers, usually wives because men are more frequently affected by liver diseases, is required. In single patients, it is necessary to identify a circle of friends or other family members who are willing to support and take care of them. (Carver, 2005; Day, 2009; De Blesser, 2009).

Considering factors that could condition differences in the evolution, future researches should assess a larger number of cases than those done to date,and implement prospective studies.

12. Conclusion

In the last few years, it has been recognized that incorporating the patient's perspective on the outcomes of interventions is highly important. While the impact of a health condition on an individual is reflected by symptoms and altered functions, these reflect only part of the total impact of a disease, hence the need to capture the effects on perceptions of his/her self well-being. Moreover, the ratings of physicians on the presence or absence and severity of symptoms or functional limitations can differ from patients' ratings and even from person to person.

Among the measures representing the health effects from the perspective of the patient, are validated questionnaires of HRQL and QOL. Three content areas are crucial, symptoms, functions, and well-being. There are well developed psychometrically generic and disease specific indices suitable for particular areas of research or practice. Chronic liver disease patients perceived a lower measured HRQOL, compared to other chronic disease patients, especially those with advanced or decompensated liver disease.

The results of QOL studies help the physicians to have a better understanding of chronic liver disease patients, some of them candidates to transplantation others post-transplant, thus enabling them to provide their patients with better support. Recognizing the goal of restoration of maximal QOL is essential to create appropriate interventions and to have the required information in order to improve treatment adherence and provide more overall satisfaction with QOL after liver transplantation.

In clinical trials, evaluating new drugs or new treatment schedules, quality-of-life questionnaires should always be added to the usual criteria of toxicity, efficacy or other

evaluations. Liver transplant outcomes have to be considered as both a life-saving intervention and also as an opportunity to improve QOL. Patient interests –reported QOL- should suggest opportunities for ongoing development and research in this area. Therefore, we conclude that psychological care should be offered in all health centers and included in the comprehensive care of patients with liver disease.

More research into the predictive and ethical aspects of psychosocial evaluation for liver transplant is needed. Further studies are necessary that include a complete evaluation of the effects of gender, age, socioeconomic status, education, and ethnicity in order to understand modifiable factors on HRQOL especially after HCV recurrence in transplanted patients that could greatly improve patient's QOL with specific interventions.

13. References

Aberg F, Rissanen AM, Sintonen H, et al. Health-related quality of life and employment status of liver transplant patients. *Liver Transpl*. 2009;15(1):64-72.

Alsatie M, Chalasani N, & Kwo PY. Management of hepatitis C infection after liver transplantation. *Drugs*. 2007;67(6):871-885.

Armstrong G L., Alter M J., McQuillan G M. et al. The past incidence of hepatitis C virus infection: implications for the future burden of chronic liver disease in the United States. *Hepatology* 2000; 31: 777–782.

Bayliss M S., Gandek B., Bungay K M., et al. A questionnaire to assess the generic and disease-specific health outcomes of patients with chronic hepatitis C. *Qual Life Res* 1998; 7(1): 39–55.

Bayliss M S. Methods in outcomes research in hepatology: definitions and domains of quality of life. *Hepatology* 1999; 29 (6 Suppl.): 3S–6S.

Beck AT, Steer RA, Brown GK. *Manual for the Beck Depression Inventory-II*. San Antonio (TX): Psychological Corporation; 1996.

Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. *Arch. Gen. Psychiatry* 1961: 4: 561–571.

Belle SH, Porayko MK, Hoofnagle JH, et al. Changes in quality of life after liver transplantation among adults. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Liver Transplantation Database (LTD). *Liver Transpl Surg*. 1997 Mar;3(2):93-104.

Bergner M., Bobbit R A., Kressel S., et al. The sickness impact profile: conceptual formulation and methodology for development of a health status measure. *Int J Health Serv* 1976; 6(3): 393–415.

Bergner MBobbitt RA, Carter WB Gilson BS The Sickness Impact Profile: development and final revision of a health status measure. *Med Care*. 1981;19(8):787-805.

Bergner M. Quality of life, health status, and clinical research. *Med Care* 1989; 27(3 Suppl.): S148–156.

Bernstein D., Kleinman L., Barker C M., et al. Relationship of health-related quality of life to treatment adherence and sustained response in chronic hepatitis C patients. *Hepatology* 2002; 35(3): 704–708.

Bianchi G, Loguercio C, Sgarbi D, et al. Reduced quality of life of patients with hepatocellular carcinoma. *Dig Liver Dis* 2003; 35(1):46-54.

Björnsson E, Verbaan H, Oksanen A, et al. Health-related quality of life in patients with different stages of liver disease induced by hepatitis C. *Scand J Gastroenterol* 2009;44(7):878-887.

Bjøro K, Friman S, Höckerstedt K, et al. Liver transplantation in the Nordic countries, 1982-1998: changes of indications and improving results. *Scand J Gastroenterol*. 1999; 34(7):714-722.

Bokemeyer M, Ding XQ, Goldbecker A, et al. Evidence for neuroinflammation and neuroprotection in HCV infection-associated encephalopathy. *Gut.* 2011;60(3):370-377.

Bolkhir A, Loiselle MM, Evon DM, et al Depression in primary caregivers of patients listed for liver or kidney transplantation. *Prog Transplant*. 2007;17(3):193-198

Borgaonkar MR, & Irvine E J. Quality of life measurement in gastrointestinal and liver disorders. *Gut.* 2000;47; 3:444-454.

Bownik H & Saab S. The effects of hepatitis C recurrence on health-related quality of life in liver transplant recipients. *Liver Int*. 2010;30:19-30.

Bownik H, Saab S. Health-related quality of life after liver transplantation for adult recipients. *Liver Transpl*. 2009;15 Suppl 2:S42-49.

Bravata D M., & Keeffe E B. Quality of life and employment after liver transplantation. *Liver Transplant* 2001; 7: s119–123.

Brazier JE , Harper R , Jones NM ,et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *BMJ* 1992; 305(6846): 160–164.

Bruns H, Krätschmer K, Hinz U, et al. Quality of life after curative liver resection: a single center analysis. *World J Gastroenterol*. 2010;16(19):2388-2395.

Carr A J., Higginson I J. Are quality of life measures patient centred. *BMJ* 2001; 322(7298): 1357–1360.

Carver, C.S. Enhancing adaptation during treatment and the role of individual differences. *Cancer Supplement* (2005), 104 (11), 2602-2607.

Casanovas T, Vallejo G., Herdman M, et al. Validation of the Spanish version of the Liver Disease Quality of Life questionnaire in transplant patients. *Transplant Proc*. 2003;35 (5):1803-1805.

Casanovas T, Jané L, Herdman M, et al. Validation of the Spanish version of the liver disease quality of life instrument among candidates for liver transplant. *Transplant Proc.* 2007;39(7):2274-2277.

Casanovas T, Jané L, Herdman M, et al. Assessing outcomes in liver disease patients: reliability and validity of the Spanish version of the Liver Disease Quality of Life Questionnaire (LDQOL 1.0). *Value Health.* 2010a;13(4):455-462.

Casanovas T,Jane L, Herdman M et al Sensitivity to change of the LDQOL Questionnaire to determination of the quality of life of liver transplanted patients prospectively followed for two years. *Hepatology,* 2010b;52, suppl 1, 846A.

Castaldo ET, Feurer ID, Russell RT, et al. Correlation of health-related quality of life after liver transplant with the Model for End-Stage Liver Disease score. *Arch Surg.* 2009;144:167-172.

Chan G., Taqi A., Marotta P.,et al. Long-term outcomes of emergency liver transplantation for acute liver failure. *Liver Transpl*. 2009;15 (12):1696-1702.

Cleemput I, & Dobbels F. Measuring patient-reported outcomes in solid organ transplant recipients: an overview of instruments developed to date. *Pharmacoeconomics*. 2007;25(4):269-286.

Consensus document of the Spanish Society of Liver Transplantation. Waiting lists, liver transplantation and quality indicators. *Gastroenterol Hepatol*.2009; 32: 702-716

Coons SJ, Rao S, Keininger DL, & Hays RD. A comparative review of generic quality-of-life instruments. *Pharmacoeconomics* 2000 Jan;17(1):13-35.

Cordoba J., Flavia M., Jacas C., et al. Quality of life and cognitive function in hepatitis C at different stages of liver disease. *J Hepatol* 2003; 39: 231–238.

Cordoba J., Reyes J., Esteban J I. et al. Labeling may be an important cause of reduced quality of life in chronic hepatitis C. *Am J Gastroenterol* 2003; 98(1): 226–227.

Cowling T, Jennings LW, Goldstein RM, et al. Societal reintegration after liver transplantation: findings in alcohol-related and non-alcohol-related transplant recipients. *Ann Surg*. 2004;239:93-98.

Cowling T., Jennings L W., Goldstein R M., et al. Liver transplantation and health-related quality of life: scoring differences between men and women. *Liver Transplant* 2004; 10(1): 88–96.

Crone CC, Gabriel GM, & DiMartini A. An overview of psychiatric issues in liver disease for the consultation-liaison psychiatrist. *Psychosomatics*. 2006;47(3):188-205. Review

Crone CC, Marcangelo MJ, & Shuster JL Jr. An approach to the patient with organ failure: transplantation and end-of-life treatment decisions. *Med Clin North Am.* 2010;94(6):1241-1254.

Curry M P.. Hepatitis B and hepatitis C viruses in liver transplantation. *Transplantation* 2004; 78(7): 955–963.

Day, E., Best, D., Sweeting, R., Russell, R, et al. . Predictors of psychological morbidity in liver transplant assessment candidates: Is alcohol abuse or dependence a factor? *Transpl Int 2009; 22* (6), 606-14.

De Bleser, L., Matteson, M., Dobbels, F., et al. Interventions to improve medication-adherence after transplantation: A systematic review. *Transpl Int.* 2009, 22 (8), 780-797.

De Bona M, Ponton P, & Ermani M . The impact of liver disease and medical complications on quality of life and psychological distress before and after liver transplantation. *J Hepatol.* 2000;33:609-615.

de Bruin AF, de Witte LP, Stevens F, et al..Sickness Impact Profile: the state of the art of a generic functional status measure. *Soc Sci Med.* 1992;35(8):1003-1014.

de Kroon L, Drent G, van den Berg AP,et al.; Liver Transplant Group Groningen. Current health status of patients who have survived for more than 15 years after liver transplantation. *Neth J Med.* 2007;65(7):252-258.

Desai R, Jamieson NV, Gimson AE, et al. Quality of life up to 30 years following liver transplantation. *Liver Transpl.* 2008;14:1473-1479.

Dew MA, Switzer GE, DiMartini AF, et al.Psychosocial assessments and outcomes in organ transplantation. *Prog Transplant.* 2000;10(4):239-259.

Dew MA, Kormos RL, DiMartini AF, et al. Prevalence and risk of depression and anxiety-related disorders during the first three years after heart transplantation. *Psychosomatics.* 2001; 42(4): 300-313.

Dew MA, DiMartini AF, Steel J, et al. Meta-analysis of risk for relapse to substance use after transplantation of the liver or other solid organs. *Liver Transpl*. 2008; 14(2) :159-172.

Dew MA, Switzer GE, Goycoolea JM, et al. Does transplantation produce quality of life benefits? A quantitative analysis of the literature. *Transplantation*. 1997; 64(9): 1261-1273.

Dias Teixeira M C, de Fatima Gomes de Sa Ribeiro M., & Strauss E. A new insight into the differences among non-cirrhotic and cirrhotic patients using the liver disease quality of life instrument (LDQOL). *Ann Hepatol* 2005; 4: 264–271.

DiMartini A., Dew MA, Fitzgerald MG & Fontes P. Clusters of Alcohol Use Disorders Diagnostic Criteria and Predictors of Alcohol Use After Liver Transplantation for Alcoholic Liver Disease. *Psychosomatics* 2008; 49:332–340.

DiMartini A., Dew M A., Chaiffetz D., et al. Early trajectories of depressive symptoms after liver transplantation for alcoholic liver disease predicts long-term survival. *Am J Transplant.* 2011;11:1287-1295.

Dobbels F, Berben L, De Geest S, et al A qualitative study exploring patients perceived quality of life following an emergency liver transplant for acute liver failure. *Transplantation.* 2010;90 (2):205-219.

Drent G, De Geest S, Dobbels F. et al. Symptom experience, nonadherence and quality of life in adult liver transplant recipients *Neth J Med*. 2009;67(5):161-168.

Duffy, JP.; Kao, K; Ko, C Y. et al. Long-Term Patient Outcome and Quality of Life After Liver Transplantation: Analysis of 20-Year Survivors. *Annals of Surgery:* 2010; 252 (4): 652-661.

Dwight M M., Kowdley K V., Russo J E., et al. Depression, fatigue, and functional disability in patients with chronic hepatitis C. *J Psychosom Res* 2000; 49(5):311–317.

Erim Y, Böttcher M, Dahmen U, et al. Urinary ethyl glucuronide testing detects alcohol consumption in alcoholic liver disease patients awaiting liver transplantation. *Liver Transpl.* 2007;13(5):757-761.

Estraviz B, Quintana JM, Valdivieso A, et al. Factors influencing change in health-related quality of life after liver transplantation. *Clin Transplant.* 2007;21(4):481-499.

European liver transplant registry www.eltr.org/

Ferrer M, Córdoba J, Garin O, et al. Validity of the Spanish version of the Chronic Liver Disease Questionnaire (CLDQ) as a standard outcome for quality of life assessment. *Liver Transpl.* 2006;12(1):95-104.

Feurer I D., Wright J K., Payne J L., et al. Effects of hepatitis C virus infection and its recurrence after liver transplantation on functional performance and health-related quality of life. *J Gastrointest Surg* 2002; 6(1):108–115.

Figueras J., Casanovas T., Rafecas A. et al. Quality of life after liver transplant. *Med Clin* (Barc). 1989;93(6):207-208.

Fisk J D, Ritvo P G Ross L, et al.,. Measuring the Functional Impact of Fatigue: Initial Validation of the Fatigue Impact Scale. *Clin Infect Dis.* 1994; 18 (Suppl 1): S79-S83.

Flamme NE, Terry CL, & Helft PR. The influence of psychosocial evaluation on candidacy for liver transplantation. *Prog Transplant.* 2008;18:89-96.

Forton DM, Hamilton G, Allsop JM, et al. Cerebral immune activation in chronic hepatitis C infection: a magnetic resonance spectroscopy study. *J Hepatol.* 2008; 49(3):316-22.

Foster GR, Goldin RD, & Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. *Hepatology*. 1998;27(1):209-212

Fowler R C, Liskow B I, & Tanna V L. Alcoholism, depression, and life events. *J Affect Disord* 1980; 2: 127–135.

Francis JJ, Wileman SM, Bekker H, et al. Beliefs about surgery: Development and validation of an instrument to assess cognitive representations about surgery, in the context of a chronic illness. *Psychol Health.* 2009;24(10):1125-1137

Frost MH, Bonomi AE, Ferrans CE, et al. Patient, clinician, and population perspectives on determining the clinical significance of quality-of-life scores. *Mayo Clin Proc.* 2002; 77(5):488-494.

Gangeri L, Tamburini M, Borreani C, et al. Candidates for liver transplantation for cancer: physical, psychological, and social conditions. *Transplantation* 2002;73(10):1627-1635.

Gangeri, L., Bosinio, M., Brunelli, C., et al. Phenomenology and emotional impact of neuropsychiatric symptoms in orthotopic liver transplant for hepatocellular carcinoma. *Transplant Proc* 2007;39(5):1564-1568.

Ghobrial RM, Steadman R, Gornbein J, et al. A 10-year experience of liver transplantation for hepatitis C: analysis of factors determining outcome in over 500 patients. *Ann Surg.* 2001;234(3):384-393; discussion 393-394.

Gifford S M, O'Brien M L, Bammer G, et al. Australian women's experiences of living with hepatitis C virus: results from a cross-sectional survey. *J Gastroenterol Hepatol* 2003; 18: 841–850.

Gifford S M, O'Brien M L, Smith A, et al. Australian men's experiences of living with hepatitis C virus: results from a cross-sectional survey. *J Gastroenterol Hepatol* 2005; 20: 79–86.

Gill TM, & Feinstein AR. A critical appraisal of the quality of quality-of-life measurements. *JAMA.* 1994;272(8):619-626.

Girgrah N, Reid G, MacKenzie S, et al. Cirrhotic cardio-myopathy: does it contribute to chronic fatigue and decreased health-related quality of life in cirrhosis? *Can J Gastroenterol*. 2003;17(9):545-551.

Goetzmann L, Klaghofer R, Wagner-Huber R, et al. Psychosocial vulnerability predicts psychosocial outcome after an organ transplant: results of a prospective study with lung, liver, and bone-marrow patients. *J Psychosom Res*. 2007;62(1):93-100.

Gotardo DR, Strauss E, Teixeira MC, et al. Liver transplantation and quality of life: relevance of a specific liver disease questionnaire. *Liver Int*. 2008;28(1):99-106.

Gralnek IM, Hays RD, Kilbourne A, et al. Development and evaluation of the Liver Disease Quality of Life instrument in persons with advanced, chronic liver disease--the LDQOL 1.0. *Am J Gastroenterol.* 2000;95(12):3552-3565.

Gross CR, Malinchoc M, Kim WR, et al. Quality of life before and after liver transplantation for cholestatic liver disease *Hepatology*. 1999;29(2):356-364.

Guillén-Riquelme A, & Buela-Casal G. Psychometric revision and differential item functioning in the State Trait Anxiety Inventory (STAI). *Psicothema.* 201123(3):510-515.

Gundy C M. & Aaronson N K. Effects of mode of administration (MOA) on the measurement properties of the EORTC QLQ-C30: a randomized study. *Health and Quality of Life Outcomes* 2010, 8:35- 42.

Gutteling JJ, de Man RA, van der Plas SM, et al Determinants of quality of life in chronic liver patients. *Aliment Pharmacol Ther*. 2006; 23(11):1629-1635.

Gutteling J J., de Man R A., Busschbach J J., et al. Overview of research on health-related quality of life in patients with chronic liver disease. *Neth J Med.* 2007;65(7):227-234.

Gutteling J J., Darlington A S., Janssen H L., et al. Effectiveness of health-related quality-of-life measurement in clinical practice: a prospective, randomized controlled trial in patients with chronic liver disease and their physicians. *Qual Life Res*. 2008;17(2):195-205.

Gutteling J J., Duivenvoorden H J., Busschbach J J., et al. Psychological determinants of health-related quality of life in patients with chronic liver disease. *Psychosomatics.* 2010;51(2):157-165

Guyatt GH, Feeny DH & Patrick DL. Measuring health-related quality of life. *Ann Intern Med*. 1993;15;118(8):622-629.

Hassoun Z., Willems B., Deslauriers J., et al. Assessment of fatigue in patients with chronic hepatitis C using the fatigue impact scale. *Dig Dis Sci* 2002; 47(12): 2674–2681.

Hauser W, Zimmer C, Schiedermaier P. et al. Biopsycosocial predictors of health-related quality of life in patients with chronic hepatitis C. *Psychosom Med* 2004; 66(6): 954–958.

Hays RD, & Woolley JM. The concept of clinically meaningful difference in health-related quality-of-life research. How meaningful is it? *Pharmacoeconomics*. 2000;18(5):419-423.

Hays R D., Kim S., Spritzer M A K. et al. Effects of mode and order of administration on generic health-related quality of life scores. *Value Health.* 2009;12(6):1035-1039.

Hilsabeck R C, Perry W, & Hassanein T I. Neuropsychological impairment in patients with chronic hepatitis C. *Hepatology* 2002; 35(2): 440–446.

Holzner B, Kemmler G, Kopp M., et al. Preoperative expectations and postoperative quality of life in liver transplant survivors. *Arch Phys Med Rehabil*. 2001; 82(1):73-79.

Hunt SM. Cross-cultural issues in the use of socio-medical indicators. *Health Policy.* 1986;6(2):149–158

Hunt SM, & McEwen J. The development of a subjective health indicator. *Sociol Health Illn*. 1980;2(3):231-246.

Hussain K B., Fontana R J., Moyer C A., et al. Co-morbid illness is an important determinant of health-related quality of life in patients with chronic hepatitis C. *Am J Gastroenterol* 2001; 96(9): 2737–2744.

Jay C L., Butt Z., Ladner D P., et al. A review of quality of life instruments used in liver transplantation. *J Hepatol.* 2009; 55(5):949-959.

J Ho J K, Ko H H, Schaeffer DF, et al. Sexual health after orthotopic liver transplantation. *Liver Transplantation*. 2006; 12 (10): 1478–1484.

Jones DE, Gray J.C. & Newton J Perceived fatigue is comparable between different disease groups *Q J Med* 2009; 102(9):617–624.

Joshi D., O'Grady J., Dieterich D., et al. Increasing burden of liver disease in patients with HIV infection. *Lancet.* 2011;377(9772):1198-1209.

Jowsey, S.G.,Taylor, M.L., Scheekloth, T.D. et al. Psychosocial challenges in transplantation. *J Psychiatr Pract* 2001; *7* (6), 404-414.

K Bjøro, S. Friman, K. Höckerstedt, P. et al. Liver Transplantations in the Nordic Countries, 1982-1998: Changes of Indications and Improving Results. *Scandinavian Journal of Gastroenterology* 1999, 34, (7): 714-722.

Kamath P S, Wiesner R H, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; 33(2): 464–470.

Kanwal F, Hays RD, Kilbourne AM, et al. Are physician-derived disease severity indices associated with health-related quality of life in patients with end-stage liver disease? *Am J Gastroenterol* 2004;99(9):1726-1732.

Kanwal F, Gralnek IM, Hays RD, et al. Impact of chronic viral hepatitis on health-related quality of life in HIV: results from a nationally representative sample. *Am J Gastroenterol.* 2005;100(9):1984-1994.

Kanwal F, Spiegel BM, Hays RD, et al.Prospective validation of the short form liver disease quality of life instrument. *Aliment Pharmacol Ther*. 2008;28 (9)1088-1101.

Kanwal F, Gralnek IM, Hays RD, et al.Health-related quality of life predicts mortality in patients with advanced chronic liver disease. *Clin Gastroenterol Hepatol.* 2009;7 (7): 793-799.

Karam VH, Gasquet I, Delvart V, et al. Quality of life in adult survivors beyond 10 years after liver, kidney, and heart transplantation. *Transplantation*. 2003;76 (12): 1699-1704

Kendall J C., Sherman M F. & Bigelow G E. Psychiatric symptoms in poly-substance abusers: relationship to race, sex, and age. *Addict Behav* 1995; 20: 685–690.

Kim S, Choi KH, Hwang SG, et al. [Validation of the Korean version of liver disease quality of life (LDQOL 1.0) instrument]. *Korean J Hepatol*. 2007;13(1):44-50.

Knott A, Dieperink E, Willenbring ML, et al. Integrated psychiatric/medical care in a chronic hepatitis C clinic: effect on antiviral treatment evaluation and outcomes. *Am J Gastroenterol*. 2006;101(10):2254-2262

Kotlyar DS, Burke A, Campbell MS, et al. A critical review of candidacy for orthotopic liver transplantation in alcoholic liver disease. *Am J Gastroenterol*. 2008;103(3):734-743.

Kotlyar DS, Campbell MS, & Reddy KR Recurrence of diseases following orthotopic liver transplantation. *Am J Gastroenterol*. 2006;101(6):1370-1378.

Kramer JR, Kanwal F, Richardson P, et al. Importance of patient, provider, and facility predictors of hepatitis C virus treatment in veterans: a national study. *Am J Gastroenterol*. 2011;106(3):483-491.

Krasnoff J B., Vintro A Q., Ascher N L., et al. Objective measures of health-related quality of life over 24 months post-liver transplantation. *Clin Transplant* 2005; 19(1): 1–9.

Lam ET, Lam CL, Lai CL, et al.Health-related quality of life of Southern Chinese with chronic hepatitis B infection. *Health Qual Life Outcomes*. 2009; 7(5):52-62.

Levy M F., Jennings L., Abouljoud M S., et al. Quality of life improvements at one, two, and five years after liver transplantation. *Transplantation*. 1995; 59(4):515-518.

Longworth L, Young T, Buxton M J, et al. Midterm cost-effectiveness of the liver transplantation program of England and Wales for three disease groups. *Liver Transpl*. 2003;9(12):1295-1307.

Lowry D, Coughlan B, McCarthy O, & Crowe J. Investigating health-related quality of life, mood and neuropsychological test performance in a homogeneous cohort of Irish female hepatitis C patients. *J Viral Hepat* 2010;17(5):352-359.

Lucey MR. Is liver transplantation an appropriate treatment for acute alcoholic hepatitis? *J Hepatol* 2002;36(6):829-831.

Malinchoc M, Kamath PS, Gordon FD, et al.A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology*. 2000; 31(4):864-871.

Mathurin P, Moreno C, Samuel D, et al. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med* 2011;365(19):1790-1800.

Marcellin F, Préau M, Ravaux I, et al. Self-reported fatigue and depressive symptoms as main indicators of the quality of life (QOL) of patients living with HIV and Hepatitis C: implications for clinical management and future research. *HIV Clin Trials*. 2007;8(5):320-327.

Marchesini G, Bianchi G, Amodio P, et al. Italian Study Group for quality of life in cirrhosis. Factors associated with poor health-related quality of life of patients with cirrhosis. *Gastroenterology*. 2001;120(1):170-178.

Miyazaki ET, Dos Santos R Jr, Miyazaki MC, et al. Patients on the waiting list for liver transplantation: caregiver burden and stress. *Liver Transpl.* 2010;16(10):1164-1168.

Morana JG. Psychological evaluation and follow-up in liver transplantation. *World J Gastroenterol.* 2009 14;15(6):694-696.

Neri, S., Bertino, G., Petralia, A., et al. A multidisciplinary therapeutic approach for reducing the risk of psychiatric side effects in patients with chronic hepatitis C treated with pegylated interferon alpha and ribavirin. *J Clin Gastroenterol* 2010, 44 (9), 210-217.

Nickel R, Wunsch A, Egle UT,et al. The relevance of anxiety, depression, and coping in patients after liver transplantation. *Liver Transpl* 2002;8:63-71

Norman GR, Sloan JA, & Wyrwich KW. Interpretation of changes in health-related quality of life: the remarkable universality of half a standard deviation. *Med Care*. 2003 May;41(5):582-592.

Osterberg L, & BlaschkeT. Adherence to medication. Patient and Treatment factors. *N Engl J Med* 2005,4;353(5):487-497.

Painter P, Krasnoff J, Paul S M., et al. Physical activity and health-related quality of life in liver transplant recipients. *Liver Transplant* 2001; 7(5): 213–219.

Pasquini M, Biondi M, Costantini A, et al. Detection and treatment of depressive and anxiety disorders among cancer patients: feasibility and preliminary findings from a liaison service in an oncology division. *Depress Anxiety.* 2006;23(7):441-448

Paterson DL, Gayowski T, Wannstedt CF, et al. Quality of life in long-term survivors after liver transplantation: impact of recurrent viral hepatitis C virus hepatitis. *Clin Transplant*. 2000;14(1):48-54.

Pfitzmann R, Schwenzer J, Rayes N, et al. Long-term survival and predictors of relapse after orthotopic liver transplantation for alcoholic liver disease. *Liver Transpl*. 2007; 13(2):197-205.

Szeifert L, Molnar MZ, Ambrus C, et al. Symptoms of depression in kidney transplant recipients: a cross-sectional study. *Am J Kidney Dis*. 2010;55(1):132-140.

Paterson D L, Gayowski T, Wanntedt C F, et al. Quality of life in long-term survivors after liver transplantation: impact of recurrent viral hepatitis C virus hepatitis. *Clin Transplant* 2000; 14(1): 48–54.

Patrick D L, & Deyo R A. Generic and disease-specific measures in assessing health status and quality of life. *Med Care* 1989; 27(3 Suppl.): S217–232.

Pelgur, H., Atak, N. & Kose, K.. Anxiety and depression levels of patients undergoing liver transplantation and their need for training. *Transplant Proc*. 2009;41(5):1743-1748.

Pérez San Gregorio MA, Martín Rodríguez A & Pérez Bernal J. Psychological differences of patients and relatives according to post-transplantation anxiety. *Span J Psychol*. 2008;11 (1): 250-258.

Pieber K, Crevenna R, Nuhr MJ et al. Aerobic capacity, muscle strength and health-related quality of life before and after orthotopic liver transplantation: preliminary data of an Austrian transplantation centre *J Rehabil Med* 2006;38(5):322-328.

Poupon R E., Chretien Y., Chazouilleres O., et al. Quality of life in patients with primary biliary cirrhosis. *Hepatology* 2004;40(2):489–494.

Poynard T., Cacoub P., Ratziu V., et al. Fatigue in patients with chronic hepatitis C. *J Viral Hepatol* 2002;9(4):295–303.

Pugh R N., Murray-Lyon I M., Dawson J L., et al. Transection of the esophagus for bleeding oesophageal varices. *Br J Surg* 1973;60(8):646–649.

Quelhas R, & Lopes A. Psychiatric problems in patients infected with hepatitis C before and during antiviral treatment with interferon-alpha: a review. *J Psychiatr Pract* 2009; 15(4):262-281.

Rannard A., Buck D., Jones D E., et al. Assessing quality of life in primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2004; 2: 164–174.

Riordan S & Williams R. Cause and prognosis in acute liver failure. *Liver Transpl* 2003; 5: 86-89

Roberts MS, Angus DC, Bryce CL, et al. Survival after liver transplantation in the United States: A disease-specific analysis of the UNOS database *Liver Transpl*. 2004; 10(7):886-897.

Rodger A J., Jolley D., Thompson S C., et al. The impact of diagnosis of hepatitis C virus on quality of life. *Hepatology* 1999;30(5):1299–1301.

Roila F, & Cortesi E Quality of life as a primary end point in oncology. *Annals of Oncology* 2001;12, suppl 3:S3-6.

Ruppert K, Kuo S, DiMartini A,& Balan V. In a 12-year study, sustainability of quality of life benefits after liver transplantation varies with pretransplantation diagnosis. *Gastroenterology*. 2010;139(5):1619-1629.

Russell RT, Feurer ID, Wisawatapnimit P, et al. The effects of physical quality of life, time, and gender on change in symptoms of anxiety and depression after liver transplantation. *J Gastrointest Surg*. 2008;12(1):138-144.

Saab S, Ibrahim AB, Shapner A, et al. MELD fails to measure quality of life in liver transplant candidates. *Liver Transpl* 2005;11(2):218-223.

Sainz-Barriga M., Baccarani U., Scudeller L., et al. Quality-of-life assessment before and after liver transplantation. *Transplant Proc* 2005;37(6):2601–2604.

Sanders C, Egger M, Donovan J, et al. Reporting on quality of life in randomised controlled trials: bibliographic study. *BMJ*. 1998;317(7167):1191-1194.

Sanders C, Rogers A, Gately C, et al. Planning for end of life care within lay-led chronic illness self-management training: the significance of 'death awareness' and biographical context in participant accounts. *Soc Sci Med* 2008;66(4):982-993.

Santos Junior, R., Miyazaki, M.C., Domingos, N.A., et al. Patients undergoing liver transplantation: psychosocial characteristics, depressive symptoms, and quality of life. *Transplant Proc* 2008;40(3):802-804.

Sargent S & Wainwright SP. A qualitative study exploring patients perceived quality of life following an emergency liver transplant for acute liver failure. Intensive *Crit Care Nurs* 2007;23(5): 272-280.

Scambler G. Stigma and disease: changing paradigms. *Lancet* 1998; 352(9133): 1054–1055.

Scambler G. Health-related stigma. *Sociol Health Illn*. 2009;31(3):441-455.

Schaffer RL, Kulkarni S, Harper A, et al.The sickest first? Disparities with model for end-stage liver disease-based organ allocation: one region's experience. *Liver Transpl.* 2003;9(11):1211-1215.

Schiano T D, Martin P. Management of HCV infection and liver transplantation. *Int J Med Sci* 2006; 3: 79–83.

Sherman KE, Sherman SN, Chenier T & Tsevat J. Health values of patients with chronic hepatitis C infection. *Arch Intern Med*. 2004;164 (21):2377-2382.

Spiegel B M., Younossi Z M., Hays R D., et al. Impact of hepatitis C on health related quality of life: a systematic review and quantitative assessment. *Hepatology* 2005;41(4):790–800.

Steel JL, Chopra K, Olek MC, & Carr BI. Health-related quality of life: Hepatocellular carcinoma, chronic liver disease, and the general population. *Qual Life Res* 2007a;16(2):203-215.

Steel, J.L., Nadeau, K., Olek, M., et al.. Preliminary results of an individually tailored psychosocial intervention for patients with advanced hepatobiliary carcinoma. *J Psychosoc Oncol*. 2007b;25(3):19-42.

Stewart A L., Greenfield S., Hays R D., et al. Functional well-being of patients with chronic conditions. Results from the medical outcomes study. *JAMA* 1989; 262: 907–913.

Stilley CS, DiMartini AF, Tarter RE,et al. Liver transplant recipients: individual, social, and environmental resources. *Prog Transplant* 2010;20(1):68-74.

Strauss E, & Dias Teixeira MC Quality of life in hepatitis C. *Liver Int* 2006;26(7):755-765.

Szeifert L, Molnar MZ, Ambrus C, et al. Symptoms of depression in kidney transplant recipients: a cross-sectional study. *Am J Kidney Dis*. 2010;55(1):132-140.

Takinella R., Kawut S M., Brown R S. Jr, et al. Health-related quality of life and survival in liver transplant candidates. *Liver Transpl* 2010;16(2):238-245.

Telles-Correia D, Barbosa A, Barroso E, & Monteiro E Psychiatric approach of liver transplant. *Acta Med Port*. 2006;19(2):165-179.

Telles-Correia, D., Barbosa, A., Barroso, E., et al. Psychiatric approach of liver transplant. *Acta Med Port*., 2006;19(2):165-179

Teixeira R, Pastacaldi S, Papatheodoridis GV., et al. Recurrent hepatitis C after liver transplantation. *Journal of Medical Virology* 2000; 61(4): 443–454.

Teixeira MC, Ribeiro Mde F, Gayotto LC. Worse quality of life in volunteer blood donors with hepatitis *C. Transfusion*. 2006;46(2):278-283.

Telles-Correia D., Barbosa A., Mega I., et al Psychiatric differences between liver transplant candidates with familial amyloid polyneuropathy and those with alcoholic liver disease. *Prog Transplant* 2008;18(2):134-139.

Telles-Correia D., Barbosa A., Mega I., et al. Mental health and quality of life in alcoholic liver disease patients after liver transplantation: a prospective controlled study. *Transplant Proc*. 2011;43(1):184-186.

Tome S, Wells JT, Said A., et al. Quality of life after liver transplantation. A systematic review. *J Hepatol*. 2008; 48(4):567-577.

Uchino B N., Cacioppo J T., & Kiecolt-Glaser J K.. The relationship between social support and physiological processes: a review with emphasis on underlying mechanisms and implications for health. *Psychol Bull* 1996;119(3):488–531.

Unal G., de Boer J B., Borsboom G J., et al. A psychometric comparison of health-related quality of life measures in chronic liver disease. *J Clin Epidemiol* 2001;54(6):587–596.

van den Berg-Emons R, Geert Kazemier G, van Ginneken B, et al. Fatigue, level of everyday physical activity and quality of life after liver transplantation *J Rehabil Med* 2006; 38: 124-129.

van den Plas S M., Hansen B E., de Boer J B., et al. Generic and disease-specific health related quality of life in non-cirrhotic, cirrhotic and transplanted liver patients: a cross-sectional study. *BMC Gastroenterol* 2003; 3: 33-46.

Varma V, Webb K., & Mirza D F. Liver transplantation for alcoholic liver disease Liver transplantation for alcoholic liver disease. *World J Gastroenterol.* 2010;16(35):4377-4393

Vazquez I., Valderrabano F., Fort I., et al. Differences in health-related quality of life between male and female hemodialysis patients. *Nefrologia* 2004; 24(2): 167–178.

Ware J E. Jr, & Sherbourne C D. The MOS 36-item short-form health survey (SF-36) I. Conceptual framework and item selection. *Med Care* 1992; 30(6): 473–483.

Ware J E .Jr, Bayliss M S., Mannocchia M., et al. Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The interventional therapy group. *Hepatology* 1999; 30(2): 550–555.

Weinrieb RM & Lucey MR Treatment of addictive behaviors in liver transplant patients *Liver Transplantation* 2007; 13, Suppl 2, S79-82.

Wiesner R, Edwards E, Freeman R, et al. United Network for Organ Sharing Liver Disease Severity Score Committee Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology. 2003;124(1):91-96.

Weissman M M., Myers J K., & Harding P S. Prevalence and psychiatric heterogeneity of alcoholism in a United States urbancommunity. *J Stud Alcohol* 1980; 41(7): 672–681.

Younossi Z M. & Guyatt G. Quality-of-life assessments and chronic liver disease. *Am J Gastroenterol* 1998; 93(7): 1037–1041.

Younossi Z M., Guyatt G., & Kiwi M. Development of a disease specific questionnaire to measure health-related quality of life in patients with chronic liver disease. *Gut* 1999; 45(2): 295–300.

Younossi Z M., Boparai N., Price L L., et al. Health-related quality of life in chronic liver disease: the impact of type and severity of disease. *Am J Gastroenterol* 2001; 96(7): 2199–2205.

Zickmund S., Ho E Y., Masuda M., et al. "They treated me like a leper". Stigmatization and the quality of life of patients with hepatitis C. *J Gen InternMed* 2003; 18(10): 835–844.

Zickmund S., Hillis S L., Barnett M J., et al. Hepatitis C virus-infected patients report communication problems with physicians. *Hepatology* 2004; 39(4): 999–1007.

Part 6

Viral Hepatitis and Liver Transplantation

9

Hepatitis C and Liver Transplantation

Miguel Jiménez Pérez, Ana Belen Sáez Gómez,
Rocío González Grande and Juan Miguel Rodrigo López
Departament of Gastroenterology and Hepatology,
Liver Transplantation Unit, University Hospital Carlos Haya, Málaga,
Spain

1. Introduction

Chronic hepatitis C virus (HCV) infection, together with chronic alcoholic liver disease, are the leading causes of hepatic cirrhosis and the development of hepatocarcinoma. Chronic HCV infection is the reason for about 50% of liver transplants in the western world; it is the second cause of liver transplants in northern Europe and and the main cause in countries such as Italy (Guillouche & Feray, 2011). In Spain, it accounts for 35% of all transplants (Registro Español de Trasplante Hepático [RETH], 2009). In fact, liver transplantation is currently the best therapeutic alternative for patients with advanced chronic liver disease due to HCV or those who develop hepatocarcinoma.

Reinfection of the graft is universal and occurs in 95% of patients transplanted due to HCV infection. This reinfection can compromise graft function and patient survival. In a few cases the histological recurrence is minimal and non progressive, though in most patients it follows a more rapid course than in immunocompetent persons, and frequently evolves to cirrhosis with graft loss. There also exists a pattern of recurrence which is very severe, but unusual (<10%) called fibrosing cholestatic hepatitis that often involves rapid graft loss (Roche & Samuel, 2010).

The elaboration of an efficient antiviral therapeutic strategy has been of paramount concern in clinical research in recent years, with questions about when, how much and at what point this treatment should be applied.

A considerable number of studies published over recent years have shown that antiviral treatment of post-transplant HCV hepatitis carried out during the late phase is the best option for improving the prognosis of these patients. Those patients who present a negative HCV serology after antiviral treatment have better survival (Picciotto et al, 2007).

The future perspectives concerning the introduction of new drugs for the treatment of chronic hepatitis C may involve therapeutic changes and, perhaps, a better prognosis for these patients.

2. Natural history of post liver transplant HCV infection

2.1 Recurrence of HCV post transplantation

The viral infection recurs in almost all cases and occurs immediately after the graft reperfusion phase. The diagnosis of viral recurrence is purely virological and is established by detection in serum of HCV RNA using polymerase chain reaction (PCR) techniques. The levels of viraemia are generally far higher than those existing before the transplant (García-Retortillo et al, 2002). The diagnosis of relapse of the hepatitis or disease in the graft, however,is based on histological findings.

Pathophysiologically, two patterns of recurrence can be distinguished: (1) a pattern of chronic HCV hepatitis similar to that seen in non-transplanted patients but with a faster course, reaching states of advanced fibrosis or cirrhosis in a shorter time (9-12 years versus 20-50 years); (2) a second pattern, called fibrosing cholestatic hepatitis, which is less common (3-5%) but very severe, and generally appears in the context of intense immunosuppression. It can present as an initial manifestation of disease relapse or, less commonly, in the context of recurrent chronic hepatitis, and is characterised by marked jaundice with cholestasis and high titres of viraemia. This form usually progresses rapidly to acute liver failure, with graft loss soon after.

Histological confirmation is necessary to establish the diagnosis of HCV recurrence, as well as to assess the degree of activity and perform a periodic follow-up of histological disease progression. This not only provides information about the prognosis, it can also establish the differential diagnosis with other complications such as rejection, biliary disease, or vascular problems (Berenguer et al., 2001a, Berenguer et al., 2003, Roche & Samuel, 2010, Samuel et al., 2006).

A new non-invasive technique, transient elastography, has recently become available that appears to correlate well with the stage of fibrosis. This technique can detect an important degree of fibrosis (F≥2) with effect from the sixth month after transplantation, and has an excellent diagnostic capacity at 12 months post-transplantation (Carrión et al, 2010a).

2.2 Evolution of the recurrence of HCV in the post-transplant liver

The histological involvement of the graft and the natural history of the recurrence both vary, with different presenting forms. Post-transplant reinfection with HCV is associated with greater aggressiveness than in immunocompetent patients (Table 1) (Berenguer et al, 2000, Berenguer et al, 2001b).

At around the fifth month after transplantation there is an acute hepatitis, which is generally asymptomatic in some 50% of patients. Its histological findings present characteristics of lobular hepatitis with varying degrees of inflammatory infiltrate in the portal space, mainly of lymphocytes and macrovesicular steatosis, similar to the histological pattern found in acute hepatitis in immunocompetent patients.

Of those patients who experience a relapse of their HCV infection after liver transplantation, 20% have histological lesions compatible with mild chronic hepatitis 5 years post-transplantation. The others experience a more important chronic evolution. The progression to hepatic cirrhosis occurs in 30% of these patients after 5 to 7 years post-transplant, and is much faster than in immunocompetent persons (Forman et al., 2002).

The progression of the fibrosis is much more accelerated in those patients who receive their transplants due to HCV infection and who have a recurrence of the disease, up to five times faster than in immunocompetent persons. Accordingly, the evolution to cirrhosis is also sooner, with the average being 10 years as opposed to 20-30 years for immunocompetent persons with chronic HCV infection (Forman et al., 2002, Firpi et al., 2009).

Once cirrhosis is reached, 40-50% of transplanted patients will experience their first decompensation within one year. Survival after this first episode of decompensation is 50% (Berenguer et al, 2001b, Firpi et al, 2009).

In immunocompetent persons, about 25% experience their first decompensation 10 years after hepatic cirrhosis is diagnosed, and of these 50% survive for five years after the decompensation episode.

	Pre-transplantation	**Post-transplantation**
Fibrosis progression	0.2 units fibrosis/year	0.3 – 0.8 units /year
Median time to cirrhosis	20-30 years	10-12 years
Decompensation after development of cirrhosis	20-25% in 10 years	50% in 3 years
Survival after decompensation	50% at 5 years	50% at 1 year

Berenguer et al, 2001b.

Table 1. Natural history of hepatitis C before and after transplantation

2.3 Factors influencing the recurrence of HCV and graft survival

The course of post-transplant hepatitis C is determined by the interaction of various factors. Certain pre-transplant factors in the recipient are associated with worse evolution, including female sex, older age, and the presence of diabetes or the metabolic syndrome (Firpi et al 2004, Gallegos-Orozco et al, 2009, Pagada et al 2009, Pérez et al 2011). Other pre-transplant factors depend on the virus, for example the genotype HCV 1b, a high pre-transplant viral load, the absence of response to antiviral therapy and coinfection with HIV have also been associated with a worse prognosis (Berenguer et al, 2005).

Other factors, related with the donor and the peri-operative period, can also affect the severity and the time to relapse of post-transplant HCV infection, such as an older donor age (>50 years), a high degree of steatosis in the donor liver, a prolonged ischaemia time, a non-heart beating donor, a living donor, preservation injury, a partial split graft, or anti-HCV positive donors, all of which have been associated with a worse evolution (Pérez-Daga et al, 2006, Machicao et al 2004;. Pine et al 2009; Humar et al 2005)

Notable among the post-transplant factors is the metabolic syndrome, present within the first 12 months in around 50% of all patients who receive a transplant due to HCV infection and which is associated with greater progression of the fibrosis. Thus, the importance of control of factors like dyslipidaemia, hypertension and, more importantly, diabetes associated with insulin resistance, all of which play a crucial role in the evolution of the recurrence of post-transplant HCV infection (Hanouneh et al., 2008, Gallegos-Orozco et al, 2009).

Recent studies appear to show that polymorphisms in the interleukin 28 B gene (*IL-28B*), in both the donor and the recipient, may influence not only the response to antiviral therapy but also the evolution of hepatitis C due to post-transplant HCV reinfection, with a worse evolution in those with the genotypes CT and TT (of the polymorphism rs12979860) as compared with the genotype CC (Charlton et al, 2011, Brocato et al, 2010).

2.3.1 Role of immunosuppression in recurrence of hepatitis C

Agreement exists that immunosuppression is significantly associated with the natural history of recurrent hepatitis C (Samuel et al, 2006). However, controversy still surrounds the role of each immunosuppressive agent on HCV replication and the evolution of hepatitis C on the graft.

Immunosuppression contributes greatly to the increased viral load that takes place in these patients during the immediate post-transplant months. The recipient's cellular immune response against the infected hepatocyte, and thus against the virus, is altered. It is almost absent in patients with fibrosing cholestatic hepatitis and severe disease recurrence (McCaughan 2004, Samonakis et al, 2005).

The role of the type of immunosuppressive regimen and its influence on the progression of recurrence are still unclear and there is no established ideal immunosuppression protocol.

The use of high-dose steroid boluses to treat episodes of acute rejection, , has been shown to be clearly prejudicial, as they condition an increase in viral replication, a more aggressive relapse and increased early post-transplant mortality (Samuel et al 2006, Berenguer et al, 2006a).

Some studies suggest the possible benefit of maintaining low-dose steroid regimens with progressive withdrawal during the first 6-12 months (Berenguer et al, 2006a). However, two recent meta-analyses found that steroid-free immunosuppression protocols are significantly better and provide benefits related with such factors as the acute graft hepatitis, HCV recurrence, cholesterol levels and the development of de novo diabetes mellitus (Sgourakis et al, 2009, Segev et al, 2008). Thus, the use of steroid-free immunosuppression protocols improves the management of metabolic complications.

Regarding the use of calcineurin inhibitors, numerous studies have compared the recurrence of HCV with the two drugs, cyclosporine and tracrolimus. However, the results are not completely conclusive, with most studies failing to find differences. Thus, it is not possible to recommend the use of a specific calcineurin inhibitor. There seems to be evidence that the use of cyclosporine during interferon treatment may be beneficial, and that tacrolimus seems to reduce episodes of acute rejection and the need for steroid boluses. A possible beneficial immunosuppressive regimen could be to start with tacrolimus and later substitute it with cyclosporine if antiviral treatment is considered necessary for HCV recurrence (Berenguer et al, 2007, Levy et al, 2006, O´Grady et al, 2007). For other immunosuppressive agents, such as mycophenolate, azathioprine, mTor inhibitors or anti-IL-2 receptor antibodies, no solid studies have yet been done from which to obtain conclusions. In summary, therefore, the only firm recommendation would be to avoid a state of over-immunosuppression, avoiding steroid boluses and full-dose triple or quadruple regimens. This could lead to improved results in these patients (Berenguer et al, 2006a).

3. HCV antiviral treatment

Although treatment is based on the administration of pegylated interferon and ribavirin, as is done in immunocompetent patients, no standardized consensus criteria exist about the ideal time to initiate such therapy, which patients should be treated or the most suitable dose or duration of the regimen. The 2010 Expert Opinion Meeting of the Italian Association for the Study of the Liver (AISF, 2010) established the following criteria for starting antiviral therapy in patients with post-transplant HCV reinfection: the presence of positive HCV-RNA serology, compatible liver histology (HCV recurrence and fibrosis stage ≥F2 (Scheuer)) and the exclusion of rejection, vascular disease or biliary obstruction, with level III evidence and grade B recommendation.

As HCV recurrence is one of the most usual causes of graft loss, it can be assumed that those patients who respond to treatment will have a better prognosis, and consequently, an improved survival. One of the main problems of antiviral therapy concerns its frequent, and sometimes severe, secondary effects, which reduce tolerability and thus efficacy. Thus, given the lack of universal methods to prevent graft reinfection, the only alternative is antiviral therapy, to be used at varying times around the transplantation.

3.1 Pre-transplant antiviral therapy

Pre-transplant therapy consists of treating patients with chronic HCV liver disease who are candidates for a liver transplant before the transplantation, in order to reduce the viral load or even make it negative, thereby avoiding later reinfection in the graft.

Studies indicate that using interferon and ribavirin up to 30% of patients manage to reach the time of transplant with negative viraemia, though a negative HCV-RNA prior to transplant does not guarantee the absence of post-transplant recurrence of infection (Charlton et alt, 1998). The main inconvenience of this regimen concerns the poor tolerance to treatment in patients with advanced cirrhosis, and the presence of more and worse side effects in relation to the evolution of the liver disease, so that it can only be used in fewer than 50% of patients. In 60% of those patients who do receive this regimen, the doses of pegylated interferon and ribavirin have to be reduced due to the high rate of undesired effects, and even occasionally stopped prematurely, which leads to the poor virological results seen. This therapeutic strategy, therefore, should be given prudently, and can be recommended for those patients on the transplant waiting list who have compensated liver disease (Child Pugh A plus hepatocarcinoma, for example), independently of the genotype and viral load, and in those patients in Child Pugh stage A-B and MELD ≤18 with a good virological response profile who have no contraindication to starting the treatment (Everson et al, 2005, Crippin et al, 2002).

3.2 Post-transplant antiviral therapy

Post liver transplant therapy can be given either shortly after the operation or some time later, when the recurrence of the hepatitis C is established.

3.2.1 Early post-transplant antiviral therapy (Pre-emptive therapy)

Early treatment is given during the first weeks after the transplant (mainly between the second and sixth weeks). The idea behind this strategy is to anticipate the infection and

eliminate the HCV before the hepatic lesion appears. Nevertheless, this early antiviral therapy has numerous limitations, such as the degree of post-transplant immunosuppression present in these patients; their clinical situation after the transplant, which affects their tolerance to treatment; their high viral load, partly related with the degree of immunosuppression, and the corresponding reduction in therapeutic success plus the high risk of episodes of rejection, which advise delaying treatment until a more suitable time from the immunological standpoint (Sheiner et al, 1998, Chalasani et al, 2005, Sugawara et al, 2004, Shergill et al, 2005).

This strategy is, however, applicable to all patients who receive a transplant due to HCV infection, including those who have no aggressive rejection episode and remain stable, with minimum hepatic lesions for whom antiviral therapy would perhaps not otherwise be indicated.

The viral response experienced, according to the various studies published, ranges from 1% to 13%. However, treatment had to be withdrawn early in 35% of the patients treated, due to adverse effects (Shergill et al, 2005). The application of this treatment regimen could perhaps be considered in those patients who receive a further transplant due to aggressive HCV infection or in coinfected (HIV) patients.

3.2.2 Late post-transplant antiviral therapy

This consists of applying treatment once the histological recurrence of the HCV is well established, with the aim of preventing rapid progression of the hepatic lesion. The period of treatment application is from 2 to 7 months after the transplant, or according to the histological lesions seen on liver biopsy. Using this strategy, the patient has a lower and better controlled degree of immunosuppression, has recovered from the surgery, and alterations present prior to the transplant have been corrected, such as anaemia, thrombocytopoenia or the nutritional status, all of which favour greater tolerance and applicability.

The rate of sustained viral response seen with this schedule is from 20% to 40% (2,21-22,25); the rate of premature interruption of treatment is around 28% and that of dose reduction 73%. The results, though, are still worse than those found in immunocompetent patients (Berenguer, 2008).

In all cases management should be personalized, and consideration given to such factors as renal function, concomitant diabetes, a prior history of rejection and genotype (Aymant et al, 2010).

3.3 Antiviral drugs and regimens

The optimal dose and duration of antiviral treatment in transplant patients are unknown, and the same regimens are usually followed as those applied to immunocompetent patients (Otón et al, 2006).

Adverse effects are the main reason for dose reduction (50%), particularly with ribavirin, and premature treatment interruption (25%). The most usual adverse effects involve haematological alterations, mainly anaemia (60-80%), neuropsychiatric disorders (around 10-15%), thyroid disorders, asthenia (60-70%) and infections (15-25%). The use of

erythropoietin and colony-stimulating factors (G-CSF) helps avoid the need for dose reduction and thus increases the possibility of reaching a sustained viral response. Though these drugs clearly permit better tolerance to antiviral treatment, no data yet exist to confirm improved efficacy. (Berenguer et al 2006b, 2008).

The development of acute cellular rejection represents another possible complication, with an incidence of around 6% (0-35%). It is related with the state of the patient's immunosuppression, the time since transplantation, the concomitant use of ribavirin and the use of pegylated interferon (more than with conventional interferon). Cases of chronic rejection (<1%) have, however, been reported in patients who achieve a viral and biochemical response. In this situation it has been attributed to improved hepatic function with the resulting change in metabolism of the immunosuppressive drugs, which could determine a reduction in their blood levels (Berenguer, 2008, Otón et al, 2006). Accordingly, close vigilance and monitoring of the immunosuppression are necessary during treatment, as well as histological study in the event of unexplained laboratory findings.

3.4 Factors predicting response to antiviral therapy

Factors predicting antiviral response in the patient who undergoes liver transplantation due to HCV infection are mostly similar to those seen in the immunocompetent patients. Factors associated with a worse response include advanced donor age, advanced fibrosis, the presence of genotype 1, a high initial viral load and the presence of the metabolic syndrome. Obtaining a rapid viral response (4 weeks after starting antiviral therapy) and an early viral response at 12 weeks of treatment predict a sustained viral response, as seen with HCV treatment in non-transplant patients (Berenguer et al, 2006; Jiménez et al, 2010).

Polymorphisms in interleukin (*IL-28B*) (chromosome 19), related with response to antiviral therapy in immunocompetent patients (Fukuhara et al, 2010), are also related to response in transplant patients. Polymorphisms in rs 1127354 (chromosome 20), which determines the activity of inosine triphosphatase, have been associated with the possibility of predicting a predisposition to the development of haemolytic anaemia in relation to ribavirin (Fellay, 2010).Another important factor to consider that is associated with a greater sustained viral response concerns treatment adherence; at least 80% compliance should be aimed for.

4. Retransplant

For those patients with recurrence of HCV who have a decompensated cirrhosis, a liver retransplant is the only curative option.The International Liver Transplantation Society Expert Panel indicates that recipients aged >55 years, donors >40 years, a bilirubin ≥10 mg/dl, creatinine clearance <40 ml/min and early recurrence of HCV-related cirrhosis after transplant are all associated with a worse prognosis after retransplant (Carrion et al, 2010b). The development of fibrosing cholestatic hepatitis also has an unfavourable prognosis after retransplant (Marti et al, 2008).

Models predicting survival after retransplantation have been validated. These include the Markmann score (Markmann et al, 1999) and the Rosen score (Rosen et al, 1999), which appear to be the most accepted and enable prediction of the prognosis in the retransplant

patient, thus improving associated survival. Generally speaking, it is recommended that a retransplant should be offered to those patients who have a likelihood of 1-year survival of at least 55%, which includes patients with a Rosen score <20.5 (Marti et al, 2008).

The cornerstone depends on reducing the number of candidates for retransplantation, identifying those patients who have accelerated recurrence and undertaking energetic measures for their management, as well as starting antiviral therapy.

5. New therapies for HCV

The advent of new drugs for the treatment of HCV infection, as well as polymerase and protease inhibitors, will considerably change the management of HCV infection due to their high antiviral power (Kwo et al, 2009, Hezode C et al, 2009). Around 50% of non-transplant patients who are difficult to treat because of the presence of factors predicting a lack of response have been shown to experience a greater sustained viral response (Aymant et, 2010). In transplanted patients, the increase in efficacy, applicability and tolerance, and the possible interactions with other drugs are as yet unknown and more studies are still required.

6. Conclusions

Despite the advances in liver transplantation, the results in patients with HCV infection are not as satisfactory as desired, due mainly to the recurrence of the primary disease and the lack of availability of an efficient prophylactic therapy. Likewise, antiviral therapy still presents important limitations, particularly its poor tolerance, which hinders its use at full doses or for a sufficient duration to achieve an adequate response. The most recommended attitude is to attempt antiviral therapy prior to the transplant, particularly for those patients with maintained liver function, in an attempt to avoid disease progression, though if this is not possible, at least reach transplantation with a negative viraemia. Strict monitoring of the progression of the fibrosis by serial biopsies and/or elastography will enable early identification of those patients who might benefit from antiviral therapy to detain the advance of the disease and thus avoid the possible need for a retransplant. Nevertheless, new therapeutic approaches are required for the treatment of hepatitis C infection that can obviate the need for liver transplantation. For this, the introduction of protease and polymerase inhibitors is opening up hope for the future treatment of this disease.

7. References

Aymant A, Kaufman M, Norah A. Terrault: Management of posttransplant hepatitis C infection. Curr Opin Organ Transplant 2010; 15: 301-309

Berenguer M, Prieto M, Rayón JM et al. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. Hepatology 2000; 32:852-858.

Berenguer M, Rayón M, Prieto M, et al. Are postransplantation protocol liver biopsies useful in the long-term? Liver Transpl 2001a; 7: 790-796.

Berenguer M, Ferrell L, Watson J et al. HCV- related fibrosis progression following liver transplantation: increase in recent years. J Hepatol 2001b; 32:673-83.

Berenguer M, Aguilera V, Prieto M, et al. Delayed onset of severe hepatitis C- related liver damage following liver transplantation: a matter of concern? Liver Transpl 2003; 9: 1152-1158.

Berenguer M. What determines the natural history of recurrent hepatitis C after liver transplantation?. J Hepatol 2005;42:448-479.

Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S et al. Significant improvement in the outcome of HCV-infected transplant recipients by avoiding rapid steroid tapering and potent induction immunosuppression. J Hepatol 2006a; 44(4):717-22.

Berenguer M, Palau A, et al. Efficacy, predictor of response, and potencial risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. Liver Transpl 2006b; 12:1067-1076.

Berenguer M, Royuela A, Zamora J. Immunosupression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. Liver Transp. 2007; 13(1):21-9.

Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. J Hepatol 2008; 49:274-87.

Brocato M, Trotter JF, Forman L et al. Genetic variation of IL-28B associated with severity of HCV recurrence following liver transplantation. AASLD 2010. Abstract poster 1158. Hepatology 2010; 52 (4 suppl): 875A.

Carrión JA, Torres F, Crespo G, et al. Liver stiffness identifies two different patterns PF fibrosis progression in patients with hepatitis C virus recurrence after liver transplantation. Hepatology 2010a; 51: 23-24.

Carrión J, Navasa M, Forns X: Retransplantation in patients with hepatitis C recurrence after liver transplantation. Journal of Hepatology 2010b; 53: 962-970.

Chalasani N, Manzarbeitia C, Ferenci P, Vogel W, Fontanal RJ et al. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. Hepatology 2005; 41: 289-298.

Charlton M, Seaberg E, Wiesner R, et al: Predictors of patient and graft survival following liver transplantation for hepatitis C. Hepatology 1998; 28: 823-830.

Charlton MR, Thompson AJ, Veldt et al. et al. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. Hepatology 2011; 53: 317-324.

Crippin JS, McCashland T, Terrault N, Sheiner P, Charlton MR. A pilot study of the tolerability and efficacy of antiviral therapy in hepatitis C virus-infected patients awaiting liver transplantation. Liver Transpl 2002; 8:350-355.

Everson GT, Trotter J, Forman L, Kugelmas M, Halprin A et al. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. Hepatology 2005; 42: 255-262.

Fellay J,Thompson AJ, Ge D, et al. ITPA gene variants protect against anemia in patients treated for chronic hepatitis C. Nature 2010; 464: 405-8.

Firpi RJ, Soldevila- Pico, et al. One year protocol liver biopsy can stratify fibrosis progressión in liver transplant recipient with recurrent hepatitis C infection. Liver Transpl 2004;10:1240-7.

Firpi RJ, Clark V, Soldevila-Pico C, Morelli G et al. The natural history of hepatitis C cirrhosis after liver transplantation. Liver Transpl 2009; 15:1063-1071.

Forman LM, Lewis JD, Berlin JA et al. The association between hepatitis C infection and survival after orthopic liver transplantation. Gastroenterology 2002, 122: 889-96.

Fukuhara T, Taketomi A, Motomura T et al. Variants in IL28B in liver recipients and donors correlate with response to peginterferon and ribavirin therapy for recurrent hepatitis C. Gastroenterology 2010; 139: 1577-1585.

Gallegos-Orozco JF; Yosephy A, Noble B et al. Natural history of post-liver transplantation hepatitis C: a review of factors that may influence its course. Liver Transpl 2009; 15:1872-1881.

García-Retortillo M, Forns X, Feliu A et al. Hepatitis C virus kinetics during and immediately after liver transplantation. Hepatology 2002; 35: 680-687.

Guillouche P, Feray, C. Systematic review: antiviral therapy of recurrent hepatitis C after liver transplantation. Aliment Pharmacol Ther 2011; 33: 163-174.

Hanouneh IA, Feldstein AE, McCullough AJ, et al. The significance of metabolic syndrome in the setting of recurrent hepatitis C after liver transplanatation. Liver Transpl 2008; 14: 1287-1293.

Hezode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009; 360: 1839-1850.

Humar A, Horn K, Kalis A, Clessing B, Payne WD, Lake J. Living donor and split-liver transplants in hepatitis C recipients: does liver regeneration increase the risk for recurrence?. Am J Transplant 2005; 5: 399-405.

Jiménez Pérez M, Sáez Gómez AB, Pérez Daga JA, Lozano Rey JM, de la Cruz Lombardo J, Rodrigo López JM. Hepatitis C virus recurrence after liver transplantation: Analysis of factors related to sustained viral response. Transplant Proc 2010; 42:666-668.

Kwo PY LE, McCone J, Schiff ER, et al. Response- guided therapy for boceprevir combination treatment; results from HCV SPRINT-1. AASLD; October 2009; Boston, MA: Hepatology; 2009.p.1035A.

Machicao VI, Bonatti H, Krishna M et al. Donor age affects fibrosis progression and graft survival after liver transplantation for hepatitis C. Transplantation 2004; 77:84-92.

Levy G, Grazi GL, Sanjuan F, Wu Y, Muhlbacher F, Samuel D et al. 12-month follow –up analysis of a multicenter, randomized, prospective trial in the novo liver transplant recipients (LIS2T) comparing cyclosporine microemulsion (C2 monitoring) and tacrolimus. Liver Transpl 2006; 12(10):1464-72.

Markmann JF, Gornbein J, Markowitz JS: A simple model to estimate survival after retransplantation of the liver. Transplantation 1999; 67: 422-430.

Marti J, Charco R, Ferrer J, et al: Optimization of liver grafts in liver retransplantation: a European single-center experience. Surgery 2008; 144:762-769.

McCaughan GW. Immunosuppression for HCV liver transplantation: enough is just enough. Transplantation 2004; 78:1413-4.

O´Grady JC, Hardy P, Burroughs AK, Elbourne D. Randomized controlled trial of tacrolimus versus microemulsifed cyclosporine (TMC) in liver transplantation: poststudy surveillance to 3 years. Am J Transplant. 2007; 7:137-41.

Otón E, Barcena R, Moreno-Planas JM, et al. Hepatitis C recurrence after liver transplantation: viral and histological response to full-dose peg-interferon and ribavirin. Am J Transpl 2006; 6: 2348-2355.

Pagada M, Dasarathy S, Eghtesad B, McCullough AJ. Posttransplant metabolic syndrome. Liver Transpl 2009; 15: 1662-1670.

Pérez-Daga JA, Santoyo J, Suárez MA et al. Influence of degree of hepatic steatosis on graft function and postoperative complications of liver transplantation. Transplant Proc 2006;38, 2468-2470.

Pérez MJ, García DM; Taybi BJ, Daga JAP, Rey JML, Grande RG, Lombardo JdlC, & López JMR. Cardiovascular risk factors after liver transplantation: Analysis of related factors. Transplant Proc 2011; 43,739-741.

Pine JK, Aldouri A, Young AL et al. Liver Transplantation following donation after cardiac death: an analysis using matched pairs. Liver Transpl 2009;15:1072-1082.

Picciotto FP, Tritto G, Lanza AG et al. Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. J Hepatol 2007; 46: 459-65.

Practice guidelines for the treatment of hepatitis C; Recommendations from an AISF/SIMIT/SIMAST/ Expert Opinion Meeting. Dig Liver Dis 42:81, 2010.

Registro Español de Trasplante Hepático. Memoria de resultados 1984-2009. http://www.ont.es/infesp/Registros/MEMORIA RETH 2009. Accessed June 2011.

Roche B, Samuel D. Hepatitis C virus: Up to the Minute. Liver Transpl 2010; 16 (suppl2): S26-S35.

Samuel D, Forns X, Berenguer M, et al. Report of the Monothematic EASL Conference on Liver Transplantation for Viral Hepatitis. J Hepatol 2006; 45: 127-143.

Samonakis DN, Triantos CK, Thalheimer U, et al. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. Liver Transpl 2005; 11:386-395

Segev DL, Sozio SM, Shin EJ et al. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. Liver Transpl 2008; 14:512-525.

Sgourakis G, Radtke A, Fouzas I et al. Corticosteroid-free immunosuppression in liver transplantation:meta-analysis and meta-regressions of outcomes.Transpl Int 2009;22:892-905.

Sheiner PA, Boros P, Klion FM et al: The efficacy of prophylactic interferon alfa -2b in preventing recurrent hepatitis C after liver transplantation. Hepatology 1998; 28:831-838.

Shergill AK, Kalili M, Straley S et al. Applicabilitiy, tolerability and efficacy of preemptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. Am J Transpl 2005; 5: 118-124.

Sugawara Y, Makuuchi M, Matsui Y et al. Preemptive therapy for hepatitis C virus after living-donor liver transplantation. Transplantation 2004; 78: 1308-1311.-Rosen HR, Madden JP, Martin P. A model to predict survival following liver retransplantation. Hepatology 1999; 29:365-370.

10

HCV-Recurrence After Liver Transplantation

Dennis Eurich, Marcus Bahra and Peter Neuhaus
Charité Campus Virchow / General, Visceral and Transplant Surgery / Berlin, Germany

1. Introduction

Hepatitis-C-virus (HCV) represents one of the most serious threats for human liver and chronic HCV-infection results in the development of liver cirrhosis and hepatocellular carcinoma (HCC). HCV is an enveloped RNA-virus belonging to the flaviviridae family. Currently six identified HCV-genotypes, different in their geographic distribution define the severity of pathogenic effect, disease course and treatment results. About 1.5% of the European population are HCV-positive. Although the natural history of HCV-infection is rather slow, highly variable disease progression may lead to a rapid loss of liver function [1, 2]. An estimated annual incidence of new infections (3-4 millions) explains 170 million HCV-positive people worldwide. 80% of all HCV-infections become chronic and fewer than 25% of HCV-positive individuals are clinically apparent presenting either in a clinically stable state with few only symptoms or with fully decompensated liver disease with a dire prognosis [1, 3, 4]. Once chronic HCV-infection is established, the rates of spontaneous viral clearance remain discouraging.

The development of liver fibrosis is the main determinant of morbidity and mortality of HCV-positive patients [1]. Fibrosis results from excessive formation of extracellular matrix (ECM). The established imbalance of fibrogenesis and fibrolysis during chronic liver damage, which leads to scarring of the liver, is accompanied by a progressive loss of liver function despite the use of antiviral or anti-inflammatory agents [1, 5]. HCV-re-infection can trigger the excess synthesis and deposition of ECM usually by activation of cytokine release [1, 5]. Activated macrophages, lymphocytes, bile duct epithelia but also endothelia and myofibroblasts are sources of fibrogenic cytokines and growth factors that can stimulate hepatic stellate cells HSCs to produce ECM-molecules leading to fibrosis during chronic liver injury [1, 5]. The most prominent fibrogenic cytokine seems to be the transforming growth factor-β1 (TGF-β1), which is released during inflammation, tissue regeneration and fibrogenesis. TGF-β1 is considered to play a pivotal role in the hepatic fibrogenesis strongly increasing the production and deposition of ECM-components [1, 6]. Fibrosis progression is influenced by a whole range of virus, host and environmental factors. Advanced age, male gender, race (black), viral co-infection (HBV), alcohol intake and genetic background seem to influence the course of the disease [7-10]. For patients with HCV-induced end-stage liver disease (ESLD) liver transplantation (LT) remains the treatment of choice according to functional (cirrhosis CHILD B-C) or neoplastic (HCC) severity of hepatic injury [11]. 30-50% of all LTs are performed due to HCV-associated ESLD thus representing one of the leading LT-indications.

Regarding the recurrence of pre-transplant diseases after LT, HCV-re-infection may represent one of the most important threats to graft and patient survival after primarily successful transplantation. Although remarkable differences in the course of HCV-infection exist between pre- and post-transplant settings, the uniform picture of liver or graft cirrhosis is similar and comparable to a certain extent.

2. HCV-recurrence after liver transplantation

HCV-recurrence after LT is one of the most important issues regarding the spectrum of current graft diseases. Despite comparable pathophysiological processes, the course of graft hepatitis is usually more progressive compared to the natural setting of HCV-infection [11-15].

2.1 Epidemiological and etiological aspects

Most of the patients either show biochemical or histological signs of inflammation, whereas 30% of all graft recipients develop graft cirrhosis within 5 years after LT, leading to an impaired patient survival and a dubious transplant success. Progressive loss of graft function may even require re-transplantation despite of consistent antiviral treatment [16].

Arbitrary in use and manifold in extent, the term "HCV-recurrence" implies the whole spectrum of graft disease such as asymptomatic infection, graft-hepatitis, fibrosis and eventually transplant cirrhosis. The uniform picture of end stage graft disease comprises scar formation and replacement of liver parenchyma by connective tissue as a result of accelerated fibrogenesis [1, 17, 18]. Clinical presentation of graft function loss is very similar to the natural setting though more rapid and progressive. Ascites, variceal bleeding, encephalopathy and jaundice are common results of graft decompensation.

Patient survival with HCV-recurrence is dramatically compromised compared to non-HCV-related transplants [15]. Several studies pointed out significantly lower survival rates in HCV-positive recipients due to accelerated fibrosis development. Survival analysis performed in a cohort of 2294 patients (Charité, Berlin, Germany since 1988) demonstrated highly significant differences (unpublished data) comparing 455 HCV-positive to 1839 HCV-unrelated transplants ($p<0.001$; fig.1).

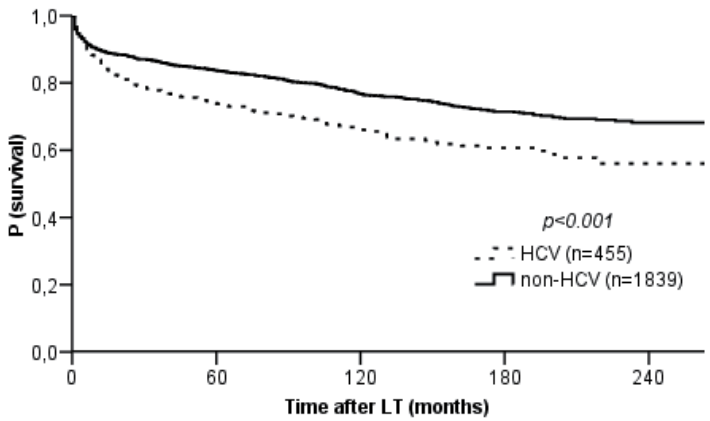


Fig. 1. Disease-related post-transplant survival

Persisting in the extrahepatic reservoir, HCV reappears as a universal post-transplant phenomenon, leading to the development of graft disease in a highly variable manner. At the moment of hepatectomy HCV-load may become even undetectable indicating the importance of the liver as major HCV-reservoir. After transplantation, HCV-redistribution arises from extrahepatic sources (lymphatic tissue) and HCV-viremia reappears within first post-transplant days [19, 20]. Molecular analysis has shown that postoperative viral strains are identical to those detected before transplantation. HCV-load literally explodes after one week achieving values of one logarithmic step higher compared to pre-transplant condition basically due to indispensable immunosuppressive medication [21]. Among currently known genetic HCV-variants (1-5), genotype-1b predominates in the post-transplant setting due to selection as the most stubborn [22].

2.2 Diagnostics

HCV-recurrence, ranging from asymptomatic viremia to rapid fibrosis progression requires an exact description and diagnostic assessment of injury extent. Clinical presentation of HCV-recurrence is frequently unspectacular, basically corresponding to HCV-infection in the natural setting. Though highly variable and unreliable, general discomfort and fatigues may appear as first symptoms of HCV-recurrence. In contrast, advanced stages may clinically result in jaundice, hemorrhage, edema, ascites, encephalopathy, infection, secondary organ failure according to the degree of functional deterioration. Therefore, standardized quantification of graft damage and disease extent must be performed, in order to identify high-risk-patients, initiate antiviral treatment and monitor further development.

2.2.1 Clinical and biochemical aspects

After successful LT, graft function is usually followed-up according to local protocols. Clinical presentation after LT is usually unremarkable unless advanced graft disease has already developed and symptoms of liver insufficiency become apparent [21]. Elevated aminotransferases (2-4-fold) are frequently observed along with normal parameters of synthesis and excretion as a biochemical expression of inflammatory activity in parenchyma. Severe HCV-recurrence may lead to variably impaired detoxification and synthesis similar to the natural course of HCV-associated liver disease [16]. However, the differentiation of HCV-associated graft hepatitis from acute cellular rejection (ACR) is frequently impossible, based on laboratory data, only. Taken a sufficient level of immunosuppression and a detectable HCV-load, HCV-recurrence seems to be probable after the exclusion of immunological, metabolic, vascular and biliary causes for biochemical abnormalities. Therefore, graft biopsy must urgently be performed as diagnostic gold standard [21].

2.2.2 Histology

As a very reliable method, histological analysis of graft tissue usually helps to determine the etiology of graft malfunction especially in combination with supportive results of other paraclinical examinations such as laboratory analysis, cholangiography and Doppler-sonography. The histological picture of HCV-re-infection usually implies a mild sinusoidal infiltration by lymphocytes and mononuclear cells resulting in a variable degree of portal

inflammation. HCV-associated inflammation may trigger an excessive synthesis of ECM-components and result in the accumulation of collagens.

The imbalance between synthesis and degradation of connective tissue defines the progression of fibrosis [1]. Once the diagnosis of HCV-related graft hepatitis is made, the indication to antiviral treatment should be evaluated in order to prevent aggravation. The development of fibrosis is not linear [21, 23]. Since the accumulation of connective tissue and pathologic alteration of histological structure are definitive endpoints of HCV- recurrence, serial biopsies and long-term follow-up are highly indispensable for the assessment of HCV-related damage (fig. 2) [21, 24, 25].

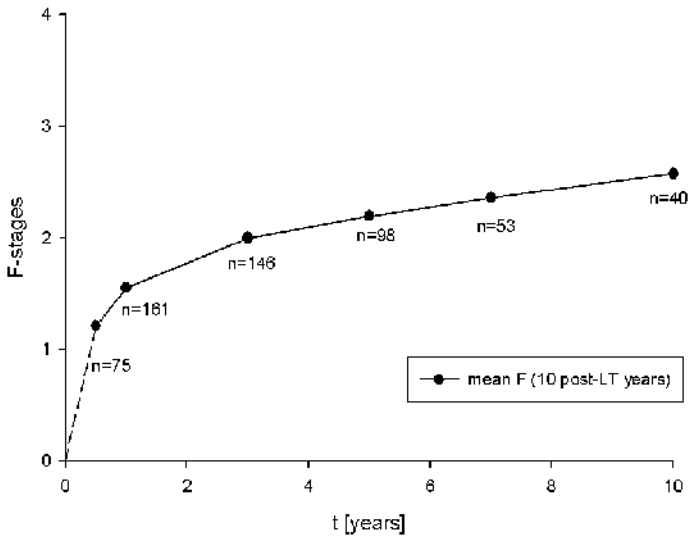


Fig. 2. Post-transplant dynamics of fibrosis

Along with several available and currently accepted scores, hepatic fibrosis is frequently characterized by a semiquantitative score, proposed by Scheuer & Desmet [26]. Using a scale (0-4), fibrosis is staged as ordinal data values (0: absent, 1: mild without septa, 2: moderate with few septa, 3: numerous septa without cirrhosis and 4: cirrhosis). Although no arithmetic procedures can be performed with ordinal data, Desmet and Scheuer-score appears to be superior in reproducibility over other semiquantitative systems in fibrosis assessment [27]. In a recent analysis according to Desmet and Scheuer, accepted time-related rates of graft cirrhosis development (30% after 5 and 50% after 10 years) could be confirmed in a representative cohort of more than 400 transplants with HCV-recurrence (Charité, Berlin, Germany). Advanced fibrosis stages (3-4) were observed in 39.2% after 5 and 47.5% after 10 post-transplant years, respectively, emphasizing the importance of a universal term definition (fig. 3).

Apart from fibrosis quantification, microscopic evaluation of inflammatory pattern helps to differentiate severe cellular rejection from HCV-infection in spite of significant biochemical similarities [28]. Low levels of immunosuppression may induce an ACR-event. Due to frequently simultaneous occurrence of acute cellular rejection and HCV-re-infection in the early post-operative period, these entities tend to be easily misdiagnosed. Classified according

to Banff-criteria, ACR may present microscopically as an accumulation of mononuclear cells (lymphocytes, eosinophil and neutrophil granulocytes) including endothelitis, portal, centrolubular inflammation and biliary alterations [28].

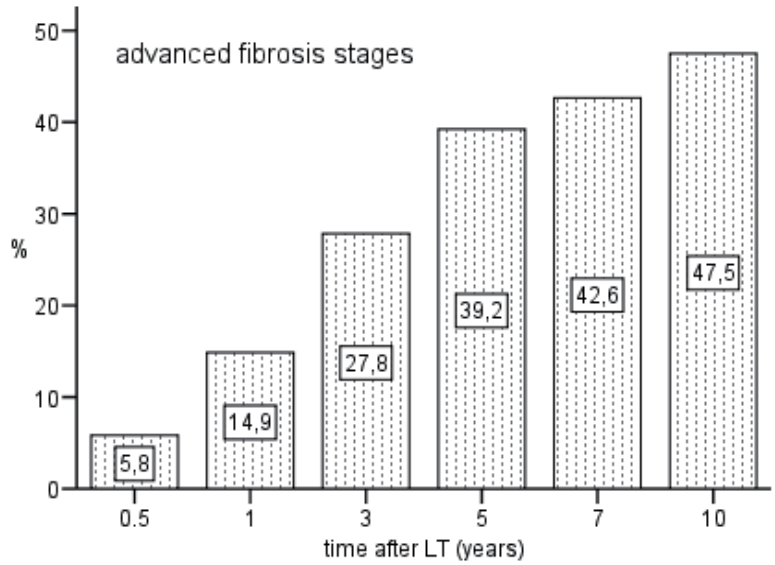


Fig. 3. Progression to advanced fibrosis stages

Currently ACR is treated by the application of intravenous steroid pulses for 3-5 days. In few cases, mono- or polyclonal antibodies are administered in case of steroid resistant rejection [21]. HCV-exacerbation is widely accepted as an inevitable side effect of ACR-treatment due to limited alternatives. Morphologically, mild forms of ACR hardly differ from HCV-re-infection due to principal differences in the pathogenesis. Regarding the danger of steroid-associated HCV-exacerbation, steroid-based treatment is recommended in moderate and severe degrees of ACR in HCV-positive transplants according to current standards. In contrast, mild ACR should be treated by the administration of higher calcineurin inhibitor doses (CNIs) and MMF-complementation as dual immunosuppressive medication. Therefore, the diagnosis must be based on the histological analysis of graft biopsy as most reliable method [21, 27].

3. Risk factors

The progression of HCV-associated graft disease is influenced by a whole range of virus, donor, recipient and environmental factors. The variety of relevant confounders exhibiting variable impact, their interaction in genetically unique living individuals resulting from a successful LT have been in the center of attention for many decades. Some risk factors for the development of graft fibrosis have been identified during the scientific attempt to unravel the mystery and to understand the substance of HCV-recurrence in detail [21, 29]. However, the majority of observations were based on low sample size. Nevertheless, the existence of virological, immunological, surgery-related and even historical confounders illustrates the complexity and variability of the issue (table 1).

Variables		
donor and surgery	age	>50 years
	warm and cold ischemia	long ischemia
	organ quality	steatosis, iron concentration
	genetics	IL28B
host	age	older age
	gender	male
	race	black
	genetics	IL28B, TGF-β1
immunology	blood group	mismatch
	histocompatibility	mismatch
	immunosuppression	type, high levels
	ACR-episodes	ACR-occurence
	ACR-treatment	corticosteroids, OKT-3
	co-infection	CMV
HCV-related	genotype	Ib
	viremia level	early peak
antiviral treatment	non-response	pre- and post-transplant

Table 1. Variables related to the severity of HCV-recurrence

3.1 Donor- and surgery-related factors

Liver graft has been quickly suspected to affect HCV-recurrence as a dominant location of pathologic events. Several studies detected a negative effect on HCV-recurrence regarding donor age, organ quality, histocompatibility matching, steatosis and iron concentration [30, 31]. Furthermore, transplant-related factors such as duration of organ harvesting, warm and cold ischemia time (transport and implantation) have been shown to contribute to HCV-related post-transplant events and processes [21]. Genetic variance of growth factors and cytokines in donor is currently suspected to impact on the progression and treatment of HCV-associated graft disease [32, 33].

3.2 Host-related factors

The development of fibrosis seems to be twice as fast in males compared to female recipients. In contrast to the controversially discussed role of recipient age, black race and male gender have been shown to negatively affect fibrosis progression [22]. Cytokines and growth factors are final effectors in the pathogenesis and may theoretically play a key role in fibrogenesis [1, 34]. Analogously to donor, genetic polymorphisms of recipient cytokines are being currently intensively investigated and seem so far to modulate the course of HCV-recurrence and antiviral treatment success. This issue will be presented below.

3.3 Viral factors

The impact of viral properties on the course of HCV-associated graft disease has been assessed by several studies, obtaining partially controversial results. Progression of graft hepatitis-C seems to be accelerated in patients with HCV-genotype-Ib, high pre-transplant HCV-RNA-load and early post-transplant peak of viremia [29, 35-37]. Interestingly, HCV-core protein has been demonstrated to promote inflammation by the release of oxidative stress and to reinforce apoptosis and steatosis. During inflammation, activated hepatic stellate cells generate ECM-components and determine the rate of graft fibrosis progression. Furthermore, CMV-coinfection seems to reinforce fibrosis progression [25, 36].

3.4 Immunologic factors

Along with histocompatibility mismatch, mode of immunosuppression, the occurrence of acute cellular rejection and its treatment have been identified as dominant confounders of HCV-related graft disease [21, 22].

3.4.1 Immunosuppressive medication

Highly complicated interaction and vulnerable balance in the immune answer to HCV is compromised by the inevitable use of immunosuppressive medication. The inappropriate T-cell mediated response to HCV-re-infection is accused to be responsible for disease progression. Stronger immunosuppressive regimen may accelerate fibrosis progression [38, 39]. Calcineurin-inhibitors (cyclosporine and tacrolimus) represent the backbone of current immunosuppressive medication and have been suspected to influence the extent of HCV-recurrence. In spite of similar pharmacological mechanisms, cyclosporine has been proposed to have a positive effect on interferon-based antiviral treatment. However, clinical significance currently remains unclear [40]. Levels of HCV-viremia seem to be the CNI-type [41].

Mycophenolate mofetil (MMF) inhibits the lymphocyte proliferation and may decrease the overall inflammatory activity in the graft. MMF was strongly suspected to have a positive impact on fibrogenesis [42]. However, the theoretically promising antiviral effect of MMF in-vitro could not be demonstrated as substantial, regarding fibrosis development. Nevertheless, dual immunosuppression based on CNIs and MMF is frequently used in patients with HCV-recurrence and is believed to exhibit a positive effect on the severity of HCV-recurrence [42].

Sirolimus as a representative of mTOR-inhibitors seems to decelerate fibrosis progression in graft re-infection by blocking post-receptor signal transduction and interleukin-2-dependant proliferation. Although, no definite statement can currently be made, sirolimus may represent a reasonable therapeutic option [43, 44].

3.4.2 Acute cellular rejection

The occurrence of acute cellular rejection (ACR), its severity and frequency have been reported to aggravate the course of HCV-related graft disease [16]. Administration of corticosteroid pulses and antibodies (OKT-3) in case of steroid-resistant rejection as ACR-therapy are associated with a significant elevation of viral load and accelerated fibrosis

development [21, 22]. The extent of immunosuppression, CNI-type and previous episodes of rejection are widely considered to affect the incidence of ACR. Furthermore, individually different genetic background of ACR-mediating cytokines might be involved in the pathogenesis [45]. Furthermore, individually different genetic background of ACR-mediating cytokines might be involved in the pathogenesis [45]. Mannose-binding-lectin-2 (MBL-2) plays an important role in the innate immune system acting as opsonine by activation antibody-independent pathway of the complement system [46, 47]. Polymorphisms of MBL-2-gene (rs7096206; G/C) have been shown to affect the occurrence of ACR in a homogenous cohort of HCV-re-infected patients (Eurich et al).

3.5 Antiviral therapy

Antiviral therapy is the cornerstone of graft cirrhosis prevention in HCV-infected recipients. The clinical and histological course of hepatitis-C is inseparably associated with antiviral treatment strategies. Recent introduction of new formulations of interferons (IFN) such as pegylated interferons (PEG-IFN) in the treatment of HCV-infection before and after LT revealed promising results. Application of pegylated interferon ⟨-2a and ribavirin (RBV) provide a sustained virologic response (SVR) in 40-50% of all treated cases with HCV-genotype 1 and in 80% with genotypes 2 or 3. In post-transplant setting, the success of antiviral therapy is significantly lower, and only a maximum of 30-40% of all patients achieve SVR [21, 39]. Some evidence exists that IFN/RBV-treatment may prevent graft cirrhosis even in unsuccessfully treated patients. Interestingly, fibrosis progression may occur in spite of successful IFN-based antiviral treatment [48]. Hence, this issue remains controversial. Moreover, immunologically active IFN may trigger rejection (5-6%) and induce chronic rejection processes during the antiviral treatment [12, 49].

3.6 Genetic diversity

HCV-re-infection can trigger the excess synthesis and deposition of ECM usually by activation of cytokine release. Activated macrophages, lymphocytes, bile duct epithelia but also endothelia and myofibroblasts are sources of fibrogenic cytokines and growth factors that can stimulate hepatic stellate cells HSCs to produce ECM-molecules leading to fibrosis during chronic liver injury [50, 51]. Genetic polymorphisms of enzymatic systems, cytokines and growth factors which are involved in the process of immunomodulation, inflammation, ECM-turnover and anti-oxidative stress defense, may explain the widely different individual extent of HCV-induced graft damage [52-54]. Highly variable rates of functional impairment defined by inflammation, tissue remodeling but also antiviral capabilities and antiviral therapy response suggest the existence of endogenous risk compounds both in natural and post-transplant settings of the disease [33]. The maximal capacity to produce different levels of cytokines in response to noxious stimulation has been shown to be under genetic control and differs among liver graft recipients [32, 55]. Genetically different backgrounds in transplant population, consisting of donor and recipient, may differently contribute to disease development. Although the exact mechanism is not yet understood in detail both, donor and recipient genetics may interact. The expression of disease-related effectors may be individual, time and tissue dependant [56]. Therefore, the interaction between two different individual backgrounds may theoretically influence post-transplant processes in the graft and be therefore pathogenetically relevant [57].

3.6.1 Genetic variants in donor

The ability to produce different levels of cytokines in response to stimulation is suspected to be gene-associated [58]. Liver graft is usually colonized by recipient cell populations such as endothelia and lymphatic tissue thus theoretically forming a functional chimerism of donor and recipient regarding biochemical processes [56, 59]. Furthermore, the impact of genetic differences in donor and recipient may vary according to the duration of post-transplant follow-up [57]. Results of intensive investigations demonstrated that donor polymorphisms of IL-28B-gene may partially predict the outcome of antiviral treatment [60].

3.6.2 Genetic variants in recipient

Genetic polymorphisms of enzymatic systems, cytokines and growth factors which are involved in the process of immunomodulation, inflammation, ECM-turnover and anti-oxidative stress defense may explain the widely different individual extent of HCV-induced graft damage [61].

3.6.2.1 Transforming growth factor-β1 (TGF-β1)

As a multifunctional polypeptide with fibrogenic, an anti-inflammatory and anti-proliferative property, TGF-β1 is considered to play a pivotal role in the hepatic fibrogenesis strongly upregulating the production and deposition of ECM-components [10, 17, 62]. Similarly to the natural setting of HCV-infection, functionally relevant polymorphisms of TGF-β1 at codon 25 are associated with the rapid development of HCV-induced graft fibrosis.

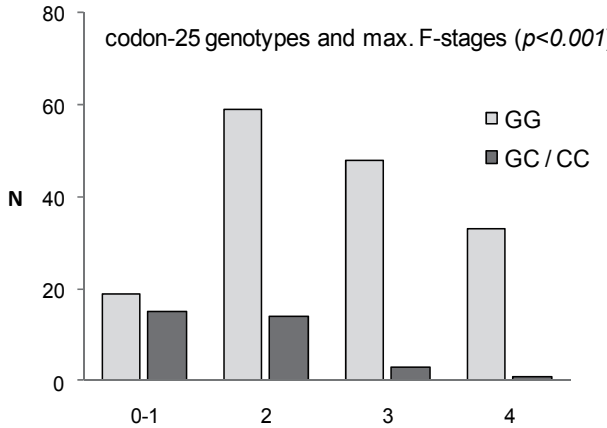


Fig. 4. Genotype distribution (TGF-β1) within maximal fibrosis stages

C-allele of the TGF-β1-gene (codon 25) has been identified as marker for graft fibrosis development and was observed significantly less frequently in advanced fibrosis stages compared to lower ones (fig. 4; p=0.001) [63].

3.6.2.2 Interleukine-28B (IL-28B)

According to numerous studies, genetic variants of IL-28B seem to be significantly involved in the pathogenesis of HCV-related graft inflammation and antiviral therapy response [60,

64, 65]. IL-28B-gene encodes an antiviral protein - IFN-λ with antiviral properties in response to IFN-α, and is upregulated by peripheral blood mononuclear cells and hepatocytes during HCV-infection [40, 66-68]. Recently, a significant association of IL-28B-genotype distribution was observed with the median grade of inflammation ($p<0.001$), mean levels of aminotransferases (ALT: $p=0.001$, AST: $p=0.003$; fig.5), median pre-treatment viremia level within 1 year after LT ($p=0.046$) and interferon-based antiviral therapy failure ($p<0.001$). IL-28B polymorphism (rs8099917) seems to influence the degree of graft inflammation at biochemical and histological levels [64].

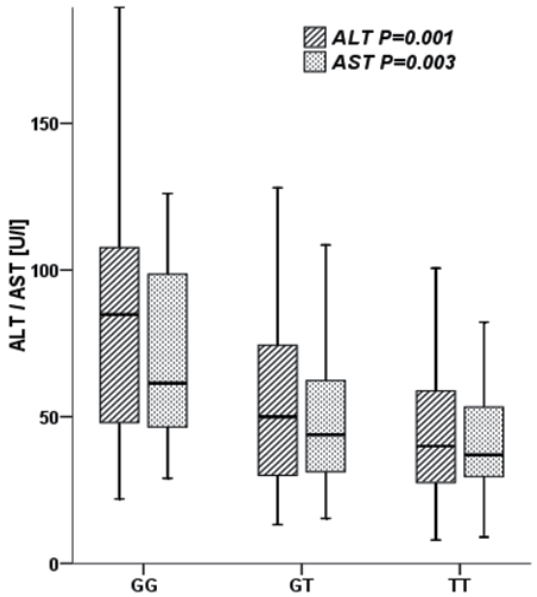


Fig. 5. Levels of aminotransferases according to IL-28B-genotypes

Among successfully treated patients G-allele was significantly less frequent and GG-genotype was not present at all [64]. G-allele might serve as a marker for graft inflammation and as predictor for unfavorable antiviral therapy outcome in HCV-re-infected LT-population [60, 64].

The identification of non-invasive inflammation and fibrosis markers might help to differentiate re-infected patients with stable graft function without significant inflammation or fibrosis progression from patients at risk for short-term graft damage and define the indication for antiviral treatment.

3.7 Organ allocation system

Methodological changes in the principals of graft allocation may affect the course of HCV-associated graft disease. Since December 2006 liver graft allocation has been carried out according to MELD-score, which primarily assesses the impairment of liver function and secondarily reflects the extent of kidney damage. Some evidence arises about the negative impact of the current allocation system regarding survival, rate of re-transplantation, fibrosis progression and success of antiviral treatment. Therefore, the MELD-score, as an

apparently reasonable attempt to improve the procedure of organ distribution, must undergo a critical analysis in future.

4. Antiviral therapy

The indication for HCV-treatment after LT depends on the individual clinical and biochemical condition after a definitive stabilization of the graft function, which is usually achieved within six post-operative months [69]. Analogously to the natural setting, therapy regimen is based on the administration of pegylated interferon-α2a (Peg-IFN-α2a) and ribavirin (RBV) for 12-18 months. Interferons are natural proteins with antiviral, antiproliferative and immunomodulatory features. They are responsible for the intracellular RNA-degradation and the inhibition of RNA-translation. Among all known interferons (IFN-α, IFN-β, Peg-IFN-α2b), pegylated IFN-α2a seems to have the highest antiviral potency, though similar to Peg-IFN-α2b, demonstrating superior treatment results in patients with HCV-re-infection [19, 70-75]. Attenuation of renal clearance and improved biochemical stability may explain prolonged half-time and therapeutical advantages observed. Ribavirin inhibits inosinmonophosphate-dehydrogenase and reduces the intracellular concentration of guanosin. RBV-monotherapy may significantly decrease the HCV-load (1 log step).

For a better comparability of results, the treatment outcome has been divided into widely accepted terms: end of treatment response (ETR: HCV-negativity at the end of treatment), sustained virologic response (SVR: HCV-RNA-negativity 6 months after therapy completion), relapse (detectable HCV-RNA after therapy completion), breakthrough (detectable HCV-RNA during treatment after initial therapy response) and non-response (persistent HCV-load under treatment). SVR before transplantation is observed in 50% of all treated cases with HCV-genotype-1 and in 80% with genotypes 2-3 [74]. As long as IFN-α remains the backbone of antiviral therapy, the identification of predictors for the therapy outcome is crucial. 50% of graft recipients survive 10 years without any significant fibrosis progression and in some cases even without antiviral treatment (own data, Charité, Berlin, Berlin). Therefore, unnecessary exposition to adverse therapy events in non-responders could be avoided by improved predictability. HCV-genotype and early viral kinetics are predominantly considered to be important for therapy performance and its potential modification [69, 76]. Apart from the identified factors (high levels of immunosuppression, corticosteroid-based ACR-treatment and HCV-genotype-1b), unfavorable host- and donor-related genetic confounders are suspected to exert a negative influence on the course and success of antiviral treatment [22, 69, 77]. According to several studies, genetic variants of IL-28B are strongly considered to affect the antiviral therapy results [64, 78, 79]. Along with other accepted predictive parameters, IL-28B-genotyping may be a useful diagnostic instrument for the indication and performance of antiviral therapy before and after LT [52, 60, 78, 80].

4.1 Current treatment standards

The aim of antiviral treatment implies the reduction or complete HCV-clearance as responsible noxious agent in the development of HCV-associated graft disease. In spite of low success rates, HCV-infection is treated by subcutaneous administration of 180µg of Peg-IFN-α2a once a week and oral intake of RBV up to three times per day [22, 35, 81]. The cumulative duration of antiviral treatment comprises 12-18 months.

The major advantage of peg-interferon α2a and ribavirin-therapy consists in the summation of the antiviral potency. However, bone marrow toxicity, psychiatric disorders and rejection frequently limit therapy success. Due to high rates of predominantly hematological adverse events (anemia, leucopenia) and a significant risk of graft decompensation, antiviral treatment should be performed under a close patient monitoring. Myelotoxic effect of IFN results in the suppression of granulocytes and thrombocytes, whereas RBV induces anemia [77, 82]. Therapeutical support of hematological and mental disorders may frequently be necessary. Frequently observed anemia may result in the reduction of the RBV-dose or in the administration of erythropoietin. Leucopenia may require a dose reduction of IFN or administration of granulocyte stimulating growth factors. Mood disorders may be handled by antidepressants or social support. While performing a strenuous, expensive and frequently futile effort of virus elimination, 60-70% of transplants with HCV-graft hepatitis are treated without sustained virologic response [72].

4.2 Alternative strategies

Demonstrating poor treatment results and serious adverse events of IFN-based therapy, reasonable alternative treatment options are needed to complement or to replace the standard therapeutical approach. Silibinin experiences its renaissance in the treatment of chronic liver diseases although it has been known for years as hepatoprotective herbal and used by patients suffering from chronic liver disease of various causes [83-85]. In contrast to unclear antiviral efficacy of oral silibinin treatment, according to the results of published studies, a significant antiviral effect could be observed after intravenous administration of silibinin [84, 86-90]. Next to three other flavonolignan isomers (isosilybin, sylidianin and silychristin) silibinin is the most pharmacologically active component of silybum marianum and has been shown to improve biochemical markers of liver function and symptoms in a range of conditions including acute and chronic viral hepatitis, alcoholic liver disease and drug-related liver injury [83, 91-94]. As a potent free radical scavenger, silibinin has been shown to reduce the initiation of pathogenetically important lipid peroxidation and to lower HCV-RNA-load [92, 95, 96]. The substance exerts a direct antiviral effect on the HCV-replicon system inhibiting RNA-dependant HCV-polymerase. Furthermore, silibinin has been shown to affect the major actors in scar tissue formation suppressing TGF-β1-synthesis and HSC-activity [92, 97, 98].

The evidence of antiviral efficacy of silibinin in patients with HCV-related liver damage is limited due to a paucity of representative clinical trials, in spite of its popularity among patients suffering from various chronic liver diseases [92, 99]. Recent reports have demonstrated significant antiviral properties of intravenously administered silibinin in IFN-non-responders in the natural setting of HCV-infection, convincingly suggesting a dose- and treatment duration-dependent antiviral effect [92]. Several observations based on unfortunately low sample size cohorts, demonstrated that intravenous administration of silibinin after LT may be an effective therapeutic approach in the treatment of HCV-re-infection, even in non-responders to IFN-based therapy [99, 100]. Rapid normalization of aminotransferases and an exponential decline of HCV-load during silibinin treatment have been reported (fig. 6) [100]. Moreover, sustained viral elimination may apparently occur even after treatment with silibinin, only. Interestingly, in patients treated directly after LT, no significant antiviral effect could be observed, probably due to initially high levels of

immunosuppression (unpublished data, Charité, Berlin). Although no large scale studies have been carried out yet, silibinin might be an effective therapeutic approach in the treatment of HCV-re-infection and should be evaluated further. Any kind of antiviral supplementation to current therapy regimen should be welcome in the age of donor organ shortage to strengthen current antiviral therapy regimen and to avoid graft loss with subsequent re-transplantation.

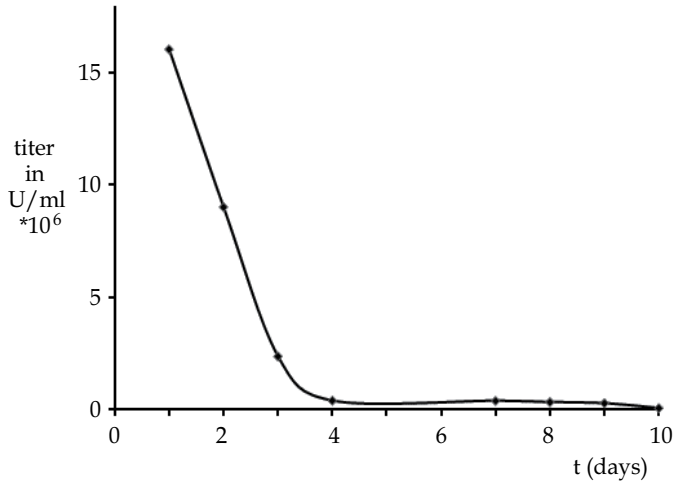


Fig. 6. HCV-load during silibinin treatment after LT

Randomized, placebo-controlled clinical trials with a representative number of graft recipients suffering from HCV-re-infection are needed to definitely answer the question of the efficacy of intravenous silybium marianum solution and its antiviral potential. As long as IFN-α remains the backbone of antiviral therapy, the identification of predictors for therapy outcome is crucial. Regarding potential therapy success, frequent severe adverse events of IFN-RBV-therapy are commonly accepted. An unnecessary exposition to adverse therapy events in non-responders could thus be avoided by an improved predictability.

4.3 Re-transplantation

Re-transplantation is frequently required (up to 10%) in patients with HCV-related graft cirrhosis as the only option of treatment [12, 15]. Significantly decreased survival rates after re-transplantation compared to first LT are in the center of scientific attention and long-term outcome has been reported to be inferior to other indications [3, 11, 39]. Physical condition, MELD-score, technical obstacles and antiviral therapy response are currently accepted as predictors for graft and patient survival after re-LT [39, 101].

5. Summary and future prospective

As long as HCV-recurrence exists as currently inevitable phenomenon, HCV will continue to endanger the success of LT. While performing a strenuous, expensive and frequently futile effort of interferon-based virus elimination, 60-70% of graft recipients are still treated without achieving SVR, thus leaving overall poor results. The natural course of HCV-

recurrence is not uniform and is influenced by a whole variety of factors. Therefore, the identification of non-invasive inflammation and fibrosis markers might help to differentiate re-infected patients with stable graft function without significant inflammation or fibrosis progression from patients at risk for short-term graft damage and define the indication for antiviral treatment. Moreover, it is indispensable to identify patients who are likely to respond to IFN-based antiviral treatment. The individual variability of disease development may be divided roughly into three different patient groups (A: no fibrosis progression, B: treatable progression and C: untreatable progression of graft dysfunction). The indication for antiviral therapy in group B seems to be more urgent in contrast to group A, which may stay stable for several years regarding their graft function, thus avoiding possibly unnecessary exposition to pharmaceutical side effects. Group-C- patients, as high risk patients may require treatment adjustment regarding its intensity, duration and mode including alternative therapeutical strategies.

Although HCV-recurrence represents an inevitable post-transplant phenomenon, the development of advanced fibrosis stages is highly variable or even individual. Furthermore, the outcome of antiviral treatment seems to depend on multiple factors, too. In spite of recent advances in HCV-graft-hepatitis treatment, prediction of therapy response and risk stratification in graft fibrosis progression, a further extensive investigation is still required. Improvement in prevention, prediction of disease course, individualized antiviral treatment and alternative antiviral medication may help to increase survival rates after LT for an HCV-associated graft disease.

6. References

[1] Schuppan D, Krebs A, Bauer M *et al.* Hepatitis C and liver fibrosis. Cell Death Differ 2003 Jan;10 Suppl 1:S59-67.

[2] Rossol S. [Chronic HCV infections. A model disease for therapy, economics and social medical aspects]. Gesundheitswesen 2007 Mar;69(3):146-150.

[3] Burra P. Hepatitis C. Semin Liver Dis 2009 Feb;29(1):53-65.

[4] Thomas DL, Astemborski J, Rai RM et al. The natural history of hepatitis C virusinfection: host, viral, and environmental factors. JAMA 2000 Jul 26;284(4):450-456.

[5] Friedman SL. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. Nat Clin Pract Gastroenterol Hepatol 2004 Dec;1(2):98-105.

[6] Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000 Jan 28;275(4):2247-2250.

[7] Svirtlih N, Jevtovic D, Simonovic J *et al.* Older age at the time of liver biopsy is the important risk factor for advanced fibrosis in patients with chronic hepatitis C. Hepatogastroenterology 2007 Dec;54(80):2324-2327.

[8] Sterling RK, Stravitz RT, Luketic VA *et al.* A comparison of the spectrum of chronic hepatitis C virus between Caucasians and African Americans. Clin Gastroenterol Hepatol 2004 Jun;2(6):469-473.

[9] Gitto S, Micco L, Conti F *et al.* Alcohol and viral hepatitis: a mini-review. Dig Liver Dis 2009 Jan;41(1):67-70.

[10] Powell EE, Edwards-Smith CJ, Hay JL *et al.* Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 2000 Apr;31(4):828-833.

[11] Neumann UP, Berg T, Bahra M *et al.* Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow-up. Transplantation 2004 Jan 27;77(2):226-231.
[12] Berenguer M. Recurrent allograft disease: viral hepatitis. Acta Gastroenterol Belg 2005 Jul- Sep;68(3):337-346.
[13] Berenguer M, Prieto M, Palau A *et al.* Severe recurrent hepatitis C after liver retransplantation for hepatitis C virus-related graft cirrhosis. Liver Transpl 2003 Mar;9(3):228-235.
[14] Prieto M, Berenguer M, Rimola A *et al.* Liver transplantation in hepatitis C. A Spanish multi-centre experience. Eur J Gastroenterol Hepatol 1998 Sep;10(9):771-776.
[15] Forman LM, Lewis JD, Berlin JA *et al.* The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002 Apr;122(4):889-896.
[16] Berenguer M. Natural history of recurrent hepatitis C. Liver Transpl 2002 Oct;8(10 Suppl 1):S14-18.
[17] Friedman SL. Stellate cells: a moving target in hepatic fibrogenesis. Hepatology 2004 Nov;40(5):1041-1043.
[18] Friedman SL. Molecular mechanisms of hepatic fibrosis and principles of therapy. J Gastroenterol 1997 Jun;32(3):424-430.
[19] Neumann UP, Neuhaus P. Course and treatment of recurrent Hepatitis C after liver transplantation. Minerva Gastroenterol Dietol 2004 Mar;50(1):61-66.
[20] Garcia-Retortillo M, Forns X, Feliu A *et al.* Hepatitis C virus kinetics during and immediately after liver transplantation. Hepatology 2002 Mar;35(3):680-687.
[21] Neumann UP, Berg T, Bahra M *et al.* Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. J Hepatol 2004 Nov;41(5):830-836.
[22] Roche B, Sebagh M, Canfora ML *et al.* Hepatitis C virus therapy in liver transplant recipients: response predictors, effect on fibrosis progression, and importance of the initial stage of fibrosis. Liver Transpl 2008 Dec;14(12):1766-1777.
[23] Schmeding M, Neumann UP, Puhl G et al. Hepatitis C recurrence and fibrosis progression are not increased after living donor liver transplantation: a single center study of 289 patients. Liver Transpl 2007 May;13(5):687-692.
[24] Desmet VJ, Gerber M, Hoofnagle JH *et al.* Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994 Jun;19(6):1513-1520.
[25] Roche B, Samuel D. Risk factors for hepatitis C recurrence after liver transplantation. J Viral Hepat 2007 Nov;14 Suppl 1:89-96.
[26] Shuhart MC, Bronner MP, Gretch DR et al. Histological and clinical outcome after liver transplantation for hepatitis C. Hepatology 1997 Dec;26(6):1646-1652.
[27] Goldin RD, Goldin JG, Burt AD *et al.* Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. J Hepatol 1996 Nov;25(5):649-654.
[28] Anonymous. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997 Mar;25(3):658-663.
[29] Zimmermann T, Otto C, Hoppe-Lotichius M *et al.* Risk factors in patients with rapid recurrent hepatitis C virus-related cirrhosis within 1 year after liver transplantation. Transplant Proc 2009 Jul- Aug;41(6):2549-2556.

[30] Lake JR, Shorr JS, Steffen BJ *et al.* Differential effects of donor age in liver transplant recipients infected with hepatitis B, hepatitis C and without viral hepatitis. Am J Transplant 2005 Mar;5(3):549- 557.

[31] Bahra M, Jacob D, Neumann UP *et al.* Influence of donor histology on outcome in patients undergoing transplantation for hepatitis C. Transplantation 2007 Jul 27;84(2):144-148.

[32] Barrett S, Collins M, Kenny C *et al.* Polymorphisms in tumour necrosis factor-alpha, transforming growth factor-beta, interleukin-10, interleukin-6, interferon gamma, and outcome of hepatitis C virus infection. J Med Virol 2003 Oct;71(2):212-218.

[33] Ben-Ari Z. Role of cytokine gene polymorphism in recurrent HCV infection after liver transplantation. Liver Transpl 2006 Nov;12(11):1723-1724.

[34] Ben-Ari Z, Mor E, Papo O *et al.* Cytokine gene polymorphisms in patients infected with hepatitis B virus. Am J Gastroenterol 2003 Jan;98(1):144-150.

[35] Shackel NA, Jamias J, Rahman W *et al.* Early high peak hepatitis C viral load levels independently predict hepatitis C-related liver failure post-liver transplantation. Liver Transpl 2009 Jul;15(7):709- 718.

[36] Gayowski T, Singh N, Marino IR *et al.* Hepatitis C virus genotypes in liver transplant recipients: impact on posttransplant recurrence, infections, response to interferon alpha therapy and outcome. Transplantation 1997 Aug 15;64(3):422-426.

[37] Prieto M, Berenguer M, Rayon JM *et al.* High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 1999 Jan;29(1):250-256.

[38] Lake JR. The role of immunosuppression in recurrence of hepatitis C. Liver Transpl 2003 Nov;9(11):S63-66.

[39] Bahra M, Neumann UP, Jacob D *et al.* Outcome after liver re-transplantation in patients with recurrent chronic hepatitis C. Transpl Int 2007 Sep;20(9):771-778.

[40] Marcello T, Grakoui A, Barba-Spaeth G et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology 2006 Dec;131(6):1887-1898.

[41] Zervos XA, Weppler D, Fragulidis GP *et al.* Comparison of tacrolimus with neoral asprimary immunosuppression in hepatitis C patients after liver transplantation. Transplant Proc 1998 Jun;30(4):1405-1406.

[42] Bahra M, Neumann UI, Jacob D et al. MMF and calcineurin taper in recurrent hepatitis C after liver transplantation: impact on histological course. Am J Transplant 2005 Feb;5(2):406-411.

[43] Kniepeiss D, Iberer F, Grasser B et al. Sirolimus and mycophenolate mofetil after liver transplantation. Transpl Int 2003 Jul;16(7):504-509.

[44] Asthana S, Toso C, Meeberg G et al. The impact of sirolimus on hepatitis C recurrence after liver transplantation. Can J Gastroenterol Jan;25(1):28-34.

[45] Fiore CE, Pennisi P, Tamborino C. YKL-40 and graft rejection. Am J Med 2000 Jun 1;108(8):688- 689.

[46] Eurich D, Boas-Knoop S, Morawietz L et al. Association of mannose-binding lectin-2 gene polymorphism with the development of hepatitis C-induced hepatocellular carcinoma. Liver Int Aug;31(7):1006-1012.

[47] Fildes JE, Shaw SM, Walker AH et al. Mannose-binding lectin deficiency offers protection from acute graft rejection after heart transplantation. J Heart Lung Transplant 2008 Dec;27(12):1353-1356.
[48] Cicinnati VR, Iacob S, Klein CG et al. Ribavirin with either standard or pegylated interferon to treat recurrent hepatitis C after liver transplantation. Aliment Pharmacol Ther 2007 Jul 15;26(2):291-303.
[49] Xirouchakis E, Triantos C, Manousou P *et al.* Pegylated-interferon and ribavirin in liver transplant candidates and recipients with HCV cirrhosis: systematic review and meta-analysis of prospective controlled studies. J Viral Hepat 2008 Oct;15(10):699-709.
[50] Cisneros L, Londono MC, Blasco C *et al.* Hepatic stellate cell activation in liver transplant patients with hepatitis C recurrence and in non-transplanted patients with chronic hepatitis C. Liver Transpl 2007 Jul;13(7):1017-1027.
[51] Friedman SL. Hepatic stellate cells. Prog Liver Dis 1996;14:101-130.
[52] Iadonato SP, Katze MG. Genomics: Hepatitis C virus gets personal. Nature 2009 Sep 17;461(7262):357-358.
[53] Bataller R. [Genetic polymorphisms and liver diseases]. Gastroenterol Hepatol 2003 May;26(5):307-309.
[54] Madsen HO, Garred P, Thiel S et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995 Sep 15;155(6):3013-3020.
[55] Mas VR, Fisher RA, Maluf DG et al. Polymorphisms in cytokines and growth factor genes and their association with acute rejection and recurrence of hepatitis C virus disease in liver transplantation. Clin Genet 2004 Mar;65(3):191-201.
[56] Sommereyns C, Paul S, Staeheli P *et al.* IFN-lambda (IFN-lambda) is expressed in a tissue- dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathog 2008 Mar;4(3):e1000017.
[57] Hove WR, van Hoek B, Bajema IM et al. Extensive chimerism in liver transplants: vascular endothelium, bile duct epithelium, and hepatocytes. Liver Transpl 2003 Jun;9(6):552-556.
[58] Cervera C, Balderramo D, Suarez B et al. Donor mannose-binding lectin gene polymorphisms influence the outcome of liver transplantation. Liver Transpl 2009 Oct;15(10):1217-1224.
[59] Moroso V, Metselaar HJ, Mancham S et al. Liver grafts contain a unique subset of natural killer cells that are transferred into the recipient after liver transplantation. Liver Transpl Jul;16(7):895-908.
[60] Charlton MR, Thompson A, Veldt BJ et al. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. Hepatology Jan;53(1):317-324.
[61] Ben-Ari Z, Pappo O, Druzd T *et al.* Role of cytokine gene polymorphism and hepatic transforming growth factor beta1 expression in recurrent hepatitis C after liver transplantation. Cytokine 2004 Jul 7;27(1):7-14.
[62] Bissell DM. Chronic liver injury, TGF-beta, and cancer. Exp Mol Med 2001 Dec 31;33(4):179-190.

[63] Eurich D, Bahra M, Boas-Knoop S et al. Transforming growth factor beta1 polymorphisms and progression of graft fibrosis after liver transplantation for hepatitis C virus--induced liver disease. Liver Transpl Mar;17(3):279-288.

[64] Eurich D, Boas-Knoop S, Ruehl M et al. Relationship between the interleukin-28b gene polymorphism and the histological severity of hepatitis C virus-induced graft inflammation and the response to antiviral therapy after liver transplantation. Liver Transpl Mar;17(3):289-298.

[65] Rauch A, Kutalik Z, Descombes P et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology Apr;138(4):1338-1345, 1345 e1331-1337.

[66] Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol 2005 Mar;79(6):3851-3854.

[67] O'Brien TR. Interferon-alfa, interferon-lambda and hepatitis C. Nat Genet 2009 Oct;41(10):1048-1050.

[68] Sheppard P, Kindsvogel W, Xu W et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 2003 Jan;4(1):63-68.

[69] Cescon M, Grazi GL, Cucchetti A *et al.* Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. Liver Transpl 2009 Jul;15(7):782-789.

[70] Brady DE, Torres DM, An JW *et al.* Induction pegylated interferon alfa-2b in combination with ribavirin in patients with genotypes 1 and 4 chronic hepatitis C: a prospective, randomized, multicenter, open-label study. Clin Gastroenterol Hepatol Jan;8(1):66-71 e61.

[71] Burra P, Targhetta S, Pevere S et al. Antiviral therapy for hepatitis C virus recurrence following liver transplantation: long-term results from a single center experience. Transplant Proc 2006 May;38(4):1127-1130.

[72] Everson GT, Kulig CC. Antiviral therapy for hepatitis C in the setting of liver transplantation. Curr Treat Options Gastroenterol 2006 Dec;9(6):520-529.

[73] Neumann U, Puhl G, Bahra M *et al.* Treatment of patients with recurrent hepatitis C after liver transplantation with peginterferon alfa-2B plus ribavirin. Transplantation 2006 Jul 15;82(1):43-47.

[74] Rodriguez-Luna H, Khatib A, Sharma P *et al.* Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. Transplantation 2004 Jan 27;77(2):190-194.

[75] Schmitz V, Kiessling A, Bahra M *et al.* Peginterferon alfa-2b plus ribavirin for the treatment of hepatitis C recurrence following combined liver and kidney transplantation. Ann Transplant 2007;12(3):22-27.

[76] Terrault NA, Berenguer M. Treating hepatitis C infection in liver transplant recipients.Liver Transpl 2006 Aug;12(8):1192-1204.

[77] Bahra M, Neumann UP, Jacob D *et al.* Fibrosis progression in hepatitis C positive liver recipients after sustained virologic response to antiviral combination therapy (interferon-ribavirin therapy). Transplantation 2007 Feb 15;83(3):351-353.

[78] Ge D, Fellay J, Thompson AJ et al. Genetic variation in IL28B predicts hepatitis C treatment- induced viral clearance. Nature 2009 Sep 17;461(7262):399-401.

[79] McCarthy JJ, Li JH, Thompson A et al. Replicated Association Between an Interleukin-28B Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin. Gastroenterology Feb 19.

[80] Tanaka Y, Nishida N, Sugiyama M et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009 Oct;41(10):1105-1109.

[81] Schmidt SC, Bahra M, Bayraktar S *et al.* Antiviral Treatment of Patients with Recurrent Hepatitis C After Liver Transplantation with Pegylated Interferon. Dig Dis Sci 2009 Oct 2.

[82] Samuel D, Bizollon T, Feray C et al. Interferon-alpha 2b plus ribavirin in patients with chronic hepatitis C after liver transplantation: a randomized study. Gastroenterology 2003 Mar;124(3):642- 650.

[83] Schumann J, Prockl J, Kiemer AK et al. Silibinin protects mice from T cell-dependent liver injury. J Hepatol 2003 Sep;39(3):333-340.

[84] Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. Dig Liver Dis 2007 Apr;39(4):293-304.

[85] Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001;61(14):2035-2063.

[86] Gordon A, Hobbs DA, Bowden DS *et al.* Effects of Silybum marianum on serum hepatitis C virus RNA, alanine aminotransferase levels and well-being in patients with chronic hepatitis C. J Gastroenterol Hepatol 2006 Jan;21(1 Pt 2):275-280.

[87] Hawke RL, Schrieber SJ, Soule TA et al. Silymarin Ascending Multiple Oral Dosing Phase I Study in Noncirrhotic Patients With Chronic Hepatitis C. J Clin Pharmacol 2009 Oct 19.

[88] Falasca K, Ucciferri C, Mancino P et al. Treatment with silybin-vitamin E-phospholipid complex in patients with hepatitis C infection. J Med Virol 2008 Nov;80(11):1900-1906.

[89] Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian J Med Res 2006 Nov;124(5):491-504.

[90] Gazak R, Walterova D, Kren V. Silybin and silymarin--new and emerging applications in medicine. Curr Med Chem 2007;14(3):315-338.

[91] Buzzelli G, Moscarella S, Giusti A *et al.* A pilot study on the liver protective effect of silybin- phosphatidylcholine complex (IdB1016) in chronic active hepatitis. Int J Clin Pharmacol Ther Toxicol 1993 Sep;31(9):456-460.

[92] Ferenci P, Scherzer TM, Kerschner H *et al.* Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. Gastroenterology 2008 Nov;135(5):1561-1567.

[93] Loguercio C, Federico A, Trappoliere M *et al.* The effect of a silybin-vitamin-e-phospholipid complex on nonalcoholic fatty liver disease: a pilot study. Dig Dis Sci 2007 Sep;52(9):2387-2395.

[94] Saller R, Brignoli R, Melzer J *et al.* An updated systematic review with meta-analysis for the clinical evidence of silymarin. Forsch Komplementmed 2008 Feb;15(1):9-20.

[95] Gazak R, Sedmera P, Vrbacky M *et al.* Molecular mechanisms of silybin and 2,3-dehydrosilybin antiradical activity--role of individual hydroxyl groups. Free Radic Biol Med 2009 Mar 15;46(6):745- 758.

[96] Dehmlow C, Murawski N, de Groot H. Scavenging of reactive oxygen species and inhibition of arachidonic acid metabolism by silibinin in human cells. Life Sci 1996;58(18):1591-1600.

[97] Trappoliere M, Caligiuri A, Schmid M *et al.* Silybin, a component of sylimarin, exerts anti- inflammatory and anti-fibrogenic effects on human hepatic stellate cells. J Hepatol 2009 Jun;50(6):1102-1111.

[98] Boigk G, Stroedter L, Herbst H *et al.* Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. Hepatology 1997 Sep;26(3):643- 649.

[99] Biermer M, Berg T. Rapid suppression of hepatitis C viremia induced by intravenous silibinin plus ribavirin. Gastroenterology 2009 Jul;137(1):390-391.

[100] Eurich D, Bahra M, Berg T *et al.* Treatment of hepatitis C-virus-reinfection after liver transplant with silibinin in nonresponders to pegylated interferon-based therapy. Exp Clin Transplant Feb;9(1):1-6.

[101] Neff GW, O'Brien CB, Nery J *et al.* Factors that identify survival after liver retransplantation for allograft failure caused by recurrent hepatitis C infection. Liver Transpl 2004 Dec;10(12):1497-1503.

11

Management of Recurrent HCV and HBV Infections after Liver Transplantation

Marta Wawrzynowicz-Syczewska
Department of Infectious Diseases and Hepatology, Pomeranian Medical University, Szczecin, Poland

1. Introduction

1.1 Hepatitis C

Sequels of chronic HCV infection such as end-stage liver cirrhosis and hepatocellular carcinoma (HCC) are the leading indications for liver transplantation (LT) in Europe and in the United States. According to the United Network for Organ Sharing (UNOS) database the proportion of transplants performed due to the decompensated cirrhosis secondary to hepatitis C infection slightly declined in the last few years from 34% in 2002 to 29% in 2007, but at the same time the increased number of candidates for LT with HCC was noted [*www.unos.org*]. This trend will be observed until year 2030. Generally, one third of LTs worldwide is performed in HCV-positive patients. Given that recurrence of HCV infection is almost universal and the natural history of HCV hepatitis in allograft is more rapid than in the immunocompetent patient, liver failure secondary to recurrent HCV infection has a significant impact on post-transplant survival and will soon become the most common cause of liver retransplantation. Organ shortage and increasing evidence of poorer outcome in retransplanted patients makes this procedure a controversial issue, not accepted in many centers. Therefore efforts of transplant physicians to manage recurrent HCV infection in order to optimize outcomes and to slow down the progression of HCV-related liver disease are the greatest challenge. Most widely explored areas of interest include timing and schedule of antiviral treatment, immunosuppression regimens and matching in donor and recipient-related factors influencing outcomes.

1.2 Natural history of HCV recurrence

Natural history of HCV infection in the immunocompetent host since the acute HCV infection until the end-stage liver cirrhosis and eventually hepatocellular carcinoma covers approximately 30 years of progressive fibrosis developing in liver parenchyma (Hu & Tong, 1999). After liver transplantation the chronic HCV disease, albeit not fully understood and highly variable in different recipients, seems to be far more aggressive and significantly impacts the overall poorer post-LT survival in comparison with HCV-negative patients. Liver transplantation performed in viremic recipients results in rapid allograft reinfection in nearly 100% of cases. In the anhepatic phase HCV viremia dramatically declines to the very low or even undetectable levels, but within a few days after transplantation increases to the

load 10 to 20-fold higher than pretransplant ones. That gives too narrow window of opportunity for any potential intervention such as passive immunization or preemptive antiviral treatment to make it effective. The higher the pretransplant viral load the bigger the chance for faster and more severe HCV reinfection. Immunosuppressive treatment is responsible for the weaker immune control of HCV infection and a distinct natural history in post-transplant setting. Typically viremia peaks 1–3 months after LT, clinically apparent acute hepatitis develops after a median time of 4 to 6 months in more than 60% of patients and almost 100% of recipients show histological evidence of chronic C hepatitis between 1 to 4 years after LT (Gane et al., 1996). Protocol liver biopsies performed every year after LT show progression of fibrosis in the reinfected liver rating from 0.3 to 0.6 points per year (Pelletier et al., 2000) whereas in the immunocompetent hosts it scores 0.1 to 0.2 points per year (Poynard et al., 1997). According to the accessible database the mean time between acute HCV reinfection and the decompensation of liver disease, presented as variceal bleeding, refractory ascites or encephalopathy, is approximately 9.5 years (Berenguer et al., 2000). Once decompensation of liver function is established, one year survival probability decreases dramatically below 50% and 3-year survival does not exceed 10% of recipients (Berenguer et al, 2000; Roche & Samuel, 2007). This is significantly inferior than survival in the immunocompetent patients. It is estimated that 20 to 30% of HCV-positive recipients will progress to the liver cirrhosis within five years after LT. A small proportion of patients (appr. 3%) will present with a particularly severe form of HCV recurrence known as fibrosing cholestatic hepatitis with a fatal outcome (or retransplantation) in the first year post-LT (Gane, 2008). According to the UNOS database 3-year survival after LT in HCV-positive recipients is inferior to the survival rate in the other etiologies (78% vs. 82%) and 5-year survival is also lower (56.7% vs. 65.6%, $p<0.05$) (Forman et al., 2002). All the above mentioned data explain why liver disease secondary to HCV infection is currently one of the worst and most challenging indications for LT.

1.3 Factors influencing severity of recurrent HCV infection

To date a number of factors has been described as having potential influence on the natural history of post-transplant recurrent C hepatitis. This debate has a practical aspect since a few of them are modifiable by the transplant team, for example optimization of immunosuppression therapy, anti-HCV therapy prior to LT, aggressive and efficient treatment of post-transplant diabetes or the appropriate donor selection. These factors are routinely divided into four groups: donor, recipient, virus and transplant procedure related (Table 1).

In the era of organ shortage careful donor-recipient matching is not always possible and poses some controversy, but there is a growing evidence that donor related variables such as younger age (< 40 years), male sex and lack or minimal steatosis in the liver (in < 30% of hepatocytes) are associated with a significantly better survival and slower progression of chronic C hepatitis (Aytaman et al., 2010). Factors related to the transplant procedure with a potential influence on hepatitis C progression include prolonged cold ischemia time, treatment of acute rejection episodes within the first months of LT, acute CMV infection post-LT, type of immunosuppressive regimen and type of liver transplantation (deceased-donor vs. living-donor LT). Initially, there was some concern that HCV recurrence was more severe with live donor transplant, but a growing experience did not confirm these findings (Gallegos-Orozco et al., 2009).

Donor factors	• older age (> 40 years) • female sex (?) • hepatocyte steatosis > 30% • donation after cardiac death • living donation (?)
Recipient factors	• female sex • concomitant HIV infection • diabetes mellitus pre- and post-LT • African American race • higher BMI (?)
Virus-related factors	• higher pre-LT viral load • HCV genotype (?) • number of quasispecies
Transplant procedure-related factors	• prolonged cold ischemia time • type of CNI (?) • immunosuppressive protocol without AZA and steroids (?) • treatment of ACR • acute CMV infection

Table 1. Predictors of severe HCV recurrence. BMI- body mass index, CNI – calcineurin inhibitor, ACR – acute cellular rejection, LT – liver transplantation, AZA – azathioprine, CMV - cytomegalovirus

Among the baseline viral characteristics some relationship was noted between genotype 1 of the virus and the patient and graft survival, but the predominance of genotype 1 among transplanted patients can be explained by the worse antiviral treatment results before and after transplantation in comparison with other genotypes, therefore negative impact of genotype 1 on the severity of HCV recurrence was not clearly determined. It was already mentioned that high pretransplant viremia, but also persistence of the same HCV variants are responsible for more severe picture of acute HCV reinfection and more progressive course of chronic HCV disease (Berenguer, 2003).

The hottest debate concerns influence of immunosuppressive drugs on the evolution of HCV infection, two issues being discussed the most: how to optimally handle corticosteroids and which calcineurin inhibitor has an advantage in HCV-positive recipients. Some transplant centers favor corticosteroid-sparing regimens whereas in the others low-dose steroid maintenance is preferred over steroids avoidance or early withdrawal. The clear benefit of one of these approaches was not concluded. The most notable influence of corticosteroids on HCV infection is seen in the case of acute cellular rejection (ACR) treatment. It was proved that pulses of methylprednisolone commonly used for such treatment are associated with a transient increase in HCV-RNA concentration of 4- to 100-fold (Gane et al., 1996). While episodes of ACR treated with steroid pulses are associated with decreased mortality in HCV-negative patients, in the group of HCV infected recipients the relative risk of mortality or graft loss increases almost three times ($p=0.04$) and is even higher when ACR is steroid refractory. Therefore, in HCV-positive recipients empiric treatment of presumed rejection episodes without histological confirmation should be avoided to avert unnecessary exposure to corticosteroids. In contrast with convincing

association of steroid pulses with increased severity of HCV recurrence, detrimental effect of low-dose steroids versus no steroids is less obvious and requires further observations. According to the study of Manousou P. et al., lack of steroids in the immunosuppressive protocol was an independent factor affecting fibrosis (Manousou et al., 2009). Some authors suggest that a low maintenance dose of prednisone (usually 5mg daily) has no deleterious effect on HCV infection, but instead, is associated with better LT outcome, while early withdrawal of steroids (3 months beyond LT) can be detrimental and should be avoided. Some other researchers favor complete avoidance of steroids in HCV infected recipients undergoing LT. Maintenance of a low-dose and no steroids options are currently best supported.

Another hot topic is a choice of calcineurin inhibitor (CNI) in HCV-positive recipients. This discussion was initiated by the clinical observation that results of LT for cirrhosis type C in the last two decades of twentieth century did not differ from results of LTs performed for other etiologies, whereas nowadays they are clearly inferior in patients with HCV infection (Forman et al., 2002; Berenguer, 2005). One of the possible explanations was introduction of more potent immunosuppressive drugs such as tacrolimus or mycophenolate mofetil which practically replaced cyclosporine and azathioprine in the immunosuppression protocols used for liver transplant patients. The role of cyclosporine in the post-transplant recurrent C hepatitis is a subject of controversy. This drug is known as having an inhibiting effect on HCV replication *in vitro* (Watashi et al., 2003). It was also used in combination with interferon alpha-2b to treat chronic HCV infection and proved to be significantly more effective than interferon monotherapy (Inoue et al., 2003). For anti-HCV effect some authors combined cyclosporine A with interferon to treat established HCV-related graft disease (Inoue & Yoshiba, 2005). Conflicting results regarding effects of CNI on HCV recurrence may be explained by the previous study design (mostly retrospective), small groups of patients, the lack of histological examinations, variety of confounding factors and multitude of immunosuppressive protocols. However, in the prospective randomized trial and in metaanalysis that were aimed to compare influence of tacrolimus and cyclosporine on HCV recurrence, no difference in the outcome was demonstrated (Berenguer et al., 2006a; Berenguer, 2007). It was concluded that the course of recurrent HCV hepatitis is not related to the CNI used after LT. Similar observation was made in HCV-positive patients with the end-stage renal disease subjected for kidney transplantation (Kahraman et al., 2011). The only difference noted by Berenguer et al. was the shorter time between LT and recurrent acute C hepatitis in tacrolimus treated patients (59 days vs. 92 days, $p=0.02$). On the other hand, introduction of tacrolimus improved results of LT (Cholongitas et al., 2011). Used in HCV-negative liver recipients, TAC is associated with fewer rejection episodes and significantly better survival. It cannot be ruled out that this effect is counterbalanced by anti-HCV effect of cyclosporine in HCV-infected recipients, therefore none of CNIs is favored in this specific group of patients.

Another type of immunosuppressive drugs that exert a specific antiviral effect on *Flaviviridae* is a group of antimetabolites such as azathioprine (AZA) and mycophenolate mofetil (MMF). As immunosuppressants they arrest T-cell proliferation by inhibiting inosine monophosphate dehydrogenase and by the same mechanism exert an anti-HCV effect (like ribavirin). AZA is one of the oldest immunosuppressive drugs, commonly used in combination with cyclosporine and steroids, but it was substituted a decade ago by more

potent MMF. However, the role of MMF in HCV-infected liver recipients is controversial. Addition of an effective immunosuppressive drug with antiviral properties to the immunosuppression protocol seemed very attractive. Some authors demonstrated that MMF not only improved allograft function but was also associated with reduction of HCV viral load and delayed histological recurrence. In a study of Fasola et al., it was shown that high doses of MMF efficiently reduced HCV-RNA concentration and liver fibrosis one year post-transplant, but after 2 years the extent of fibrosis did not differ along all studied groups (Fasola et al., 2002). Other groups demonstrated no superiority of MMF over AZA in decreasing HCV viremia, histological slow down and better survival. Moreover, it was reported that AZA decreased replication of flaviviruses 10 times more effectively than MMF and was as potent as ribavirin in HCV inhibition in a replicon model (Stangl et al., 2004). Lately, use of AZA in liver transplant recipient, chronically infected with HCV, was reevaluated. It was concluded that MMF shows little, if any, clinical benefit in LT versus AZA and that HCV recurrence was less severe in patients treated with AZA in contrast to MMF (Germani et al., 2009). Further randomized controlled trials are warranted to solve this issue.

mTOR inhibitors such as sirolimus and everolimus are not licensed in liver transplantation. Sirolimus, however, due to its antifibrotic, antiproliferative and renal sparing properties has been recently used by some transplant teams with encouraging results (Harper et al., 2011). Different mode of action by inhibiting proangiogenic factors (i.e. VEGF, vascular endothelial growth factor) is associated with a decreased risk of cancer recurrence or *de novo* development. The main indications for sirolimus in liver recipients are CNI nephrotoxicity, hepatocellular carcinoma (as an indication for LT, *de novo* or recurrent) and fibrosis related to chronic HCV infection. Some authors suggest conversion from CNI to sirolimus-based immune suppression in case of fibrosis progression > 2. Further trials are necessary to confirm the beneficial role of sirolimus in this indication.

In conclusion, the best immunosuppressive regimen for recurrent C hepatitis is not known. Despite some reports on beneficial effect of cyclosporine in this setting, the type of CNI does not seem to matter (Berenguer, 2011), and tacrolimus remains the major immunosuppressant in the protocol. Temporary conversion to CsA is suggested during antiviral treatment to combine cyclosporine with interferon and ribavirin. As soon as fibrosis progresses to the moderate–severe stages, switching to sirolimus-based suppression can be considered, but currently this is an off-label approach. A role of MMF in the HCV recurrence is unclear. As grafted liver usually does not require very potent immunosuppression, replacement of MMF by AZA is feasible and recommended. As far as steroids are considered, steroid-free protocols or low-dose maintenance steroids seem to be the best option.

1.4 Treatment of recurrent HCV infection

Because many of the above mentioned strategies aiming to slow down progression of post-transplant HCV disease fail, the best option is to introduce anti-HCV treatment in order to attempt eradication of the virus prior to LT or after surgery to prevent recurrence or liver damage. Three approaches are possible: pre-transplant anti-viral therapy, early post-LT treatment (preemptive or in the acute phase) and treatment of the established disease. Each strategy has its pros and cons (Table 2), but the overall outcomes are rather disappointing.

There are several reasons for worse results in transplant patients in comparison with the non-transplant setting: history of unsuccessful antiviral treatment pre-LT, predominance of genotype 1 patients, significant increase in viral load following transplantation, concomitant immunosuppressive treatment, frequent dose reductions due to numerous side effects, mostly cytopenias and infections, frequent peritransplant renal impairment, limiting ribavirin dosing, and ocassionally poor general status of the patient.

Firm conclusions on the role of interferon and ribavirin in the transplant setting are hard to be driven from clinical studies due to many methodological limitations such as a small number of patients, mostly retrospective character of the trials, lack of randomization and control, differences in immunosuppressive protocols, variability in patient selection, different doses, schedules and types of anti-viral therapy, different study end-points and scarce number of control liver biopsies. Nevertheless, in a limited and carefully selected number of patients anti-viral treatment can be strongly considered and is currently recommended (Wiesner et al., 2003).

Timing of treatment	Advantage	Disadvantage
Pre-transplant	• Elimination of HCV recurrence • Stopping of disease progression • Stabilization of the general clinical status of the recipient	• Poor tolerance • Risk of life-threatening decompensation • Very low SVR rate
Preemptive	• Treatment at the low HCV RNA level • Minimal or no disease in liver biopsy	• Very low SVR rate • Poor tolerance • Higher risk of ACR and infection • Maximal immunosuppression
Established recurrent HCV disease	• Stable clinical status of the recipient • Lower doses of immunosuppression • Lower risk of ACR	• More advanced disease in liver biopsy • High viral load • Low SVR rate

Table 2. Advantages and disadvantages of anti-HCV treatment in the transplant setting

1.4.1 Pre-transplant antiviral therapy

Treating patients on the waiting list is an attractive option, because there is a body of evidence that viral clearance at the time of transplantation can minimize the risk of recurrent HCV infection post-LT and improve outcomes. However, only few candidates are suitable for the treatment. In a vast majority signs and symptoms of liver decompensation (jaundice, variceal bleeding, encephalopathy, tense ascites) and cytopenias (platelet count below 50 000/μL, absolute neutrophil count < 1500/μL) are the most common exclusion criteria. Those who are eligible, constitute a difficult-to-treat group of patients usually requiring

frequent administration of hematopoietic growth factors or decompensating on the treatment with the necessity for urgent transplantation. Expert panel recommends that antiviral treatment is worth considering in clinically stable patients with MELD score < 18 or Child-Pugh score < 7 points (Wiesner et al., 2003). Careful monitoring by the experienced team and local donor availability are mandatory. A special group of HCV-positive patients listed for LT are transplant candidates with HCC. They are often upgraded on the waiting list not due to the impaired hepatic synthetic function, but the risk of cancer growth. As having a well-compensated cirrhosis they should be strongly considered for antiviral treatment.

Duration of the treatment is not clearly defined, because cirrhotic patients can become HCV-RNA negative with a delay (if at all) and LT is not a scheduled procedure (with the exception of living donor LT). Some authors suggest keeping patients on treatment until viral clearance is achieved and continue at least for three months before LT. To avoid serious side effects, a low maintenance dose or a low accelerating dose regimen (LADR) was proposed (Everson, 2000; Everson et al., 2005). LADR was initially based on the recombinant interferon which is no more used as a standard of care (SOC). Encouraging results have been achieved by Everson (overall sustained viral response in 24% of patients and no recurrence after LT in most cases), but other trials were far less enthusiastic (Forns et al., 2003; Martinez-Bauer et al., 2006). In a low maintenance dose regimen 1 MU of standard interferon and 400 mg of ribavirin daily have been used; in LADR interferon was given 3 times a week with a starting dose of 1 MU and ribavirin was administered daily with a starting dose of 200mg, both drugs being subsequently increased fortnightly to a standard dose of 3 x 3 MU of IFN weekly and 800 mg of RBV daily.

Although combination therapy with pegylated interferons (peg-IFN) and ribavirin has limited efficacy in patients with advanced fibrosis and cirrhosis, especially if decompensated, results with peg-IFN can be better in comparison with the standard formula. Based on the current literature patients with compensated cirrhosis receive SOC therapy with peg-IFN α in a routine dose of 1.5μg/kg weekly with RBV in a routine dose of 1000-1200 mg daily for genotype 1 and 4, and in a dose of 800-1000 mg for another genotypes. In decompensated cirrhosis patients are more likely to develop various side effects and they cannot tolerate SOC easily. The suggested dose in this setting appears to be 1μg/kg/week of peg-IFN and 10.6 mg/kg/daily of RBV. In case of cytopenias, dose reductions are recommended in the first instance. If this strategy fails, hematopoetic growth factors can be used. For neutropenia G-CSF may be considered in a starting dose of 480 μg weekly, then adjusted according to the response rate to a maximum dose of 480 μg 3 times a week. Once adequate neutrophil count is attained, IFN dose can be increased to the optimum level. EPO may be considered if hemoglobin falls below 8 g/dL or by 4 g/dL. The starting dose is 20.000 IU weekly to a maximum dose of 60.000 IU weekly or, according to another study suggesting lower dosing, 4.000 IU thrice weekly with increase upon the response.

Although decompensated cirrhosis is no more an absolute contraindication to antiviral treatment, it must be used with caution. A chance to achieve sustained viral response (SVR) is rather low and patients experience numerous side effects, including life-threatening complications such as sepsis and liver function deterioration. Patients must be closely monitored and treated in experienced transplant centers. Treatment indications should be

individualized and very sick patients should be ruled out. Still the ideal candidate for pre-LT treatment is a patient in Child-Pugh class A to B, or MELD below 18 points listed because of HCC or history of variceal bleeding. Hopefully, novel therapies with combination of direct antiviral agents (DAA) such as protease or polymerase inhibitors will be more beneficial in decompensated HCV-related cirrhosis.

1.4.2 Early post-transplant antiviral treatment

1.4.2.1 Pros and cons of preemptive therapy

Similarly to the idea of treating decompensated cirrhosis, early post-LT anti-HCV treatment has a theoretical rationale. It is well documented that the lower the HCV viremia, the more effective the antiviral therapy. Also treatment of acute hepatitis C gives extremely favorable results with almost 90% chance for viral clearance. However, transition of experience from immunocompetent patients onto the post-transplant setting is not possible due to several reasons. Indeed, HCV-RNA level rapidly decreases after reperfusion, but within one-two weeks reaches pretransplant load and tends to increase by 1 to 2 logs thereafter. Hence, time for antiviral intervention, optimal at the lowest viremia, is very narrow and falls to the moment of greatest clinical instability of the recipient (renal impairment, risk of rejection, risk of bacterial infection related to the surgical procedure, deep cytopenias, etc.). During early post-LT period patients are under the strongest immunosuppression and cannot spontaneously clear the virus as it happens in a significant proportion of immunocompetent patients with the acute C hepatitis. Moreover, immunological responses to HCV that were unable to clear the virus in the past, remain the same. These factors make early post-transplant anti-viral treatment a mission almost impossible. No more than two third of liver recipients are eligible for early treatment, but even if they start therapy, dose reductions as well as rate of discontinuation are very high (Sheiner et al., 1998; Verna & Brown, 2008).

1.4.2.2 Treatment regimes

Experience with interferon monotherapy, either standard or pegylated, is scarce and disappointing. Patients were started on therapy at a mean time of 2–3 weeks post LT. Results obtained with standard interferon did not show any SVR cases (Sheiner et al., 1998; Singh et al.,1998). According to Singh et al. prophylactic treatment with IFN did not have any influence on the severity of recurrent C hepatitis, whereas in the study of Sheiner et al. treated patients less frequently developed recurrent hepatitis on liver biopsy or had abnormal liver tests. In both studies discontinuation rate was high and IFN did not influence patient and graft survival. There was the only one well designed trial published to date with peg-IFN alone given prophylactically. According to Chalasani et al., SVR was a rare event (8% of treated patients vs. no treatment), but discontinuation from the study, rejection episodes, adverse events and life threatening complications were similar in both groups. One third of treated patients were withdrawn from the study (Chalasani et al., 2005).

Slightly better results were obtained using combined non-pegylated or pegylated IFN with ribavirin. In a study of Mazzaferro et al., SVR was achieved in 33.3% of patients treated with IFN and RBV in comparison with 13% of SVR in patients on IFN alone (Mazzaferro et al., 2003). Interestingly, those who cleared the virus, did not show recurrent hepatitis C. Less encouraging effects were shown by Terrault. Only 11% obtained SVR and there was no

difference in the frequency of recurrent hepatitis between responders and non-responders (Terrault, 2003). In the latter trial therapy was initiated a bit later – within 6 weeks post LT – and almost 50% of recipients did not meet inclusion criteria. Only minority (23%) received a full-dose RBV, haemolytic anemia being the main reason for significant dose reduction or discontinuation, what may explain worse results. Experience with pegylated IFN in combination with RBV in preemptive anti-HCV treatment is very limited and further studies are necessary to draw conclusions.

Given that early post-LT antiviral treatment in not efficacious and requires further studying, the expert panel consensus conference recommends that it should be limited to rapidly progressing recurrent C hepatitis and *de novo* acute C hepatitis in recipients who received grafts from HCV-positive donors (Wiesner et al., 2003).

1.4.2.3 Immunoprophylaxis

Another strategy that theoretically could be implemented in the early post-transplant period is immune globulin prophylaxis to prevent HCV recurrence similarly to highly successful use of hyperimmune anti-HBV globulin (HBIG). Farci et al., demonstrated the existence of neutralizing anti-HCV antibodies, at least in the animal model (Farci et al., 1996), and Krawczynski K. showed that hyperactive anti-HCV globulin can delay hepatitis C onset in chimpanzees (Krawczynski, 1999). It was also shown that HBIG used in liver recipients before 1990, hence before HCV discovery, also reduced graft reinfection with HCV and recurrent C hepatitis in patients coinfected with HBV and HCV (Feray et al., 1998). These results suggested that at that time HBIG possibly contained antibodies with the anti-HCV properties. Unfortunately, clinical trials with high doses of human hepatitis C antibody enriched immune globulin product (Civacir) failed to prove any beneficial effects in HCV-positive recipients in terms of HCV suppression (Davis et al., 2005) and this strategy has been abandoned. There are several reasons for failure including unclear neutralizing properties of HCV antibodies, high genetic variability of HCV allowing easy escape from immune control and lack of small animal models to test various antibody preparations.

1.4.3 Treatment of established recurrent HCV hepatitis

Treating significant HCV recurrence that has been confirmed in liver biopsy is currently the best option to manage post-LT HCV infection. With the exception of fibrosing cholestatic hepatitis (FCH) antiviral treatment should be initiated after the first year of transplantation. Decrease in the doses of immunosuppressive drugs result in the lower HCV-RNA levels and better tolerance. Patients become clinically stable and have fewer contraindications for IFN and RBV. This strategy requires ease in performing protocol or clinically driven liver biopsies with repeated frequency, or implementation of reliable non-invasive methods to detect liver fibrosis. Current policy is to treat well established HCV recurrence defined by grade 3 or 4 of inflammation or by at least grade 2 of fibrosis. In such way patients with mild and non-progressing disease avoid unnecessary treatment, related drug toxicities and possible serious complications. Despite these issues, study results show that the efficacy of anti-HCV therapy in transplant setting is poor and SVR can be achieved in around 20% of treated patients (Samuel et al., 2003). In randomized controlled trials and in trials with pegylated interferon SVR seems to be higher and reaches rates of 38-50% (Berenguer et al. 2006b). The target dose of peg-IFN is 1.5 μg/kg/week for α-2b and 180μg/week for α-2a in

combination with ribavirin in a standard dose of 800–1200 mg/kg daily (or at least 10.6mg/kg/day). Duration of treatment is 48 weeks, but in previous relapsers or non-responders can be extended to 72 weeks or longer provided that there is an early viral response (EVR) at the end of the third month. Only a small proportion of patients are able to continue therapy without initial dose reductions and/or discontinuation mostly due to severe anemia or another cytopenia. The best predictors of SVR are non-1 genotype, achievement of viral clearance after 3 months of therapy, good compliance (>80% of IFN and >80% of RBV received) and less advanced fibrosis. EVR seems to have the strongest impact on treatment outcome. The most important concern regarding antiviral treatment in transplant setting is an increased risk of either acute or chronic rejection. Treatment of ACR episode requires otherwise unwanted high doses of steroids, and chronic rejection may lead to retransplantation. Recent studies suggest that ACR develops due to decreasing levels of immunosuppressive drugs after viral clearance and subsequent improvement of hepatic microsomal function. Reported rejection rates vary in respect to the study design, being lower in randomized controlled trials (0-5%) [Chalasani et al., 2005; Samuel et al., 2003) and as high as 35% in uncontrolled trials (Dumortier et al., 2004; Sharma et al., 2007; Stravitz et al., 2004). Berenguer M et al. reported trend towards higher rejection rate on pegylated IFN in comparison with standard treatment (Berenguer et al., 2006b). Also *de novo* autoimmune hepatitis due to immunomodulatory properties of IFN and RBV may develop in 0.4 to 3.4 % of treated patients (Selzner et al., 2011).

1.5 Retransplantation in HCV recurrence

It is estimated that approximately 10% of HCV-positive transplant patients will require retransplantation (reLT) due to graft decompensation (Carrion et al., 2010). Similarly to the indications for the primary LT, hepatitis C may soon become the major indication for reLT. Many patients will not be able to survive until reLT is feasible as the mortality on the waiting list varies between 50 and 80%, and many transplant centers hesitate to retransplant patients with recurrent HCV disease due to inferior results of reLT in comparison with non-HCV candidates (Pelletier et al., 2005). However, there is no firm evidence that the unfavorable scenario after primary LT is going to repeat after reLT in every HCV-positive recipient. Moreover, other recent studies do not identify HCV recurrence as a predictor of increased mortality in comparison with other etiologies with the exception of reLT performed during the first year after primary LT (Ghobrial et al., 2002). Based on multivariate analysis it was shown that early reLT performed in HCV-positive patients is an independent predictor of morality after reLT, indicating that severe hepatitis C recurrence (such as FCH or another reason for early graft dysfunction) should be a contraindication for retransplantation (Ghabril et al., 2008). Multiple prognostic scores were implemented to facilitate decisions which reLT would be unreasonable due to compromised graft and patient survival. Many of them are routinely used for urgent LT, and therefore are not appropriate for candidates with recurrent HCV disease who need an elective retransplantation. Prognostic criteria, traditionally used in patients with cirrhosis, such as MELD and Child-Pugh scores, turned out to be more accurate in the exclusion of high risk candidates. The ILTS expert panel concluded that bilirubin > 10mg/dL, creatinine > 2mg/dl (or EGFR < 40 ml/min), recipient age > 60 years, donor age > 40 years and early HCV-related cirrhosis (< 1 year post-LT) were the variables significantly associated with poorer outcome and with increased mortality (Wiesner et al., 2003). Lately, a MELD score >28 was

added to that list (Zimmerman & Ghobrial, 2005). With use of these prognostic measurements as screening tools survival in HCV-infected patients after reLT reached similar rates as survival in non-HCV patients.

2. Hepatitis B

Liver transplantation for hepatitis B is a true success story. In the early 1990s HBV infection was a relative contraindication for LT as the risk of recurrence was greater than 80% (depending on pre-transplant viral load) and the mortality rate was approximately 50% at 2 years. That was comparable with results of LT in malignancies (O'Grady et al., 1992). Introduction of effective immunoprophylaxis and very potent oral antivirals revolutionized this area in the last two decades and made HBV the best etiology for LT in terms of patient and graft survival (Lake et al., 2005). The number of LTs performed for this indication steadily declines in the Western countries due to effective vaccination program and good results of anti-HBV treatment, but in Asia the majority of patients undergoing LT still has HBV related end-stage liver disease or fulminant hepatitis. Overall, 5 to 10% of LTs worldwide are performed in HBsAg-positive patients (Terrault et al., 2005). By dint of long-term use of hepatitis B immune globulin (HBIG) combined with highly effective and well-tolerated nucleoside/nucleotide analogues (NUCs) HBV reinfection rate decreased below 10% (Angus et al., 2000; Marzano et al., 2001). Together with these favorable results there are, however, some concerns. According to current understanding of HBV pathogenesis complete withdrawal of reinfection prophylaxis is not feasible. Life-long prophylaxis makes LT for hepatitis B a very expensive procedure. Economical pressure stimulates studies on various alternatives which are cheaper and more convenient for the patient. Moreover, long-term use of HBIG is associated with some side-effects and the development of escape mutants in HBsAg region. Indefinite use of NUCs plus surface antigen mutations during long-term HBIG administration pose a great risk of multidrug resistance. Novel strategies need to be developed to optimize outcomes in this setting.

2.1 Pre-transplant HBV management

There are a few therapeutic options in chronic hepatitis B including indirectly acting interferons and directly acting anti-HBV molecules such as nucleotide/nucleoside analogues. Interferons were the first drugs used for this indication, but limited efficacy and poor tolerability in cirrhotic patients hampered successful management of HBV-related liver decompensation and preparation for LT for many years. Chronic HBV infection in the replicative phase was considered by many transplant teams a contraindication for transplantation because of a great risk of recurrence under immunosuppression. A turning point was discovery of the first potent viral polymerase inhibitor that allowed effective HBV suppression and clinical improvement, and in consequence permitted LT. Without LT survival in HBV-related decompensated cirrhosis is very poor and does not exceed 14% at 5 years (Zoulim et al., 2008). Independent factors associated with survival are hepatitis B e antigen (HBeAg) positivity, bilirubin level, age, transaminase activity, presence of oesophageal varices and Child-Pugh score (Zoulim et al. 2008). In another study in addition to age, bilirubin and HBeAg status, platelet count, albumin level and splenomegaly were found to be significantly related to survival. In patients with signs and symptoms of

decompensation (jaundice, increased bilirubin, low albumin level, low platelet count, prothrombin time prolongation), use of interferon alpha was associated with further deterioration and high risk of life-threatening flares in case of a minimal hepatic reserve. Its use was therefore restricted to experienced centers and was generally contraindicated. Notable improvement has been achieved in the recent years together with introduction of lamivudine (LAM), the first nucleoside analogue inhibiting HBV DNA polymerase, followed by the availability of new potent drugs with direct antiviral effect. In some patients excellent replication control and clinical stabilization allow removal from the waiting list. But these treatments do not eradicate HBV infection. HBV DNA polymerase can be suppressed and viremia effectively controlled only when patients take medications. As soon as treatment is stopped (or the patient is not compliant), the virus recurs in blood in most cases. That means the necessity for life-long treatment to maintain viral suppression and counteract decompensation.

The major concern connected with prolonged HBV therapy is a risk of drug resistance. Knowledge of the past antiviral treatment (if relevant), baseline parameters and patterns of mutations conferring resistance is essential in the management of candidates for LT. Patients require careful monitoring and prompt interventions as soon as resistance emerges. Determination of pretreatment HBV-DNA level is obligatory, because this value will be used for further comparisons and treatment efficacy evaluation. Quantitative HBV-DNA testing should be repeated in three to six month intervals, preferably using the same diagnostic assay. Primary non-response to treatment is defined by HBV-DNA decrease below 1 log after 24 weeks of a given therapy. Patients with primary failure require prompt switch to an alternative treatment. Increase in serum HBV-DNA level by at least 1 log above nadir is defined as virological resistance (or viral breakthrough). This can be related to genotypic resistance which means emergence of amino acid substitutions in the reverse transcriptase region of HBV polymerase gene during treatment. Suspicion of mutations conferring resistance require confirmation with genotypic testing, especially as the main reason for viral breakthrough is medication non-compliance and should be considered in the first instance to avoid unnecessary modification in therapy. If the patient denies medication negligence, one of the tests for the detection of resistant mutants should be ordered and if antiviral resistance confirmed, a rescue therapy has to be implemented (Table 4). If it is not done in time, clinical (or biochemical) resistance, defined as a significant liver enzymes elevation on treatment, can occur within months to years after development of polymerase gene mutations. It can be potentially life-threatening in patients with decompensated cirrhosis, and should be strictly avoided.

A question is when and with which drug to initiate antiviral treatment in patients awaiting LT to avoid prolonged administration and development of drug resistance. There is a consensus panel agreement that each patient with HBV DNA > 2000 IU/mL is in danger of disease progression and HCC development, therefore requires antiviral treatment (Chen et al., 2006; Iloeje et al., 2006). It is especially relevant in patients with liver cirrhosis, as viral suppression may lead to significant clinical improvement and withdrawal from transplant waiting list. It is also commonly accepted that in decompensated cirrhosis any HBV viremia preceding transplantation is harmful and should be treated. If a patient is HBV DNA repeatedly negative by one of commercially available sensitive PCR assays, they can be commenced on antiviral therapy at the time of transplantation.

Therapeutic decision should be based on drug potency and high genetic barrier to resistance. Several oral NUCs with different antiviral properties are currently available and can be considered for treatment (Table 3).

Drug	Class	Resistance	Potency
Lamivudine	nucleoside	high	high in the first year of treatment
Adefovir	nucleotide	low	moderate
Telbivudine	nucleoside	medium	high in naïve patients
Entecavir	nucleoside	low	very high in naïve patients
Tenofovir	nucleotide	low	very high

Table 3. Antivirals against hepatitis B virus

Careful consideration of the past medical history, resistance pattern (if detected) and cross-resistance data is mandatory (Table 4). Lamivudine was the first NUC used in patients with decompensated cirrhosis, initially with a great success. It is a relatively cheap, very well tolerated and potent drug showing effective suppression of HBV viremia within a few weeks of treatment. It improves hepatic function in more than a half of patients with decompensated cirrhosis within the first year of treatment and is associated with better survival. Usual daily dose is 100–150 mg. A significant disadvantage is a low genetic barrier of resistance making long-term treatment with LAM impossible in a considerable number of patients. It was shown that drug-resistant mutants emerge in 20% of patients treated with LAM per year. Therapy with LAM requires frequent determinations of HBV viremia, preferably every three months, and prompt initiation of rescue therapy in case of genetic breakthrough (Table 4). LT performed in patients on lamivudine with LAM resistance mutations can give inferior results and should be avoided (Perillo et al., 2001).

Another disadvantage is cross-resistance with other NUCs, considerably limiting rescue treatment options. Albeit LAM provided an important progress in LT for hepatitis B, now it is not indicated as a first line therapy in decompensated cirrhosis type B. The same concerns telbivudine, another L-nucleoside, which is even more potent than LAM, but relatively quickly selects for mutations at the same sites as LAM and entecavir. For these reasons it is neither recommended as a first line therapy in cirrhotic patients nor as a rescue therapy in LAM or entecavir resistance.

Adefovir (ADV), a nucleotide analogue of adenosine monophosphate, is effective as a first line treatment of wild type HBV infection as well as a rescue therapy in LAM-resistant patients. It is a slowly acting molecule, and in some patients delayed decrease in viremia can be mistaken with a primary non response to treatment or with a breakthrough if the baseline HBV DNA level was not determined. Its use as a drug of choice in decompensated cirrhosis is limited due to a relatively weak inhibition of HBV DNA polymerase and slow viral suppression at the approved dose (10 mg daily). A potential nephrotoxicity also limits indication for ADV in patients with cirrhosis and concomitant renal insufficiency. Dose adjustment in case of renal impairment is necessary. Despite a few disadvantages, it was

discovered that ADV lacks cross-resistance with LAM and can be used as a rescue therapy in LAM-resistant patients. However, it was also reported that to avoid sequential resistance to ADV (resistance develops only if LAM is stopped), it is better to add ADV to LAM than to switch LAM on ADV (Villeneuve et al., 2003). In very sick patients who would not be able to tolerate hepatic flares related to the selection of resistant strains, the best option is to use *de novo* combination of LAM and ADV. In case of resistance to ADV (cumulative probability appr. 2% in 2 years), the best option is to add LAM, telbivudine or entecavir.

NUC	**Primary antiviral resistant mutation**	**Preferred management**
lamivudine	rtM204V/I rtA181T	• add adefovir • add or switch to tenofovir or tenofovir + emtricitabine
telbivudine	rtM204I	• add adefovir • add or switch to tenofovir or tenofovir + emtricitabine
adefovir	rtA181V/T rtN236T rtI233V*	• add or switch to entecavir • or tenofovir • or tenofovir + emtricitabine
entecavir	rtL180M and rtM204V plus rtI169T and rtM250V or rtT184G and rtS202I	• add or switch to tenofovir • or tenofovir + emtricitabine
tenofovir	none	?

*primary non-response

Table 4. HBV mutations associated with drug resistance and rescue treatment options

Entecavir, launched in 2005, is a very potent and well tolerated nucleoside analogue with a high genetic barrier of resistance. Used as a first line therapy in a daily dose of 0.5mg dramatically reduces HBV DNA viremia within a few weeks irrespectively on HBeAg status, and currently is a drug of choice in the naïve patients with decompensated HBV-related cirrhosis. Results obtained at 5 years of treatment showed practically negligible resistance rate (Colonno et al., 2006). However, in LAM-resistant patients the efficacy of entecavir is markedly reduced even at higher doses (1 mg daily), and resistance develops in more than one third of patients after 4-year treatment (Sherman et al., 2006). It can be explained by a selection of rtM204V/I mutants already being developed during LAM treatment and less susceptible to entecavir in comparison with wild-type HBV, and the emergence of another mutation at codons 184, 202 and 250 under entecavir pressure (Table 4). If at least three mutations develop together, a viral breakthrough occurs. Therefore, entecavir should not be used as a rescue therapy in LAM-resistant (or telbivudine-resistant) patients. Such sequence may select for multidrug resistant virus. In case of entecavir resistance the only possibility is to add (better than to switch on!) adefovir or tenofovir.

Tenofovir alone or in combination with emtricitabine is a nucleotide analogue successfully used in HIV-positive patients. In HIV/HBV coinfection it also showed high potency against HBV virus. To date resistant strains have not been discovered. In comparison with ADV it is far more potent and can be used as a rescue therapy in the majority of resistance situations.

The daily dose is 300 mg. The drug is potentially nephrotoxic and should be used with caution in renal insufficiency. Tenofovir has been only recently registered in Europe to treat patients with chronic hepatitis B, therefore the experience in decompensated cirrhosis is very limited. Because of its excellent antiviral activity and lack of mutations associated with drug resistance it is reasonable to restrict its use to the patients who require rescue therapy and failed previous treatments.

In conclusion, the best option in treatment-naïve patients with decompensated HBV-related cirrhosis, especially if they await LT and will continue antiviral treatment after transplantation, is entecavir in a standard dose. In case of LAM-experienced patients, either with or without LAM-resistance, the best option is to add adefovir and to keep patients on the combination therapy until transplantation. To ensure ongoing viral susceptibility frequent, preferably in 3 month intervals, testing for HBV-DNA level is mandatory.

2.2 Prevention of HBV recurrence after LT

Two-three decades ago HBV recurrence after liver transplantation was very frequent. Although HBV replicates almost exclusively in hepatocytes, reinfection can be caused by the circulating HBV particles or, less frequently, by HBV harvested from peripheral blood mononuclear cells. A good evidence for that was a close relation of reinfection risk with the pre-transplant viral load. Viremic patients developed reinfection almost inevitably. The course of recurrent HBV hepatitis was accelerated in comparison with HBV infection in non-transplant setting resulting in liver failure and premature death in the majority of patients. It could be explained by high doses of steroids, routinely included to the immunosuppression protocol in all transplanted patients (there is a glucocorticoid-susceptible element in HBV genome), loss of immune control over HBV replication together with a potent immunosuppression and sudden availability of new hepatocytes for viral replication. Due to the very poor results, retransplantation for recurrent HBV infection was performed reluctantly and in many transplant centers was contraindicated. There was a new histological finding in recurrent HBV reinfection characterized by cholestasis, marked inflammation and fibrosis, described as fibrosing cholestatic hepatitis (FCH). This particular form of reinfection, believed to be a direct cytopathic effect of HBV on hepatocytes, resulted in rapid development of hepatic decompensation and death, usually within one year post-LT (Lau et al., 1992). Before implementation of successful strategies to prevent reinfection, it was reasonable to withdraw steroids from immune suppression in HBV-positive patients or maintain them at a low dose, and to reduce immunosuppression strength to the lowest possible levels. There was no evidence that any of CNI inhibitors had an advantage or disadvantage in this particular group of recipients by stimulating or suppressing HBV replication (McMillan et al., 1995). Neither it was confirmed for mycophenolate mofetil (Maes et al., 2001). Rather the overall potency compromising immune system mattered. New approaches were essential to change these disappointing results and to make transplantation of HBV-related end-stage liver disease an acceptable procedure.

The first change came together with the use of human immune globulin containing high titers of anti-HBsAg antibodies (HBIG) to neutralize circulating virions and to prevent virus entry to the hepatocytes. Different schedules were used (Table 5), but it was soon proved that HBIG started at the anhepatic phase in a dose of 10 000 IU followed by high doses during the first days after transplantation (10 000 IU daily for a week) and administered

long-term thereafter to maintain anti-HBs titer >100 IU/L was associated with a significant reduction risk of recurrence from 74 to 36% and far better prognosis (Samuel et al., 1993). The question arose why HBIG was not effective in all patients, but the answer is not entirely clear. Some centers tried to overcome this problem by administering larger and more frequent doses of HBIG in order to keep anti-HBs titer above 500 IU/L, but the costs of such approach were extremely high. Moreover, it was noticed that long-term use of HBIG results in selection of the escape mutants. Mutations occur in the coding region of 'a' determinant of the surface protein. In such cases vaccination failure is also present. Protective threshold of anti-HBs was not established. Some transplant teams preferred keeping anti-HBs at a level of 500 IU/L, whereas others accepted 300 IU/L or titers as low as 100 IU/L. A schedule of administration was either fixed (i.e. 2000 IU monthly or 5000 IU every second month) or individualized (on-demand) according to the anti-HBs titer. With HBIG monotherapy,

Type of HBIG	**Lead-in dose**	**Maintenance dose**	**Recurrence**
High-dose (iv.) HBIG	10 000 IU at LT followed by 10 000 IU daily for 7 days (80 000 IU in the first month)	10 000 IU monthly	0
	46 500 IU in the first month	5 000 IU monthly	4%
	40 000 IU in the first week	To keep anti-HBs > 500 IU/L for the second week and > 100 IU/L thereafter	9.5%, only in LAM-resistant patients
	10 000 IU daily until anti-HBs > 1000 IU/L	To keep anti-HBs > 100 IU/L	20%, only in LAM-resistant patients
	10 000 IU daily until HBsAg is cleared	To keep anti-HBs > 100 IU/L	8%, mostly in LAM-resistant patients
	80 000 IU in the first month	2 000 IU to keep anti-HBs > 100 IU/L	18%, only in LAM-resistant patients
Low- dose (im.) HBIG	800 IU at LT and daily for one week	800 IU monthly	3.1%
	80 000 IU iv. in the first week post-LT	1 200 IU monthly to keep > 100 IU/L	3.6%
	4 000 IU at LT and then 2 000 daily until anti-HBs > 200 IU/L	To keep anti-HBs > 100 IU/L	5.7%, only in LAM-resistant patients
	2 000 IU at LT and 800 IU daily for 6 days, weekly for 3 weeks	800 IU monthly	14%
	800 IU at LT and daily for 6 days	800 IU monthly	4%

Table 5. Different HBIG protocols and recurrence rate

however the recurrence rate was high when HBV DNA was detectable in blood at the time of transplantation. Lower recurrence was observed in negative HBV DNA patients, in the concomitant delta virus infection and in fulminant B hepatitis.

High costs of HBIG, the necessity of frequent testing and inconvenient immune globulin administration led transplant centers to study some other options. Early results with lamivudine showing recurrence rate of 10% at the first year post-LT were very promising and gave hope to abandon costly immunoprophylaxis, but further observations were less enthusiastic, as 43-50% of patients developed recurrence within 3 years after LT largely due to cumulative LAM-resistance and high pre-transplant viral load [Mutimer et al., 1999; Perillo et al., 2001)]. Because monotherapy either with HBIG or with LAM was not satisfactory, a combination of these two agents was soon proposed. As both drugs have different mode of action and exert additive prophylactic effect, it was quickly proved that such strategy is very effective and prevents HBV recurrence in more than 90% of recipients. Combination of LAM with intravenous high dose anti-HBV immune globulin (IV HBIG) is currently a standard of care in terms of anti-HBV prophylaxis. LAM can be replaced with another NUC (or combination of NUCs), usually the one that was started before transplantation. Some centers try to reduce IV HBIG use by switching on the intramuscular formula or by administering HBIG only when anti-HBs titer falls below 10 IU/L. The latter approach turned out to be safe and no recurrence was noted despite low cumulative dose of HBIG. The authors suggested that in the concomitant use of NUC it is not necessary to keep anti-HBs at higher levels (Takaki et al., 2007). Recent publication from Australia and New Zealand on the efficacy of low-dose IM HBIG in combination with LAM is very promising and supports efforts to reduce high costs of anti-HBV prophylaxis. IM HBIG is given at a dose of 800 IU daily for 7 days post-transplant and in a dose of 400–800 IU monthly thereafter. With this strategy the rate of recurrence was only 4% at 5 years (Gane et al., 2007). The authors suggest that in case of low risk of recurrence (HBV-DNA < 2 000–20 000 IU/mL at baseline, HDV coinfection, fulminant hepatitis) HBIG can be withdrawn within one-two years post-LT with NUC to be continued life-long. In high recurrence risk (HBV-DNA > 2 000–20 000 IU/mL before treatment commence, history of drug resistance) IM HBIG in combination with NUC should be administered indefinitely. According to Gane et al. IM HBIG can be possibly replaced by the combination of two potent analogues. Further studies on this issue are warranted.

Another strategy to provide an effective anti-HBV prophylaxis without immunoglobulin administration is a vaccination program. The idea was to start active immunization at an anti-HBs titer around 100 IU/mL hoping to maintain this level after indefinite HBIG withdrawal. First generation vaccines did not prove to be useful. Some success has been achieved with the pre-S vaccines in lamivudine treated recipients. In one series anti-HBs response was obtained in 50% of vaccinated, two third of whom managed to maintain titers (Lo et al., 2007). Spontaneous antibody production was also noted in a small number of patients and development of a novel vaccine that is able to sustain that production would be of great importance.

2.3 Management of *de novo* or recurrent HBV hepatitis

As it was already mentioned, in the absence of an effective prevention, recurrent B hepatitis, defined as detectable HBsAg in blood after LT, occurs in a substantial proportion of patients

preoperatively positive for HBsAg. In the nineties of the last century recurrence rate was reported to be as high as 70 to 90% among HBsAg-positive recipients (Lake & Wright, 1991). Nowadays these proportions are notably better and vary between 26 and 53%, possibly due to more accurate selection of HBV DNA negative candidates (Yeo et al., 2004). To a lesser extent HBsAg may reappear in circulation as a result of reactivation in patients with previously resolved infection, that is in HBsAg-negative but anti-HBc positive individuals. Berger et al. found reactivation in 0.9% of anti-HBc positive liver recipients, whereas other authors reported a bit higher risk of HBsAg reappearance reaching approximately 3% of transplant patients (Barclay et al., 2008). In addition to reactivation, *de novo* HBV infection following LT has been reported in HBsAg-negative individuals who received livers from anti-HBc-positive donors (Dickson et al., 1997; Prieto et al., 2001). Currently, livers from anti-HBc-positive donors can only be transplanted to the HBsAg-positive recipients or to the anti-HBc-positive recipients with high anti-HBs titers. The same applies to kidney donors and recipients. Anti-HBc-positive heart and lung donors do not pose a significant risk of HBV transmission. In most transplant centers there is a policy to administer pre-emptively one of the NUCs (usually lamivudine for at least one year post-LT) to the anti-HBc-positive recipient to prevent reactivation of an occult HBV infection. It is needless to say that harvesting organs from HBsAg-positive donors is not allowed.

However, post-LT HBV infection still occurs either as recurrent hepatitis B due to the unsuccessful prophylaxis or as *de novo* community acquired infection, but most frequently because of use of an organ from anti-HBc-positive/HBsAg-negative donor. In case of recurrent B hepatitis or newly acquired HBV infection recipients have to be treated promptly with one of the potent NUCs, preferably the one with high genetic barrier of resistance, i.e. entecavir or tenofovir (in LAM-experienced patients). Adefovir should be considered with caution as the potency of the drug is moderate and there are reports on nephrotoxicity. Antiviral therapy should be continued until the end-point is reached (preferably HBsAg loss and seroconversion to anti-HBs) or life-long. HBV-DNA level and aminotransferase activity should be monitored every 12 to 24 weeks to seek for primary non-response, partial virological response (HBV-DNA decrease >1 log but <2 logs without resistance) and virological breakthrough. In primary and partial non-response a rapid switch to another NUC is recommended. In case of genotypic resistance-related virological breakthrough (after excluding non-compliance) adding-on a second drug is the optimal strategy. Knowledge of cross-resistance patterns is obligatory (European Association for the Study of the Liver, 2009). Clinical experience with interferons in the recurrent or *de novo* hepatitis B in liver transplant recipients is scarce.

3. References

Angus, P.W., McCaughan, G.W., Gane, E.J., Crawford, D.H. & Harley, H. (2000). Combination low-dose hepatitis B immune globulin and lamivudine therapy provides effective prophylaxis against post-transplantation hepatitis B. *Liver Transplantation*, Vol.6, No.4, (July 2000), 429–33

Aytaman, A., Kaufman, M. & Terrault N.A. (2010). Management of posttransplant hepatitis C infection. *Current Opinion on Organ Transplantation,* Vol.15, No.3, (June 2010), 301–9

Barclay, S., Pol, S., Mutimer, D., Benhamou, Y., Mills, P.R., Haves, P.C., Cameron, S. & Carman, W. (2008). The management of chronic hepatitis B in the immunocompromised patients: recommendations from a single topic meeting. *Journal of Clinical Virology,* Vol.41, No.4, (April 2008), 243–54

Berenguer, M. (2003). Host and donor risk factors before and after liver transplantation that impact HCV recurrence. *Liver Transplantation*, Vol. 9, No. 11, (November 2003), S44–S47

Berenguer, M. (2005). What determines the natural history of recurrent hepatitis C after liver transplantation? *Journal of Hepatology*, Vol.42, No.4, (April 2005), 448–56

Berenguer, M. (2007). Recurrent hepatitis C: worse outcomes established, interventions still inadequate. *Liver Transplantation*, Vo.13, No. 5, (May 2007), 641–3

Berenguer, M. (2011). "Hot topic debate on HCV: the type of immunosuppression does not matter". *Liver transplantation,* (June 2011), DOI: 10.1002/lt.22347

Berenguer, M., Aguilera, V., Prieto, M., San Juan, F., Rayon, J.M., Benlloch, S. & Berenguer, J. (2006). Effect of calcineurin inhibitors on survival and histologic disease severity on HCV-infected liver transplant recipients. *Liver Translantation,* Vol.12, No.5, (May 2006), 762–7

Berenguer, M., Palau, A., Fernandez, A., Benlloch, S., Aguilera, V., Prieto, M., Rayon, J.M. & Berenguer J. (2006). Efficacy, predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. *Liver Transplantation*, Vol.12, No. 7, (July 2006), 1067–76

Berenguer, M., Prieto, M., Rayon, J.M., Mora, J., Pastor, M., Ortiz, V., Carrasco, D., San Juan, F., Burgueno, M.D., Mir, J. & Berenguer, J. (2000). Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology,* Vol. 32, No. 4 Pt 1, (October 2000), 852–8

Carrion, J.A., Navasa, M. & Forns, X. (2010). Retransplantation in patients with hepatitis C recurrence after liver transplantation. *Journal of Hepatology*, Vol. 53, No.5, (November 2010), 962–70

Chalasani, N., Manzarbeitia, C., Ferenci, P., Vogel, V., Fontana, R.J., Voigt, M., Riely, C., Martin, P., Teperman, L., Jiao, J. & Lopez-Talavera, J.C. (2005). Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized controlled trials. *Hepatology*, Vol. 41, No.2, (February 2005), 289–98

Chen, C.J., Yang, H.I., Su, J., Jen, C.L., You, S.L., Lu, S.N., Huang, G.T. & Iloeje, U.H. (2006). Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *Journal of American Medical Association*, Vol. 295, No. 1, (January 2006), 65–73

Cholongitas, E., Shusang, V., Germani, G., Tsochatzis, E., Raimondo, M.L., Marelli, L., Senzolo, M., Davidson, B.R., Patch, D., Rolles, K., & Burroughs A.K. (2011). Long-term follow-up of immunosuppressive therapy in liver transplantation: tacrolimus and microemulsified cyclosporin. *Clinical Transplantation*, Vol.25, No. 4, (July 2011), 614–24

Colonno, R.J., Rose, R., Baldick, C.J., Levine, S., Pokornowski, K., Yu, C.F., Walsh, A., Fang, J., Hsu, M., Mazzucco, C., Eggers, B., Zhang, S., Plym, M., Klesczewski, K. & Tenney, D.J. (2006). Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology*, Vol. 44, No. 6, (December 2006), 1656–65

Davis, G.L., Nelson, D.R., Terrault, N.A., Pruett, T.L., Schiano, T.D., Fletcher, C.V., Sapan, C.V., Riser, L.N., Li, Y., Whitley, R.J. & Gnann J.W. Jr. (2005). A randomized, open-

label study to evaluate the safety and pharmacokinetics of human hepatitis C immune globulin (Cicavir) in liver transplant recipients. *Liver Transplantation*, Vol.11, No.8, (August 2005), 941–49

Dickson, R.D., Everhart, J.E., Lake, J.R., Wei, Y., Seaberg, E.C., Wiesner, R.H., Zetterman, R.K., Pruett, T.L., Ishitani, M.B. & Hoofnagle J.H. (1997). Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Gastroenterology*, Vol.113, No.5, (November 1997), 1668–74

Dumortier, J., Scoazec, J.Y., Chevallier, P. & Boillot, O. (2004). Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. *Journal of Hepatology*, Vol. 40, No.4, (April 2004), 669–74

European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. (2009). *Journal of Hepatology*, Vol.50, No.2, (February 2009), 227–42

Everson GT. (2000). Maintenance interferon for chronic hepatitis C: more issues than answers? *Hepatology*, Vol.32, No.2, (August 2000), 436–8

Everson, G.T., Trotter, J., Forman, L., Kugelmas, M., Halprin, A., Fey, B. & Ray, C. (2005). Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology*, Vol.42, No.2, (August 2005), 255–62

Farci, P., Shimoda, A., Wong, D., Cabezon, T., De Gioannis, D., Strazzera, A., Shimizu, Y., Shapiro, M., Alter, H.J. & Purcell, R.H. (1996). Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proceedings of the National Academy of Sciences USA*, Vol.93, No.26, (December 1996), 15394–9

Fasola, C.G., Netto, G.J., Jennings, L.W., Christensen, L.L., Molmenti, E.P., Sanchez, E.Q., Levy, M.F., Goldsterin, R.M. & Klintmalm, G.B. (2002). Recurrence of hepatitis C in liver transplant recipients treated with mycophenolate mofetil. *Transplantation Proceedings*, Vol.34, No.4, (August 2002), 1563–4

Feray, C., Gigou, M., Samuel, D., Ducot, B., Maisonneuve, P., Reynes, M., Bismuth A. & Bismuth H. (1998). Incidence of hepatitis C in patients receiving different preparations of hepatitis B immunoglobulins after liver transplantation. *Annals of Internal Med,* Vol.128, No.10, (May 1998), 810–16

Forman, L.M., Lewis, J.D., Berlin, J.A., Feldman H.I. & Lucey M.R. (2002). The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology*, Vol.122, No.4, (April 2002), 889–896

Forns, X., Garcia-Retortillo, M., Serrano, T., Feliu, A., Suarez, F., de la Mata, M., Garcia-Valdecasas, J.C., Navasa, M., Rimola, A. & Rodes, J. (2003). Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *Journal of Hepatology*, Vol.39, No.3, (September 2003), 389–96

Gallegos-Orozco, J.F., Yosephy, A., Noble, B., Agel, B.A., Byrne, T.J., Carey, E.J., Douglas, D.D., Mulligan, D., Moss, A., de Petris, G., Williams, J.W., Rakela, J. & Vargas, H.E. (2009). Natural history of post-liver transplantation hepatitis C: a review of factors that may influence its course. *Liver Transplantation*, Vol.15, No.12, (December 2009), 1872–81

Gane, E.J. (2008). The natural history of recurrent hepatitis C and what influences this. *Liver Transplantation*, Vol.14, Suppl. 2, (October 2008), S36–S44

Gane, E., Naoumov, N., Qian, K., Mondelli, M.U., Maertens, G. & Portmann, B.C. (1996). A longitudinal analysis of hepatitis C virus replication following liver transplantation. *Gastroenterology*, Vol. 110, No.1, (January 1996), 167–77

Gane, E.J., Angus, P.W., Strasser, S., Crawford, D.H., Ring, J., Jeffrey, G.P. & McCaughan, G.W. (2007). Lamivudine plus low-dose hepatitis B immunoglobulin to prevent recurrent hepatitis B following liver transplantation. *Gastroenterology*, Vol.132, No.3, (March 2007), 931–7

Germani, G., Pleguezuelo, M., Villamil, F., Vaghijani, S., Tsochatzis, E., Andreana L. & Burroughs A.K. (2009). Azathioprine in liver transplantation: a reevaluation of its use and comparison with mycophenolate mofetil. *American Journal of Transplantation*, Vol.9, No.8, (August 2009), 1725–31

Ghabril, M., Dickson, R. & Wiesner, R. (2008). Improving outcomes of liver retransplantation: an analysis of trends and the impact of hepatitis C infection. *American Journal of Transplantation,* Vol. 8, No. 2, (February 2008), 404–11

Ghobrial, R.M., Gornbein, J., Steadman, R., Danino, M., Markmann, J.F., Holt, C., Anselmo, D., Amersi, F., Chen, P., Farmer, D,G., Han, S., Derazo, F., Saab, S., Goldstein, L.I., McDiarmid, S.V. & Busuttil, R.W. (2002). Pretransplant model to predict posttransplant survival in liver transplant patients. *Annals of Surgery*, Vol.236, No.3, (September 2002), 315–22

Harper, S.J., Gelson, W., Harper, I.G., Alexander, G.J., Gibbs P. (2011). Switching to sirolimus-based immune suppression after liver transplantation is safe and effective: a single-center experience. *Transplantation*, Vol.91, No.1, (January 2011), 128–32

Hu, K.Q. & Tong, M.J. (1999). The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology,* Vol.29, No.4, (April 1999), 1311–6

Iloeje, U.H., Yang, H.I., Su, J., Jen, C.L., You, S.L. & Chen, C.J. (2006). Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology*, Vol. 130, No.3, (March 2006), 678–86

Inoue, K. & Yoshiba, M.(2005). Interferon combined with cyclosporine treatment as an effective countermeasure against hepatitis C virus recurrence in liver transplant patients with end-stage hepatitis C virus related disease. *Transplantation Proceedings,* Vol.37, No.2, (March 2005), 1233–4

Inoue, K., Sekiyama, K., Yamadi, M., Watanabe, T., Yasuda, H. & Yoshiba, M. (2003). Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. *Journal of Gastroenterology*, Vol.38, No. 6, (June 2003), 567–72

Kahraman, A., Witzke, O., Scherag, A., Putter, C., Miller, M., Dechene, A., Ross, S.R., Gerken, G. & Hilgard, P. (2011). Impact of immunosuppression therapy on hepatitis C infection after renal transplantation. *Clinical Nephrology*, Vol.75, No.1, (January 2011), 16–25

Krawczynski K. (1999). Passive transfer of anti-HCV in chronic and acute HCV infection in chimpanzees – trials of experimental immune treatment [abstract]. *Hepatology,* Vol.30, Suppl. 1, 423A

Lake, J.R. & Wright, T.W. (1991). Liver transplantation for patients with hepatitis B: what have we learned from our results? *Hepatology,* Vol.13, No.4, (April 1991), 796–8

Lake, J.R., Shorr, J.S., Steffen, B.J., Chu, A.H., Gordon R.D. & Wiesner R.H. (2005). Differential effects of donor age in liver transplant recipients infected with hepatitis B, hepatitis C and without viral hepatitis. *American Journal of Transplantation*, Vol.5, No.3, (March 2005), 549–57

Lau, J.Y., Bain, V.G., Davies, S.E., O'Grady, J.G., Alberti, A., Alexander G.J. & Williams, R. (1992). High level expression of hepatitis B viral antigens in fibrosing cholestatic hepatitis. *Gastroenterology*, Vol.102, No.3, (March 1992), 956 – 62

Lo, C.M., Lau, G.K., Chan, S.C., Fan, S.T. & Wong, J. (2007). Efficacy of a pre-S containing vaccine in patients receiving lamivudine prophylaxis after liver transplantation. *American Journal of Transplantation*, Vol.7, No.2, (February 2007), 434–9

Maes, B.D., van Pelt, J.F., Peeters, J.C., Nevens, F., Evenepoel, P., Kuypers, D., Messiaen, T., Fevery, J. & Vanrenterghem, Y.F. (2001). The effect of mycophenolate mofetil on hepatitis B viral load in stable renal transplant recipients with chronic hepatitis B. *Transplantation*, Vol.72, No.6, (September 2001), 1165–6

Manousou, P., Samonakis, D., Cholongitas, E., Patch, D., O'Beirne, J., Dhillon, A.P., Rolles, K., McCormick, A., Hayes, P. & Burroughs, A.K. (2009). Outcome of recurrent hepatitis C virus after liver transplantation in a randomized trial of tacrolimus monotherapy versus triple therapy. *Liver Transplantation*, Vol.15, No.12., (December 2009), 1783–91

Martinez-Bauer, E., Carrion, J.A., Ramirez, S., Massaguer, A., Garcia-Valdecasas J.C., Sanchez-Tapias J.M. & Forns, X. (2006). Antiviral therapy of patients with decompensated cirrhosis to prevent hepatitis C recurrence after liver transplantation [abstract]. *Journal of Hepatology*, Vol.44, Suppl. No.2, (April 2006), S64

Marzano, A., Salizzoni, M., Debernardi-Venon, W., Smedile, A., Franchello, A., Ciancio, A., Gentilcore, E., Piantino, P., Barbui, A.M., David, E., Negro, F. & Rizzetto, M. (2001). Prevention of hepatitis B virus recurrence after liver transplantation in cirrhotic patients treated with lamivudine and passive immunoprophylaxis. *Journal of Hepatology*, Vol.34, No.6, (June 2001), 903–10

Mazzaferro V, Schiavo M, Caccamo L, et al. (2003). Prospective randomized trial on early treatment of HCV infection after liver transplantation in HCV-RNA positive patients [abstract]. *Liver Transplantation*, Vol.9, Suppl., 141A.

McMillan, J.S., Shaw, T., Angus, P.W. & Locarnini, S.A. (1995). Effect of immunosuppressive and antiviral agents on hepatitis B virus replication in vitro. *Hepatology*, Vol.22, No.1, (July 1995), 36–43

Mutimer, D., Pillay, D., Dragon, E., Tang, H., Ahmed, M., O'Donnell K., Shaw, J., Burroughs, N., Rand, D., Cane P., Martin, B., Buchan, S., Boxall, E., Barnat, S., Gutekunst, K., McMaster, P. & Elias, S. (1999). High pre-treatment serum hepatitis B virus titer predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. *Journal of Hepatology*, Vol.30, No.4, (April 1999), 715–21

O'Grady, J.G., Smith, H.M., Davies, S.E., Daniels, H.M., Donaldson, P.T., Tan, K.C., Portmann, B., Alexander, G.J. & Williams, R. (1992). Hepatitis B virus reinfection after orthotopic liver transplantation. Serological and clinical implications. *Journal of Hepatology*, Vol.14, No.1, (January 1992), 104–11

Pelletier, S.J., Iezzoni, J.C,, Crabtree, T.D., Hahn, Y.S., Sawyer, R.G. & Pruett, T.L. (2000). Prediction of liver allograft fibrosis after liver transplantation for hepatitis C virus. *Liver Transplantation*, Vol.6, No.1, (January 2000), 44–53

Pelletier, S.J., Schaubel, D.E., Punch, J.D., Wolfe, R.A., Port, F.K. & Merion, R.M. (2005). Hepatitis C is a risk factor for death after liver retransplantation. *Liver Transplantation*, Vol.11, No.4, (April 2005), 434–40

Perillo, R.P., Wright, T., Rakela, J., Levy, G., Schiff, E., Gish, R., Martin, P., Dienstag, J., Adams, P., Dickson, R., Anschuetz, G., Bell, S., Condreay, L. & Brown, N. (2001). A multicenter United States – Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. *Hepatology*, Vol.33, No.2, (February 2001), 424–32

Poynard, T., Bedossa, P. & Opolon, P. (1997). Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet*, Vol.349, No.9055, (March 1997), 825–32

Prieto, M., Gomez, M.D., Berenguer, M., Cordoba J., Rayon, J.M., Pastor M., Garcia-Herola, A., Nicolas, D., Carrasco, D., Orbis, J.F., Mir, J. & Berenguer, J. (2001) De novo hepatitis B after liver transplantation from hepatitis B core antibody-positive donors in an area with high prevalence of anti-HBs positivity in the donor population. *Liver Transplantation*, Vol.7, No.1, (January 2001), 51–8

Roche, B. & Samuel, D. (2007). Risk factors for hepatitis C recurrence after liver transplantation. *Journal of Viral Hepatitis*, Vol.14, Suppl.1, (November 2007), 89–96

Samuel, D., Bizollon, T., Feray, C., Roche, B., Ahmed, S.N., Lemonnier, C., Cohard, M., Reynes, M., Chevallier, M., Ducerf, C., Baulieux, J., Geffner, M., Albrecht, J.K., Bismuth, H. & Trepo, C. (2003). Interferon alpha 2b plus ribavirin with chronic C after liver transplantation: a randomized study. *Gastroenterology*, Vol.124, No.3, (March 2003), 642–50

Samuel, D., Muller, R., Alexander, G., Fassati, L., Ducot, B., Benhamou, J.P. & Bismuth, H. (1993). Liver transplantation in European patients with the hepatitis B surface antigen. *New England Journal of Medicine*, Vol.329, No.25 (December 1993), 1842–7

Selzner, N., Guindi, M., Renner, E.L. & Berenguer, M. (2011). Immune-mediated complications of the graft in interferon-treated hepatitis C positive transplant recipients. *Journal of Hepatology*, Vol.55, No.1, (July 2011), 207–17

Sharma, P., Marrero, J.A., Fontana, R.J., Greenson, J.K., Conjeevaram, H., Su, G.L., Askari, F., Sullivan, P. & Lok, A.S. (2007). Sustained virological response to therapy of recurrent hepatitis C after liver transplantation is related to early virological response and dose adherence. *Liver Transplantation*, Vol.13, No.8, (August 2007), 1100–8

Sheiner, P.A., Boros, P., Klion, F.M., Thung, S.N., Schluger, L.K., Lau, J.Y., Mor, E., Bodian, C., Guy, S.R., Schwartz, M.E., Emre, S., Bodenheimer, H.C.Jr. & Miller, C.M. (1998). The efficacy of prophylactic interferons alfa-2b in preventing recurrent hepatitis C after liver transplantation. *Hepatology*, Vol.28, No.3, (September 1998), 831–8

Sherman, M., Yurdaydin, C., Sollano, J., Silva, M., Liaw, Y.F., Cianciara, J., Boron-Kaczmarska, A., Martin, P., Goodman, Z., Colonno, R., Cross, A., Denisky, G., Kreter, B. & Hindes, R. (2006). Entecavir for treatment of lamivudine-refractory, HBeAg positive chronic hepatitis B. *Gastroenterology*, Vol.130, No.7, (June 2006), 2039–49

Singh, N., Gayowski, T., Wannstedt, C.F., Shakil, A.O., Wagener, M.M., Fung, J.J. & Marino, I.R. (1998). Interferon-alpha for prophylaxis of recurrent viral hepatitis C in liver transplant recipients: a prospective, randomized controlled trial. *Transplantation*, Vol.65, No.1 (January 1998), 82–6

Stangl, J.R., Carroll, K.L., Illichmann, M. & Striker, R. (2004). Effect of antimetabolite immunosuppressants on Flaviviridae, including hepatitis C virus. *Transplantation*, Vol.77, No.4, (February 2004), 562–7

Stravitz, R.T., Shiffman, M.L., Sanyal, A.J., Luketic, V.A., Sterling, R.K., Heuman, D.M., Ashworth, A., Mills, A.S., Contos, M., Cotterell, A.H., Maluf, D., Posner, M.P. & Fisher, R.A. (2004). Effects of interferon treatment on liver histology and allograft rejection in patients with recurrent C following liver transplantation. *Liver Transplantation*, Vol.10, No.7, (July 2004), 850–8

Takaki, A., Yagi, T., Iwasaki, Y., Sadamori, H., Matsukawa, H., Matsuda, H., Shinoura, S., Umeda, Y., Miyake, Y., Terada, R., Kobashi, H., Sakaguchi, K., Tanaka N. & Shiratori, Y. (2007). Short-term high dose followed by long-term low-dose hepatitis B immunoglobulin and lamivudine therapy prevented recurrent hepatitis B after liver transplantation. *Transplantation*, Vol.83, No.2 (January 2007), 231–3

Terrault, N., Roche, B. & Samuel, D. (2005). Management of the hepatitis B virus in the liver transplantation setting: a European and an American perspective. *Liver Transplantation*, Vol.11, No.7, (July 2005), 716–32

Terrault, N.A. (2003). Prophylactic and preemptive therapies for hepatitis C virus-infected patients undergoing liver transplantation. *Liver Transplantation*, Vol.9, No. 11, (November 2003), S95 – S100

Verna, E.C. & Brown, R.S.Jr. (2008). Hepatitis C and liver transplantation: enhancing outcomes and should patients be retransplanted. *Clinics in Liver Disease*, Vol.12, No.3, (August 2008), 637–59

Villeneuve, J.P., Durantel, D., Durantel, S., Westland, C., Xiong S., Brosgart C.L., Gibbs, C.S., Parvaz, P., Werle, B., Trepo, C. & Zoulim, F. (2003). Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. *Journal of Hepatology*, Vol.39, No.6, (December 2003), 1085–9

Watashi, K., Hijikata, M., Hosaka, M., Yamaji, M. & Shimotohno, K. (2003). Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology*, Vol.38, No.5, (November 2003),1282–8

Wiesner, R.H., Sorrell, M. & Villamil, F. (2003) Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transplantation*, Vol.9, No.11, (November 2003), S1–S9

Yeo, W., Hui, E.P., Chan, A.T., Wong, W.L., Ho, W.M., Lam, K.C. & Johnson, P.J. (2004). Comprehensive analysis of risk factors associating with Hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy. *British Journal of Cancer*, Vol.90, No.7, (April 2004), 1306–11

Zimmerman, M.A. & Ghobrial, R.M. (2005). When should't we retransplant? *Liver Transplantation*, Vol.11, Suppl.2, (November 2005), S14–S20

Zoulim, F., Radenne, S. & Ducerf, C. (2008). Management of patients with decompensated hepatitis B virus association cirrhosis. Liver Transplantation, Vol.14, Suppl. 2, (October 2008), S1–S7

Part 7

Other Indications for Liver Transplantation

12

Role of Liver Transplantation in Acute Liver Failure

Saleh A. Alqahtani[1] and Anne M. Larson[2]
[1]*University of Texas Southwestern Medical Center*
[2]*Swedish Medical Center*
USA

1. Introduction

Orthotopic liver transplantation (OLT) was initially developed in the 1960s as treatment for individuals dying of end-stage liver disease. It began to be utilized in the 1980s as salvage therapy in the setting of acute liver failure (ALF). Prior to the use of OLT, ALF mortality rates reached 80-85%, and the early post-transplant survival rates were much lower than those following transplantation for chronic liver diseases (Bernuau et al., 1986a). Over the past thirty years, however, with advances in critical care management and in the field of liver transplantation, 1-year survival rates following OLT for ALF have improved to 60-80% (Bismuth et al., 1995; DeVictor et al., 1992; Hoofnagle et al., 1995; W Lee, 2003; O'Grady et al., 1988). ALF is one of the few conditions for which a patient can be listed as a United Network for Organ Sharing (UNOS) status 1A (urgent) patient in the United States and "super urgent" in the United Kingdom. Although about half of ALF patients undergo OLT, ALF accounts for less than 10% of US transplants and approximately 11% in Europe (Freeman et al., 2008).

2. Prognosis and prognostic models

It is essential to quickly and accurately identify those patients most likely to benefit from emergent OLT. In the setting of organ shortage, it is also important to identify and even delist patients who are too ill to benefit from OLT. To minimize the risk of unnecessarily committing individuals to lifelong immunosuppression, one must balance the desire to delay transplantation to allow for the potential of spontaneous recovery against the risk of death with that delay and the risk of the surgery itself. Many ALF patients who have been listed for OLT will recover spontaneously without transplantation and it is estimated that as many as 20% of patients may be transplanted needlessly. In addition, a significant number of ALF patients listed for OLT will die awaiting a donor organ. In the United Kingdom, about 30% of patients initially considered for OLT ultimately become untransplantable following the development of complications (i.e., cerebral edema, sepsis, hemodynamic abnormalities, multiorgan system failure) (Bernal et al., 1998). Additionally, many patients have medical or psychosocial contraindications to transplantation, including irreversible brain injury, underlying cardiovascular disease, infection/sepsis, alcohol or drug abuse, poorly controlled psychiatric disease, or inadequate family support (Simpson

et al., 2009). Thus, it is important to identify and delist patients who are too ill to benefit from OLT.

It is crucial that reliable predictive models of survival and the need for OLT be developed. Successfully predicting outcome would allow more judicious use of scarce organs and spare those who will ultimately recover the need for lifelong immunosuppression. There are at present no standardized criteria to predict who should be listed for OLT. Several prognostic models have been developed to help identify appropriate patients for transplantation. Unfortunately, these prognostic models have limitations, and their predictive accuracy varies (Blei, 2005; Anand et al., 1997).

2.1 Predictors of prognosis

Many factors may help determine which ALF patients are more likely to die; however, they are generally unreliable in predicting who will ultimately survive or require transplantation.

2.1.1 Etiology

The etiology of ALF is one of the most important predictors of spontaneous outcome (Ostapowicz et al., 2002). The lowest mortality is seen with ALF secondary to acetaminophen (N-acetyl-p-aminophenol; APAP) toxicity (~30%), hepatitis A virus infection (~50%), shock liver, and pregnancy-related ALF (Larson et al., 2005; Ostapowicz et al., 2002; Schiodt et al., 2003). In contrast, the non-transplant mortality for the remainder of causes, including ALF secondary to drug-induced liver injury, remains abysmal (80% to 100%) (O'Grady et al., 1988; Ostapowicz et al., 2002). Therefore, understanding which causes of ALF predominate in a particular region can help lead to the early evaluation and listing of these latter cases for OLT.

ALF secondary to drug-induced liver injury (DILI) predominates in Europe and North America, with a high prevalence of APAP-induced ALF in the US and United Kingdom (Hoofnagle et al., 1995; Larson et al., 2005; Ostapowicz et al., 2002; Schiodt et al., 1999; Williams, 1996). Viral hepatitis predominates in developing countries. The US ALF study group looked at 1198 patients with ALF over an 11 year period and a total of 133 (11.1%) subjects were deemed by expert opinion to have DILI ALF. Transplant-free (3-week) survival was poor (27.1%), but with successful transplantation in 42.1%, overall survival was 66.2%. Transplant-free survival in DILI ALF is determined by the degree of liver dysfunction, specifically baseline levels of bilirubin, prothrombin time/international normalized ratio (PT/INR), and Model for End-Stage Liver Disease (MELD) scores (Reuben et al., 2010).

In the United Kingdom, the number of patients with APAP ALF has declined due to legislative changes in drug packaging, leading to an increase in the relative number of cryptogenic or seronegative cases (16% of all ALF). The majority (88%) of these latter ALF patients met King's College transplant criteria (see below), reflecting the low likelihood of spontaneous recovery (Wigg et al., 2005). A recent study from the UK suggested that OLT is a more favorable approach to managing patients with non-APAP induced ALF compared to patients with APAP induced ALF. This was predominantly due to the frequent psychosocial contraindications in patients with APAP induced ALF (Simpson et al., 2009).

2.1.2 Clinical and laboratory criteria

The clinical criteria most commonly used to exclude a patient from OLT vary by transplant center but may include age older than 70 years, the presence of certain malignancies outside of the liver, severe cardiac, lung, or multiple organ failure, severe infection, uncontrolled septic shock and brain death (Table 1) (Samuel & Bismuth, 2001). Patients with grade 3-4 encephalopathy and fixed pupils, cerebral perfusion pressure <40 mmHg, sustained elevation in intracranial pressure >50 mmHg, or seizures are at high risk for postoperative neurologic complications or brain death. They are usually not deemed candidates for OLT (Bismuth et al., 1995). As long as the pupils remain active and the patient does not have posturing movements, liver transplantation can still be considered (Daas et al., 1995). The degree of serum aminotransferase elevation and the rate of its recovery do not predict prognosis. In fact, improvement of aminotransferase levels in conjunction with worsening bilirubin, hepatic encephalopathy, and coagulopathy (INR) signals complete liver failure and is a particularly ominous sign.

Common Exclusion Criteria
Age >70 years old (relative)
Certain malignancies outside of the liver
Severe cardiac, lung, or multiple organ failure
Severe infection
Uncontrolled septic shock
Brain death
UNOS Status 1a Listing Criteria for ALF
Age ≥ 18 years
Life expectancy without a liver transplant of <7 days
Onset of encephalopathy within 8 weeks of the first symptoms of liver disease
Absence of pre-existing liver disease (except for the diagnosis of fulminant Wilson's disease)
Residence in the intensive care unit
At least one of the following: ventilator dependence, renal replacement therapy, or INR >2.0

Table 1. Exclusion and Listing Criteria for Transplantation for Acute Liver Failure. INR-international normalized ratio

2.1.3 Multiorgan failure

The severity of multiorgan failure at the time of OLT is also a predictor of post-transplant survival. Decreased renal function is associated with worse spontaneous survival in non-APAP-induced liver injury. In a multivariate analysis of UNOS data (1988–2003), four risk factors predicting post-transplant survival were identified: pretransplant use of life support, recipient age >50 years, recipient body mass index ≥30 kg/m2, and serum creatinine >2 mg/dL. If an individual had all of these risk factors, the 5-year post-transplant survival was only 44–47%. Whereas, if none of these features were present, the 5-year post-transplant survival was 82–83% (Barshes et al., 2006).

2.1.4 Hepatic encephalopathy

Mortality rates correlate with the severity of hepatic encephalopathy (HE), reported at 30% for grade 2, 45-50% for grade 3 and 80-90% for grade 4 HE (Daas et al., 1995; Hoofnagle et al., 1995). A multicenter US series, in which 39% participants had APAP hepatotoxicity, showed a 52% 3-week transplant-free survival in patients with grade 1-2 encephalopathy, but only 33% with grade 3-4 HE survived without transplant (Ostopowicz et al., 2002). Conversely, 85% of patients with non-APAP ALF without HE experienced spontaneous recovery (Elinav et al., 2005). Paradoxically, those with more rapid development of HE (i.e., APAP-induced) appear to have a better outcome than those with a longer interval between the development of symptoms and HE (i.e., DILI) (Bernuau et al., 1986a; O'Grady et al., 1989; O'Grady et al., 1993). A distinctive feature ALF-induced HE is the development of cerebral edema, the complete pathophysiology of which remains poorly understood. Cerebral edema develops in nearly 80% of patients who progress to grade 4 HE, leading to intracranial hypertension with subsequent ischemic brain damage or brainstem herniation, accounting for up to 50% of ALF mortality (Clemmensen et al., 1999; Jalan et al., 2003). Intracranial pressure (ICP) monitoring is more often utilized in patients who are deemed candidates for OLT, and ICP may be more aggressively managed in these cases. ICP monitors may also be of significant value during the transplant operation, when fluctuations in ICP are common (Philips et al., 1998). ICP monitoring is associated with up to a 10% risk of intracranial hemorrhage, and it has not been shown to change 30 day post-OLT survival (Gasco et al., 2010). Thus, the indication and timing of use of ICP monitoring devices remain controversial (Vaquero et al., 2005). Intracranial hypertension may persist during the first 10-12 hours following liver transplantation, thus ICP monitoring, if utilized, should continue during and after surgery (Bismuth et al., 1995; Jalan et al., 2003).

2.1.5 Infection

ALF-induced hemodynamic changes can be difficult to distinguish from infection and sepsis and are complicated by the fact that ALF patients may not develop leukocytosis or fever. Bacterial infection is the cause of death in up to 37%, with the most common sites of infection being pulmonary (47%), blood (26%), and urine (23%) (Bernal et al., 2003). Fungal infections, especially Candida sp., are seen in up to 32%, occur later in the course of disease, particularly after use of antibiotics or in the setting of renal dysfunction, and are often associated with bacterial infection (Rolando et al., 1991; Vaquero et al., 2003). Active infection is a contraindication to OLT. The empiric use of antibiotics is controversial. Prophylactic antibiotics decrease the number of infections, but do not change overall outcome (Rolando et al., 1990; Rolando et al., 1996; Stravitz et al., 2007). Some centers administer anti-infectives (antibacterial and antifungal) to patients who have significant isolates on surveillance cultures, have progression to Stage 3-4 HE, have refractory hypotension, or have clinical evidence of systemic inflammatory response syndrome (Stravitz et al., 2007). Periodic surveillance cultures and frequent chest radiographs can help detect bacterial and fungal infections early.

2.1.6 Psychosocial predictors

The burden of medical follow-up after OLT can be substantial, and quality of life can be significantly affected. Therefore, the decision to offer OLT to an individual patient also

needs to consider more controversial issues such as psychosocial factors (i.e., adequacy of social support and substance and/or alcohol abuse), and adequacy of medical insurance coverage. For example, in one study, four patients (12%) died in the post-transplant follow-up period from deliberate self-harm (Bernal et al., 1998).

2.2 Prognostic models

Multiple prognostic models have been proposed to help determine the likelihood of spontaneous survival (Table 2) (Antoniades et al., 2007; Bailey et al., 2003; Bernuau et al., 1986b; Bernuau, 1993; Craig et al., 2010; Harrison et al., 1990; Itai et al., 1997; O'Grady et al., 1989; Pereira et al., 1992; Rolando et al., 2000; Schiodt et al., 2005; Van Thiel, 1993). However, many of these models are methodologically flawed and subject to bias. In addition, many equate OLT with death, which falsely elevates the positive predictive value of these prognostic systems (Craig et al., 2010).

Variable	Clichy	King's Criteria APAP	King's Criteria non-APAP	APACHE II	MELD
Factor V Level	X				
Age	X		X	X	
Hepatic Encephalopathy	X	X		X	
Arterial pH		X		X	
INR		X	X		X
Serum Creatinine		X		X	X
Etiology			X		
Serum Bilirubin			X		X
Duration of Jaundice			X		
Vital Signs (T, BP, HR, RR)				X	
Oxygenation				X	
Serum Na & K				X	
WBC				X	
Hematocrit				X	

Table 2. Comparison Between the Various Prognostic Scoring Systems for Acute Liver Failure APAP-acetaminophen; MELD-model for end-stage liver disease; INR-international normalized ratio; T-temperature; BP-blood pressure; HR-heart rate; RR-respiratory rate; Na-sodium, K-potassium; WBC-white blood cell count. Clichy (Bernuau et al, 1986); King's Criteria (Bernal et al., 2002; O'Grady et al., 1989), Apache II (Mitchell et al., 1998); MELD (Schmidt & Larsen, 2007; Villamil et al., 2007; Wiesner, 2004; Yantorno et al., 2004; Zaman et al., 2006)

2.2.1 King's college hospital criteria

The most widely applied prognostic system are the King's College Hospital criteria (King's criteria) developed from a retrospective cohort of nearly 600 patients (Bernal et al., 2002;

O'Grady et al., 1989). The Kings criteria incorporate both the etiology of ALF (APAP- versus non-APAP induced ALF) and clinical parameters of disease (O'Grady et al., 1989). In a meta-analysis of studies using the Kings criteria, the pooled sensitivity and specificity was 69% and 92%, respectively (Bailey et al., 2003). The Kings criteria appear to have high positive predictive values (80% in APAP induced ALF and 70-90% in non-APAP induced ALF) but poorer negative predictive values (70-90% and 25-50%, respectively). A recent meta-analysis found that the Kings criteria for non-APAP induced ALF have good specificity, especially for patients with high grade encephalopathy (McPhail et al., 2005). The Kings criteria are helpful in identifying those who may need OLT, but up to 20% of those meeting criteria potentially could have survived without OLT, and those not meeting criteria may still require transplantation. The addition of arterial blood lactate levels to the model has improved its sensitivity (Bernal et al., 2002; MacQuillan et al. 2005).

2.2.2 Other models and predictors

Other Models and Predictors. The Clichy criteria were developed in a cohort of French patients with acute hepatitis B virus infection (Bernuau et al., 1986b). These criteria suggest that a serum factor V level of <20% in patients younger than 30 years or <30% in any patient with grade 3-4 HE has validity as a marker of mortality. The criteria predicted a poor outcome with a sensitivity and specificity of 86% and 76%, respectively. Factor V level measurements are less readily available to the clinician than are the measures in the Kings criteria, therefore, this prognostic model is not commonly utilized (Izumi et al., 1996; Pauwels et al., 1993). In addition, this model has not been validated in the non-HBV population. A factor V <10% has been shown to predict a poor outcome with a sensitivity of 91% and a specificity of 100%; while a factor VIII : V ratio of >30 similarly predicts outcome (91% sensitivity, 91% specificity) (Pereira et al., 1992). The admission Acute Physiology and Chronic Health Evaluation (APACHE) II is ineffective in predicting who will survive without transplantation, since many patients who do not meet the severity criteria will ultimately die of subsequent complications (Mitchell et al., 1998).

Elevated arterial ammonia levels increase the risk of developing intracranial hypertension. A level of >150 μmol/L predicts development of intracranial hypertension with a sensitivity of 60% and a specificity of 84% (Kitzberger et al., 2009). Concentrations of more than 100-150 μmol/L have been positively correlated with cerebral herniation (Bernal et al., 2007; Bhatia et al., 2006; Clemmensen et al., 1999; Toftent et al., 2006).

Serum alpha-fetoprotein (AFP) is generally considered a marker of hepatocellular regeneration. There has been no consistent correlation seen between the absolute AFP level and outcome in ALF (Tofteng et al., 2006). However, an increasing AFP level has been strongly associated with a more favorable outcome (Schiodt et al., 2006; Yang et al., 2002). A threshold AFP of ≤3.9 μg/L at 24 hours following the peak ALT identified nonsurvivors with a sensitivity and specificity of 100% and 74%, respectively, and a negative predictive value of 100% (Schmidt et al., 2005). In addition, it has been shown that a rising AFP level between day 1 and day 3 from presentation predicted survival without transplantation, whereas a decreasing level was seen in 80% of those who died (Schiodt et al., 2006).

Persistently elevated phosphate levels may be associated with a poorer prognosis in the setting of acetaminophen-induced ALF. A serum phosphate level >1.2 mmol/L on day 2 or

3 following APAP overdose carries a sensitivity of 89% and a specificity of 100% for predicting poor outcome (Baquerizo et al., 2003; Chung et al., 2003; Schmidt et al., 2003). The level of Gc-globulin, a protein which is markedly reduced in the setting of tissue injury, has not been shown to reliably predict survival in those with APAP-induced ALF. In non-APAP ALF; however, using a cutoff value of ≤80 mg/L, the Gc-globulin level carries a positive predictive value of 74% and a negative predictive value of 81% (Schiodt et al., 2005; Schiodt et al., 2007)). An elevated arterial blood lactate level following volume resuscitation predicts worse survival in APAP-induced ALF (Bernal et al., 2002; Cholongitas et al., 2008; MacQuillan et al. 2005).

The model for end-stage liver disease (MELD) scoring system, is an excellent prognostic model for chronic liver disease (Schmidt & Larsen, 2007; Villamil et al., 2007; Wiesner, 2004; Yantorno et al., 2004; Zaman et al., 2006). However, it has been limited as a prognostic model in ALF, because it does not account for most of the extremely important outcome predictors in ALF, including age, etiology of ALF and duration of jaundice. The MELD score has a sensitivity and specificity of <75% for predicting outcome in all forms of ALF (Bernal et al., 2007; Dhiman et al., 2007; Riorden & Williams, 2003). Whether modification of the MELD with these important factors would improve the MELD as standard scoring system in prognosis of ALF remains to be seen. Dhiman and colleagues compared clinical predictors of MELD and Kings criteria in patients with ALF. Clinical predictors were superior to both MELD and Kings criteria in predicting prognosis of ALF. Significant factors included age >50 years, jaundice to encephalopathy time greater than 7 days, grade 3-4 encephalopathy, cerebral edema, prothrombin time ≥35 seconds, and serum creatinine ≥1.5mg/dL) are associated with a poor prognosis. The presence of 3 or more of these factors is associated with a poorer prognosis (Dhiman et al., 2007; O'Grady et al., 2007). Molecular markers of cell apoptosis have also been found to be helpful prognostic factors. Bechmann looked at replacing the bilirubin value in the MELD score with the ratio of CK18/M65, a marker of cell death. This model was found to be associated with higher sensitivity and specificity. Although this study was limited by a small number of patients, the idea of using molecular markers of cell death in predicting prognosis of ALF is promising and needs to be studied in larger cohorts (Bechmann et al., 2010). Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury.

In effort to develop a functional scoring model for non-APAP induced ALF, Miyaki and colleagues looked at 4 prognostic factors – etiology of ALF, hepatic coma grade (III or IV), systemic inflammatory response syndrome, and ratio of total to direct bilirubin (>2.0). The authors found these factors to be predictors of 2-week outcome with high positive and negative predictive values, 93.3%, and 81.8%, respectively (Miyake et al., 2005). This prognostic model would help the clinician predict prognosis and consideration for liver transplantation for patients with non-APAP ALF, however it requires validation before it can be widely clinically applied.

A liver volume of <1000 mL on computed tomography (CT) imaging is also associated with a high mortality rate, a finding which has been validated (Shakil et al., 2000; Yamagishi et al., 2009). Based upon these findings, a prognostic formula has been proposed, but is not widely utilized. Liver biopsy may also be helpful in determining the cause of the ALF and, theoretically, the severity and extent of liver damage. Hepatic necrosis of more than 70% was associated with a transplant free survival of <10% in one analysis (Scotto et al., 1973).

However, there is a great degree of sampling error, and more recently, a multivariate analysis of 97 consecutive patients found that the amount of necrosis was not predictive of mortality (Miraglia et al., 2006; Voigt et al., 2007).

Based upon the available data, the current prognostic scoring systems have not consistently demonstrated reliable accuracy in predicting outcome from ALF and the subsequent need for OLT. Therefore, the American Association for the Study of Liver Diseases (AASLD) does not recommend reliance on any one of these systems (W Lee & Larson, 2005).

3. Liver transplantation

As previously noted, advances in critical care management of ALF patients has improved the spontaneous survival from 10-20% to about 40% without transplantation (Ostapowicz et al., 2002). For those who will not spontaneously recover; however, OLT remains the only treatment modality that improves survival. With the advent of use of OLT in this setting, overall survival rates have further improved to about 60%.

3.1 Transplant listing criteria

Candidacy for liver transplantation must be determined quickly in the setting of ALF, given the rapid progression of the syndrome. In the US, ALF is one of the few conditions for which a patient can be listed as a United Network for Organ Sharing (UNOS) status 1A (urgent) patient (available at http://www.unos.org) (Table 1). ALF patients may be listed in the "super urgent" category in the United Kingdom. Approximately half of ALF patients undergo liver transplantation; however, ALF accounts for less than 10% of US transplant and 11% in Europe (Freeman et al., 2008; Organ Procurement & Transplantation Network [OPTN], 2009).

3.2 Types of liver transplantation

In addition to whole-organ deceased donor liver transplantation (DDLT), which is preferred, various types of liver transplantation may be considered depending on the situation: living donor liver transplants, ABO-compatible transplants, ABO-incompatible transplants and auxiliary liver transplants. In the setting of organ shortage, the risk of mortality awaiting an organ should be weighed against the risk of complications or failure using an alternative graft (Table 3).

	OLT	LDLT	ABO-compatible	ABO-incompatible	Heterotopic auxiliary LT	Auxiliary partial LT
Graft	75	56-90	49-54	39-52		
Patient	82	59-90		30	33	71

Table 3. One-Year Post-Transplant Sruvival Rates for Acute Liver Failure (percent). OLT-orthotopic liver transplantation; LDLT-living donor liver transplantation, LT-liver transplantation. OLT (O'Mahony et al., 2007); LDLT (Ichida et al., 2000; S Lee et al., 2007; Miwa et al., 1999; Uemoto et al., 2000); ABO-Compatible (Bismuth et al., 1996b); ABO-incompatible (Bismuth et al., 1996b; Farges et al., 1995); Heterotopic (Van Hoek et al., 1999); Auxillary Partial (Van Hoek et al., 1999)

3.2.1 Living donor liver transplantation (LDLT)

The use of LDLT in this setting remains controversial (Campsen et al., 2008; Liu et al., 2002; Nishizaki et al., 2002; Uemoto et al., 2000). It is imperative to consider the need for an adequately sized graft for the recipient with the requirement of a sufficient residual liver mass for the donor. Grafts over 40% of the standard liver volume are necessary in the setting of ALF, and outcomes are better with a graft-to-recipient weight ratio greater than 0.8, with 1.0 being ideal (Kawasaki et al., 1998Kiuchi et al., 1999). A graft of <40% of standard liver weight is at risk for the development of small-for-size syndrome – portal hypertension following reperfusion leading to sinusoidal damage and graft injury (Man et al., 2003). In the absence of small-for-size syndrome following OLT, the graft and donor livers regenerate to full size in a matter of 4 weeks (Marcos et al., 2000). Despite these risks, right lobe LDLT improves survival in patients with ALF, with overall 1-year survival rates of between 60-90%, averaging about 75% (Campsen et al., 2008; Ichida et al., 2000; S Lee et al., 2007; Miwa et al., 1999; Uemoto et al., 2000). For children undergoing LDLT, the 1-year survival was 67-89% and death on the waiting list was decreased to 9% (Casas et al., 1999; Emre et al., 1999). In the SPLIT experience of pediatric transplantation, 57% of the recipients with ALF received partial grafts, without a difference in outcome compared to recipients of whole grafts (Baliga et al., 2004).

Unique ethical issues exist in the setting of LDLT. Given the urgent need for an organ in this setting, the donor evaluation must be expedited. The time required for thorough donor medical and psychosocial evaluation may be truncated in the setting of rapid clinical deterioration of the intended recipient (Abouna, 2001). This carries the risk of an incomplete evaluation and the possibility of donor coercion. The 1997 Council of European Recommendations argued against the use of LDLT for ALF due to the theoretical risk of coercion, with the assumption that patients were undergoing transplantation without significant waiting times (Committee of Ministers, 1997). In regions where cadaveric organs are not as readily available, the risk of the recipient's death while waiting for a cadaveric organ must be weighed against the risk to the living donor, including a 0.2% mortality (Ghobrial et al., 2008; Yasutomi et al., 2000). Protocols will likely need to be established to address these concerns (Carlisle et al., 2011; Reding, 2005).

It has been suggested that instead of comparing the donor risks to the recipient benefits, one should compare the donor risks to the donor benefits. Some individuals may feel rewarded by being a donor (such as parent to child donation) (Spital, 2005). Mathematical modeling suggests the sickest patients or those with highest risk of death while on the waiting list would receive more benefit from living donation than those who are less sick (Durand et al., 2006). Donors surveyed in the year following donation (two thirds responded), appeared to be doing well from a psychosocial perspective, but their well-being was linked to recipient outcomes (Kim-Schluger et al., 2002). A prospective German study evaluated the psychological impact on potential donors during evaluation for urgent indications for LDLT. They found that there was more mental stress compared to the general population, explained by the recipient's severity of illness. Donors had more postoperative pain, particularly somatoform pain, and decreased vitality. Three months after LDLT, donor mental quality of life, depression, and anxiety scores were again normal, although they were somewhat linked to recipient outcomes (Erim et al., 2007). The US Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) group reported that 4.1% of all donors

(392) had experienced one or more psychiatric complication. Three had severe psychiatric complications, including suicide, accidental drug overdose, and suicide attempt, despite the well-being of the recipients. Although there was no clear explanation why these donors, despite detailed screening, would be at increased risk for psychiatric problems, they suggested that donors need careful preoperative assessments and perhaps prolonged post-operative monitoring (Trotter et al., 2007).

3.2.2 ABO-incompatible grafts

Although ABO-identical grafts are preferred, ABO-compatible grafts (e.g., O graft; A recipient) have comparable 1-year patient survival following OLT and 49-54% 1-year graft survival (Bismuth et al., 1996a). However, ABO-incompatible grafts (e.g., A graft, B recipient) have less favorable outcomes and experience diminished 1-year graft survival rates of about 30% (Bismuth et al., 1996a). Patient survival was not affected by ABO compatibility for patients who had ALF, but the grafts suffered a greater incidence of hyperacute rejection (20%), vascular thrombosis, and/or biliary injury (56%). A Canadian group reported overall 5-year graft survival rates of 54–60% and 5-year patient survival rates of 61–77%, although the number of study subjects was small (Toso et al., 2007). The Birmingham group published their experience in liver transplantation for 29 children of < 5 kg weight, five of whom underwent for ABO-incompatible grafts. They found no difference in transplant outcome or survival between ABO- incompatible vs. ABO-compatible graft (Gelas et al., 2011). A recent meta-analysis found that ABO-incompatible grafts have excellent outcome in children but not in adult liver transplantation (Wu et al., 2011). At present, up to 60% survival of the graft is generally seen in ABO-incompatible transplants, likely related to intensive management (use of quadruple immunosuppression, postoperative plasmapheresis, splenectomy, methylprednisolone, or prostaglandin E1) (Egawa et al., 2004; Farges et al., 1995; Hanto et al., 2003; Sugawara & Makuuchi, 2006). Overall, controversy remains as to whether ABO-compatible and ABO-identical OLT leads to equivalent post-transplant outcomes. Some studies have reported no statistical difference in graft survival, while others have demonstrated that patient survival is less in ABO-compatible recipients compared to ABO-identical recipients. ABO-identical OLT is still preferred (Aladag et al., 2006; Bjoro et al., 2003; Koukoutsis et al., 2007); Smith et al., 2000).

3.2.3 Auxiliary transplantation

Auxiliary transplantation leaves the recipient's liver in place and utilizes a partial left or right lobe from the donor which acts as temporary support for the recipient's injured liver. Ideally, once the native liver recovers, immunosuppression may be withdrawn and the graft is either surgically removed or is allowed to atrophy naturally (Bismuth et al., 1996b; Chenard-Neu et al., 1995; Chenard-Neu et al., 1996). The partial graft is placed below the native liver (heterotopic auxiliary transplantation) or replaces a resected right or left native lobe (auxiliary partial liver transplantation.) While easier to perform, implantation of the heterotopic graft onto the infrahepatic vena cava may induce venous outflow obstruction, resulting in slower hepatocyte regeneration, presumably due to cytokine release from residual necrotic liver tissue. There is also an increased incidence of primary graft non-function and portal vein thrombosis with heterotopic auxiliary transplantation compared to auxiliary partial or whole graft OLT (Van Hoek et al., 1999). Despite a similar patient

survival rate compared to conventional OLT, unique postoperative complications may develop following auxiliary transplantation, including biliary and neurologic problems (Azoulay et al., 2001). Portal blood flow is partially diverted from the native liver to the auxiliary graft, therefore, regeneration of the native liver and graft function may be impaired. Moreover, due to the smaller mass of the transplanted liver, cerebral edema and neurologic dysfunction may continue to progress (Bismuth et al., 1996b). In addition, leaving the necrotic graft in situ following immunosuppression withdrawal may lead to the development of multi-system organ failure, or over time, cirrhosis may develop in the native liver (Chenard-Neu et al., 1996; Pereira et al., 1997).

The best outcomes with auxiliary transplantation are in young patients with hyperacute presentations due to a viral or autoimmune disorder, but this group also has the greatest chance of spontaneous recovery (Chenard-Neu et al., 1995; Chenard-Neu et al., 1996; Brandsaeter et al., 2002). Overall patient survival rate for auxiliary transplantation is reported to be between 60% and 65% and up to 85% of these survivors were able to discontinue immunosuppressive therapy by one year following transplantation (Bismuth et al., 1996b; Boudjema et al., 2002; Chenard-Neu et al., 1995; Van Hoek et al., 1999). However, those who had auxiliary partial transplantation have the greater 1-year survival rate, whereas those who underwent heterotopic transplantation had a diminished 1-year survival rate of only 33% (Van Hoek et al., 1999). Fifteen percent of the patients who underwent auxiliary transplantation had to undergo retransplantation for a variety of reasons. More recently, Faraj et al. looked at the long term outcome of 20 children who underwent auxiliary liver transplantation in the UK, the 1 and 10 year survival in this group of children was 85% (Faraj et al., 2010).

4. Transplant outcomes

Unfortunately, medical contraindications may develop quickly over the course of illness, thereby preventing OLT. This was demonstrated in patients with APAP-induced ALF who fulfilled KCH criteria. Thirty percent were not listed due to the rapid development of pre-operative contraindications to surgery and 35% of those who were listed were eventually delisted or not transplanted because of rapid clinical deterioration. The majority (90%) who met transplantation criteria but did not undergo OLT died (Bernal et al., 1998). In the largest US study, 29% of ALF patients underwent OLT but 25% of those listed (10% of the entire group) died prior to receiving an organ (Ostapowicz et al., 2002). In general, about 15-30% of patients die before OLT can be performed, usually due to brain death but other causes include sepsis, hemodynamic instability, multiple organ failure, and gastrointestinal bleeding (Bismuth et al., 1995; Castells et al., 1993).

4.1 Survival with transplantation

There are unique postoperative issues that afflict ALF patients. Despite OLT, elevated ICP and cerebral edema can persist for up to a day or more. In ALF patients who die post-OLT, as many as 13% succumbed to brain death (Barshes et al., 2006). Protective strategies, such as continued ICP monitoring, may be helpful through this period of risk. Although renal function often improves dramatically, patients may require renal replacement therapy for many weeks post-OLT, particularly in the setting of APAP-ALF. Immunosuppressive

strategies that attempt to minimize nephrotoxic agents, such as calcineurin inhibitors, in this critical recovery period may be necessary. Nearly one third of post-OLT deaths in this setting are from bacterial or fungal infections (Barshes et al., 2006). The majority of these deaths occur within the first 2–3 months following the transplantation, usually due to neurologic complications or sepsis (Bismuth et al., 1995; DeVictor et al., 1992; Hoofnagle et al., 1995; W Lee, 2003; O'Grady et al., 1988; Russo et al., 2004; Wigg et al., 2005). In the largest Canadian study (60 patients transplanted between 1994-2007); the wait-list mortality rate was 6% with mean waiting time of 2.7 days. The perioperative mortality rate was 15%, and complications included neurological problems (13%), biliary problems (10%), and hepatic artery thrombosis (5%) (Chan et al., 2009). The Canadian data suggested that cerebral edema and extended criteria donor graft are associated with worse outcome.

The severity of multi-organ failure at the time of OLT is a good predictor of post-transplant survival (Devlin et al., 1995). Decreased renal function is also associated with worse spontaneous survival in non-APAP induced liver injury (Moore et al., 1991). In a multivariate analysis of UNOS data (1988-2003), four risk factors predicting post transplant survival were identified: history of life support, recipient age >50 years, recipient body mass index ≥30 kg/m2, and serum creatinine >2 mg/dL. If an individual had all of these risk factors, the 5-year post transplant survival was only 44-47%. Whereas, if none of these features were present, the 5-year post transplant survival was 82-83% (Barshes et al., 2006). The quality of the graft also impacts post-transplant outcome (Bismuth et al., 1995). Graft steatosis, reduced graft size, and ABO-incompatible grafts have all been shown in multivariate analyses to lead to decreased patient and graft survival (Bernal et al., 1998; Bismuth et al., 1995). On multivariate analysis of data from the United Kingdom, the strongest predictor of early mortality in seronegative ALF was higher donor body mass index (BMI), which may be a marker for donor graft steatosis (Wigg et al., 2005). This group found an odds ratio (OR) of 1.2 for every unit increase in donor BMI relative to a normal donor (BMI 25 kg/m2). For example, the OR for early death following OLT with a liver from an obese donor (BMI 35 kg/m2) is 1.2 to the power of 10 or 1.2^{10} which is an OR of 6.2. The next most predictive variables were recipient age >50 years (OR 4.2) and non-Caucasian ethnicity (OR 4.9) Additional factors which have been reported to influence survival in ALF include recipient age >60 years, donor age >60 years, and mechanical ventilation at the time of transplant (O'Mahony et al., 2007; Mas et al., 2010). Unfortunately, graft quality needs to be weighed against the time factor, since patients may deteriorate while waiting for optimal grafts, sometimes to the point when they are no longer feasible candidates. Suboptimal grafts may fail; however, leading to the need for retransplantation.

Some advocate the use of venovenous bypass during the operation, but this is not uniformly practiced. Bypass is thought to minimize changes in cerebral perfusion pressure during the clamping of the inferior vena cava and portal vein as well as during reperfusion (Bismuth et al., 1996a; Jalan et al., 2003). Hepatectomy of the native liver with temporary portocaval anastamosis in certain patients may achieve temporary hemodynamic stabilization, with the expectation that a suitable graft will be available within the next 24-28 hours (Ejlersen et al., 1994; Ringe et al., 1993).

In infants with ALF transplanted between 1986 and 2000, only 24% had spontaneous recovery. Nearly half (47%) succumbed to sepsis or multiorgan failure, and 29% underwent

OLT, of which half were still alive at a mean follow-up of 5 years. The authors concluded that infants had worse prognosis with ALF, since the etiology was more commonly an inborn error of metabolism. Extrahepatic disease sometimes excluded OLT as a means of treatment (Durand et al., 2001). The Studies of Pediatric Liver Transplantation (SPLIT) Research Group found that 13% of all primary transplants performed between 1995 and 2002 in children were done for ALF and that the majority of these cases were from unknown (indeterminate) causes (89%). The 3-month spontaneous survival was markedly diminished for children with ALF compared to those without (59% vs. 96%) and 6-month post-transplant survival was lower (76% vs. 91%, respectively). The majority of children with ALF (80%) die from brainstem herniation. On multivariate evaluation, risk factors for post-transplant mortality included grade 4 HE, age less than 1 year, and use of pre-transplant dialysis (Baliga et al., 2004).

Over the past thirty years, however, with advances in the field of liver transplantation and critical care management, the US 1-year survival rates following OLT for ALF have improved to 60-80% and 1-year post-transplant graft survival rates have improved from 63% to 75% (Bismuth et al., 1995; DeVictor et al., 1992; Hoofnagle et al., 1995; W Lee, 2003; O'Grady et al., 1988; Wigg et al., 2005). In Spain, Portugal, Belgium, and Italy, where the majority of ALF cases are induced by hepatitis B infection or cryptogenic causes, 1-year post-transplant survival is 61-79% (Areia et al., 2007; Detry et al., 2007; Escorsell et al., 2007; Montalti et al., 2005). These 1-year survival rates are less than the 1-year survival seen in patients who have been transplanted for chronic liver failure (80–90%) (Farmer et al., 2003; Freeman et al., 2008). However, by 1–4 years following transplantation this trend has reversed, and ALF patients have a better survival than those transplanted for chronic liver disease. Chan et al. reported the Canadian experience with 5- and 10-year patient survival rates of 76% and 69%, respectively, and graft survival rates of 65% and 59% (Chan et al., 2009). Poorer outcomes are seen in centers performing less than 25 liver transplants per year and less than 20 split-liver grafts per year for those doing living donor liver transplantation (Adam et al., 2000).

4.2 Retransplantation

Retransplantation occurs more frequently following emergent OLT (13%) compared to elective OLT (7%). The cause of graft failure is usually secondary to acute cellular rejection, primary graft nonfunction, or intrahepatic biliary strictures, all of which may be related to the quality of graft used or the use of an ABO incompatible graft (Adam et al., 1991; Farges et al., 1995; Gugenheim et al., 1990).

5. Quality of life

Overall, the quality of life and long-term survival among ALF transplant survivors is good, but some differences have been identified. When ALF patients were compared to a matched control group who had undergone OLT for chronic liver disease, both groups complained of memory difficulties but more ALF patients complained of concentration difficulties and as a group scored lower on neuropsychological tests (Jackson et al., 2002). The King's College group initially sent out questionnaires to small sample of ALF and chronic liver disease OLT recipients about 2-3 years following surgery. The ALF patients tended to be younger (35 vs.

59 years), so age may have influenced results. More ALF patients were employed or were in full-time education (50 vs. 26.5%). The mental health scores were slightly lower for those who had ALF (68 vs. 79; p=0.022), which was attributed to the fact that the ALF recipients did not undergo typical preoperative education and psychological support prior to OLT. There was no significant difference in parasuicide quality of life scores between the two groups (Sargent et al., 2006).

ALF patients scored slightly lower in the physical function and role emotion areas compared to normal values, but the values were similar to those who were transplanted for chronic liver disease (Sargent et al., 2006). When more carefully interviewed, six ALF recipients described significant physical inactivity and fatigue for the first 3-6 months following OLT due to weight loss and loss of muscle tone. They also noted a health transition lasting between 3-6 months, during which time dependence on others was present. Pretransplant lifestyles were changed in order to regain independence. Support groups or role models were deemed extremely helpful in coping with the ordeal. The majority felt that they had been given a "second chance at life" and were willing to reciprocate support to other going through the same process (Sargent et al., 2007).

Following spontaneous recovery, ALF patients with psychiatric illness who had taken a deliberate APAP overdose are at risk of repeated overdoses. Risk of repeated overdose appears to be less common; however, if the patient was transplanted, perhaps due to the intensity of postoperative care. In two series from the United Kingdom, APAP-ALF patients who underwent OLT showed similar long-term survival (median 5 years and 9 years) compared with patients transplanted for chronic liver disease (Cooper et al., 2009; Karvellas et al., 2010). Less than 5% of those transplanted for APAP overdose reattempted overdose. There was worse 30-day mortality for the APAP-ALF patients, and a greater probability of post-OLT medical nonadherence and adverse events in those who had taken APAP for deliberate self-harm compared with both non-APAP-ALF patients and chronic liver disease patients (Cooper et al., 2009).

6. Future directions

Hepatocyte transplantation has been studied predominantly in patients with chronic metabolic disorders. There is evidence, however, that partial liver engraftment is possible and there may be improvement in neurological status, as noted in small groups of patients with ALF who have undergone hepatocyte transplantation (Bilir et al., 2000; Habibullah et al., 1994; Strom et al., 1997). Xenotransplantation is an intriguing concept and porcine livers have been used for ex vivo perfusion but in vivo use has not yet proven effective due to problems with transspecies rejection. Bioartificial livers and extracorporeal liver assist devices (ELAD) have been used to bridge patients to transplantation and have demonstrated improved neurologic outcomes. There are two cell-based devices. One uses porcine hepatocytes and did not show survival advantage in one large multicenter study and another uses hepatoblastoma cells which was noted to decrease severity of encephalopathy without change in survival in one study (Demetriou et al., 2004; Ellis et al., 1996). Non-biological systems also exist such as albumin dialysis (MARS) and plasmapheresis, and no survival advantage was found on meta-analysis (Khuroo et al., 2004).

7. Conclusion

Many advances have occurred that have significantly improved outcomes following transplantation for ALF. Prognostic models are helpful, but they are not entirely predictive of which individuals need OLT and which will survive without OLT. In the setting of organ shortage, alternatives to conventional OLT are being increasingly used, including living donor split grafts, ABO incompatible grafts, and auxiliary grafts, with variable outcomes. The risks and benefits to both the donor and the recipient must be considered. Long term outcomes and quality of life for both donors and recipients are good but prolonged monitoring may be helpful to identify those in distress. Newer technologies are being developed and enhanced to improve short term and long term survival after acute liver injury.

8. References

2009 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1994-2003. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI.

Abouna G. (2001) Emergency adult to adult living donor liver transplantation for fulminant hepatic failure--is it justifiable? *Transplantation* Vol. 71, No. 10, (May 2001), pp. 1498-1500.

Adam, R., Reynes, M., Johann, M., Morino, M., Astarcioglu, I., et al. (1991) The outcome of steatotic grafts in liver transplantation. *Transplant Proc* Vol. 23, No. 1 (Pt 2), (February 1991), pp. 1538-1540.

Adam, R., Cailliez, V., Majno, P., Karam, V., McMaster, P., et al. (2000) Normalised intrinsic mortality risk in liver transplantation: European Liver Transplant Registry study. *Lancet* Vol. 356, No. 9230, (August 2000), pp. 621-627.

Aladag, M., Gurakar, A., Camci, C., Yong, Y., Wright, H., et al. (2006) Compatible ABO mismatch and liver transplantation: a single center's experience. *Exp Clin Transplant* Vol. 4, No. 1, (June 2006), pp. 467-469.

Anand, A., Nightingale, P., Neuberger, J. (1997) Early indicators of prognosis in fulminant hepatic failure: an assessment of the King's criteria. *J Hepatol* Vol. 26, No. 1, (January 1997), pp. 62-68.

Antoniades, CG., Berry, PA., Bruce, M., Cross, TJ., Portal, AJ., *et al.* (2007) Actin-free Gc globulin: a rapidly assessed biomarker of organ dysfunction in acute liver failure and cirrhosis. *Liver Transpl* Vol. 13, No. 9, (September 2007), pp. 1254-1261.

Areia, M., Romãozinho, JM., Ferreira, M., Amaro, P., Leitão, MC. (2007) Fulminant hepatic failure: a Portuguese experience. *Eur J Gastroenterol Hepatol* Vol. 19, No. 8, (August 2007), pp. 665-669.

Azoulay, D., Samuel, D., Ichai, P., Castaing, D., Saliba, F., et al. (2001) Auxiliary partial orthotopic versus standard orthotopic whole liver transplantation for acute liver failure: a reappraisal from a single center by a case-control study. *Ann Surg* Vol. 234, No. 6, (December 2001), pp. 723-731.

Bailey, B., Amre, DK., Gaudreault, P. (2003) Fulminant hepatic failure secondary to acetaminophen poisoning: A systematic review and meta-analysis of prognostic criteria determining the need for liver transplantation. *Crit Care Med* Vol. 31, No. 1, (January 2003), pp. 299-305.

Baliga, P., Alvarez, S., Lindblad, A., Zeng, L., et al. (2004) Posttransplant survival in pediatric fulminant hepatic failure: the SPLIT experience. *Liver Transpl* Vol. 10, No. 11, (November 2004), pp. 1364-1371.

Baquerizo, A., Anselmo, D., Shackleton, C., Chen, TW., Cao, C., et al. (2003) Phosphorus as an early predictive factor in patients with acute liver failure. *Transplantation* Vol. 75, No. 12, (June 2003), pp. 2007-2014.

Barshes, NR., Lee, TC, Balkrishnan, R., Karpen, SJ, Carter, BA., et al. (2006) Risk stratification of adult patients undergoing orthotopic liver transplantation for fulminant hepatic failure. *Transplantation* Vol. 81, No. 2, (January 2006), pp. 195-201.

Bechmann, LP., Jochum, C., Kocabayoglu, P., Sowa, JP., Kassalik, M. et al. (2010) Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. *J Hepatol* Vol. 53, No. 4, (Otober 2010), pp. 639-647.

Bernal, W., Wendon, J., Rela, M., Heaton, N., Williams, R. (1998). Use and outcome of liver transplantation in acetaminophen-induced acute liver failure. *Hepatology* Vol. 27, No. 4, (Aprl 1998), pp.1050-1055

Bernal, W., Donaldson, N., Wyncoll, D., Wendon, J. (2002) Blood lactate as an early predictor of outcome in paracetamol-induced acute liver failure. *Lancet* Vol. 359, No. 9306, (February 2002), pp. 558-563.

Bernal W. (2003) Changing patterns of causation and the use of transplantation in the United Kingdom. *Semin Liver Dis* Vol. 23, No. 3, (August 2003), pp. 227-237.

Bernal, W., Hall, C., Karvellas, CJ., Auzinger, G., Sizer, E., et al. (2007) Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. *Hepatology* Vol. 46, No. 5, (December 2007), pp. 1844-1852.

Bernuau, J., Rueff, B., Benhamou, J. (1986a). Fulminant and subfulminant liver failure: definition and causes. *Semin Liver Dis* Vol. 6, No. 2, (May 1986), pp. 97-106.

Bernuau, J., Goudeau, A., Poynard, T., Dubois, F., Lesage, G., et al. (1986b) Multivariate analysis of prognostic factors in fulminant hepatitis B. *Hepatology* Vol. 6, No. 4, (July-August 1986), pp. 648-651.

Bernuau J. (1993) Selection for emergency liver transplantation. *J Hepatol* Vol. 19, No. 3, (November 1993), pp. 486-487.

Bhatia, V., Singh, R., Acharya, SK. (2006) Predictive value of arterial ammonia for complications and outcome in acute liver failure. *Gut* Vol. 55, No. 1, (January 2006), pp. 98-104.

Bilir, BM., Guinette, D., Karrer, F., Kumpe, DA., Krysl, J., et al. (2000) Pilot study of hepatocyte transplantation in acute liver failure. *Liver Transpl* Vol. 6, No. 1, (January 2000), pp. 32-40.

Bismuth, H., Samuel, D., Castaing, D., Adam, R., Saliba, F., et al. (1995). Orthotopic liver transplantation in fulminant and subfulminant hepatitis. The Paul Brousse experience. *Ann Surg* Vol. 222, No.2, (August 1995), pp. 109-119.

Bismuth, H., Samuel, D., Castaing, D., Williams, R., Pereira, SP. (1996a) Liver transplantation in Europe for patients with acute liver failure. *Semin Liver Dis* Vol. 16, No. 4, (November 1996), pp. 415-425.

Bismuth, H., Azoulay, D., Samuel, D., Reynes, M., Grimon, G., et al. (1996b) Auxiliary partial orthotopic liver transplantation for fulminant hepatitis. The Paul Brosse experience. *Ann Surg* Vol. 224, No. 6, (December 1996), pp. 712-726.

Bjøro, K., Ericzon, BG., Kirkegaard, P., Höckerstedt, K., Söderdahl, G., et al. (2003) Highly urgent liver transplantation: possible impact of donor-recipient ABO matching on the outcome after transplantation. *Transplantation* Vol. 75, No. 3, (February 2003); pp. 347-353.

Blei, A. (2005) Selection of acute liver failure: have we got it right? *Liver Transpl* Vol. 11, No. Suppl 2, (November 2005), pp. S30-S34.

Boudjema, K., Bachellier, P., Wolf, P., Tempé, JD., Jaeck, D. (2002) Auxiliary liver transplantation and bioartificial bridging procedures in treatment of acute liver failure. *World J Surg* Vol. 26, No. 2, (February 2002), pp. 264-274.

Brandsaeter, B., Höckerstedt, K., Friman, S., Ericzon, BG., Kirkegaard, P., et al. (2002) Fulminant hepatic failure: outcome after listing for highly urgent liver transplantation--12 years experience in the Nordic countries. *Liver Transpl* Vol. 8, No. 11, (November 2002), pp. 1055-1062.

Campsen, J., Blei, AT., Emond, JC., Everhart, JE., Freise, CE., et al., (2008) Outcomes of living donor liver transplantation for acute liver failure: the adult-to-adult living donor liver transplantation cohort study. *Liver Transpl* Vol. 14, No. 9, (September 2008), pp. 1273-1280.

Carlisle, EM., Angelos, P., Siegler, M., Testa, G. (2011) Adult living-related liver donation for acute liver failure: is it ethically appropriate? *Clin Transplant* (February 2011) [Epub ahead of print].

Casas, A., Falkenstein, K., Gallagher, M., Dunn, SP. (1999) Living donor liver transplantation in critically ill children. *Transplant* Vol. 3, No. 2, (May 1999), pp. 104-108.

Castells, A., Salmerón, J., Navasa, M., Rimola, A., Saló, J., et al. (1993) Liver transplantation for acute liver failure: analysis of applicability. *Gastroenterology* Vol. 105, No. 2, (August 1993), pp. 532-538.

Chan, G., Taqi, A., Marotta, P., Levstik, M., McAlister, V., et al. (2009) Long-term outcomes of emergency liver transplantation for acute liver failure. *Liver Transpl* Vol. 15, No. 12, (December 2009), pp. 1696-702.

Chenard-Neu, MP., Boudjema, K., Bernuau, J., Degott, CC., Belghiti, J., et al. (1995)Auxiliary liver transplantation: regeneration of the native liver and outcome in 30 patients with fulminant hepatic failure. A multicenter study. *Hepatology* Vol. 23, No. 5, (May 1995), pp. 1119-1127.

Cholongitas, E., O'Beirne, J., Betrossian, A., Senzolo, M., Shaw, S. et al. (2008) Prognostic impact of lactate in acute liver failure. *Liver Transpl* Vol. 14, No. 1, (January 2008); pp. 121-122; author reply 123.

Chung, PY., Sitrin, MD., Te, HS. (2003) Serum phosphorus levels predict clinical outcome in fulminant hepatic failure. *Liver Transpl* Vol. 9, No. 3, (March 2003), pp. 248-253.

Clemmesen, JO., Larsen, FS., Kondrup, J., Hansen, BA., *et al.* (1999) Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology* Vol. 29, No. 3, (March 1999), pp. 648-653.

Cooper, SC., Aldridge, RC., Shah, T., Webb, K., Nightingale, P., et al. (2009) Outcomes of liver transplantation for paracetamol (acetaminophen)-induced hepatic failure. *Liver Transpl* Vol. 15, No. 10, (October 2009), pp. 1351-1357.

Committee of Ministers. (1997) Recommendation No R(97)16 of the Committe of Ministers to Member States on liver transplantation from living related donors. In: Council of Europe; 1997. http://www.coe.int/t/dg3/health/recommendations_en.asp (accessed September 15, 2011)

Craig, DG., Ford, AC., Hayes, PC., Simpson, KJ. (2010) Systematic review: prognostic tests of paracetamol-induced acute liver failure. *Aliment Pharmacol The*r Vol. 31, No. 10, (May 2010), pp. 1064-1076.

Daas, M., Plevak, DJ., Wijdicksc EF., Rakela, J., Wiesner, RH., et al. (1995) Acute liver failure: Results of a 5-year clinical protocol. *Liver Transpl Surg* Vol. 1, No. 4, (July 1995), pp. 210-219.

Demetriou, AA., Brown, RS., Jr, Busuttil, RW., Fair, J., McGuire, BM., et al. (2004) Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. *Ann Surg* Vol. 239, No. 5, (May 2004), pp. 660-667.

Detry, O., De Roover, A., Coimbra, C., Delwaide, J., Hans, MF., et al. (2007) Cadaveric liver transplantation for non-acetaminophen fulminant hepatic failure: a 20-year experience. *World J Gastroenterol* Vol. 13, No. 9, (March 2007), pp. 1427-1430.

DeVictor, D., Desplanques, L., Debray, D., Ozier, Y., Dubousset, AM., et al. (1992). Emergency liver transplantation for fulminant liver failure in infants and children. *Hepatology* Vol. 16, No. 5, (November 1992), pp. 1156-1162.

Devlin, J., Wendon, J., Heaton, N., Tan, KC, Williams, R. (1995) Pretransplant clinical status and outcome of emergency transplantation for acute liver failure. *Hepatology* Vol. 21, No. 4, (April 1995), pp. 1018-1024.

Dhiman, RK., Jain, S., Maheshwari, U., Bhalla, A., Sharma, N., et al. (2007) Early indicators of prognosis in fulminant hepatic failure: an assessment of the Model for End-Stage Liver Disease (MELD) and King's College Hospital criteria. *Liver Transpl* Vol. 13, No. 6, (June 2007), pp. 814-821.

Durand, P., Debray, D., Mandel, R., Baujard, C., Branchereau, S., et al. (2001) Acute liver failure in infancy: A 14-year experience of a pediatric liver transplantation center. *J Pediatr* Vol. 139, No. 6, (December 2001), pp. 871-876.

Durand, F., Belghiti, J., Troisi, R., Boillot, O., Gadano, A., et al. (2006) Living donor liver transplantation in high-risk vs. low-risk patients: optimization using statistical models. *Liver Transpl* Vol. 12, No. 2, (February 2006), pp. 231-239.

Egawa, H., Oike, F., Buhler, L., Shapiro, AM., Minamiguchi, S., et al. (2004) Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. *Transplantation* Vol. 77, No. 3, (February 2004), pp. 403-411.

Ejlersen, E., Larsen, FS., Pott, F., Gyrtrup, HJ., Kirkegaard, P., et al. (1994) Hepatectomy corrects cerebral hyperperfusion in fulminant hepatic failure. *Transplant Proc* Vol. 26, No. 3, (June 1994), pp. 1794-1795.

Elinav, E., Ben-Dov, I., Hai-Am, E., Ackerman, Z., Ofran,Y. (2005) The predictive value of admission and follow up factor V and VII levels in patients with acute hepatitis and coagulopathy. *J Hepatol* Vol. 42, No. 1, (January 2005), pp. 82-86.

Ellis, AJ., Hughes, RD., Wendon, JA., Dunne, J., Langley, PG., et al. (1996) Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. *Hepatology* Vol. 24, No. 6, (December 1996), pp. 1446-1451.

Emre, S., Schwartz, ME, Shneider, B., Hjosak, J., Kim-Schluger, L., et al. (1999) Living related liver transplantation for acute liver failure in children. *Liver Transplant Surg* Vol. 5, No. 3, (May 1999), pp. 161-165.

Erim, Y., Beckmann, M., Kroencke, S., Valentin-Gamazo, C., Malago, M., et al. (2007) Psychological strain in urgent indications for living donor liver transplantation. *Liver Transpl* Vol. 13, No. 6, (June 2007), pp. 886-895.

Escorsell, A., Mas, A., de la Mata, M., Spanish Group of the Study of Acute Liver Failure. (2007) Acute liver failure in Spain: analysis of 267 cases. *Liver Transpl* Vol. 13, No. 10, (October 2007), pp. 1389-1395.

Faraj, W., Dar, F., Bartlett, A., Melendez, HV., Marangoni, G., et al. (2010) Auxiliary liver transplantation for acute liver failure in children. *Ann Surg* Vol. 251, No. 2, (February 2010), pp. 351-356.

Farges, O., Kalil, AN., Samuel, D., Saliba, F., Arulnaden, JL., et al. (1995) The use of ABO-incompatible grafts in liver transplantation: a life-saving procedure in highly selected patients. *Transplantation* Vol. 59, No. 8, (April 1995), pp. 1124-1133.

Farmer, DG., Anselmo, DM., Ghobrial, RM., Yersiz, H., McDiarmid, SV., et al. (2003) Liver transplantation for fulminant hepatic failure: experience with more than 200 patients over a 17-year period. *Ann Surg* Vol. 237, No. 5, (May 2003), pp. 666-75; discussion 675-6.

Freeman, RB. Jr., Steffick, DE., Guidinger, MK., Farmer, DG., et al. (2008). Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* Vol. 8, No. 4, Pt. 2, pp. 958-976.

Gasco, J., Rangel-Castilla, L., Franklin, B., Thomas, PG., Patterson, JT. State-of-the-art management and monitoring of brain edema and intracranial hypertension in fulminant hepatic failure. A proposed algorithm. *Acta Neurochir Suppl* Vol. 106 (2010), pp. 311-314.

Gelas, T., McKiernan, PJ., Kelly DA., Mayer, DA., Mirza, DF., et al. (2011) ABO-incompatible pediatric liver transplantation in very small recipients: Birmingham's experience. *Pediatr Transplant* (July 2011) [Epub ahead of print].

Ghobrial, RM., Freise, CE., Trotter, JF., Tong, L., Ojo, AO., et al. (2008) Donor morbidity after living donation for liver transplantation. *Gastroenterology* Vol. 135, No. 2, (August 2008), pp. 468-476.

Gugenheim, J., Samuel, D., Reynes, M., Bismuth, H. (1990) Liver transplantation across ABO blood group barrier. *Lancet* Vol. 336, No. 8714, (September 1990), pp. 519-523.

Habibullah, CM., Syed, IH., Qamar, A., Taher-Uz, Z. (1994) Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. *Transplantation* Vol. 58, No. 8, (October 1994), pp. 951-952.

Hanto, DW., Fecteau, AH., Alonso, MH., Valente, JF., Whiting, JF. (2003) ABO-incompatible liver transplantation withno immunological graft losses using total plasma exchange, splenectomy, and quadruple immunosuppresion: evidence for accomodation. *Liver Transpl* Vol. 9, No. 1 (January 2003), pp. 22-30.

Harrison, PM., O'Grady, JG., Keays, RT., Alexander, GJ., Williams, R. (1990) Serial prothrombin time as prognostic indicator in paracetamol induced fulminant hepatic failure. *BMJ* Vol. 301, No. 6758, (October 1990), pp. 964-966.

Hoofnagle, J., Carithers, RJ., Shapiro, C., Ascher, N. (1995). Fulminant hepatic failure: summary of a workshop. *Hepatology* Vol. 21, No. 1, (January 1995), pp. 240-252.

Ichida, T., Todo, S., Fujiwara, K., et al. (2000) Living related-donor liver transplantation for adult fulminant hepati failure [abstract]. *Hepatology* Vol. 32, (October 2000), pp. 340A.

Itai, Y., Sekiyama, K., Ahmadi, T., Obuchi, M., Yoshiba, M. (1997) Fulminant hepatic failure: observation with serial CT. *Radiology* Vol. 202, No. 2, (February 1997), pp. 379-82.

Izumi, S., Langley, PG., Wendon, J., Ellis, AJ., Pernambuco, RB., et al. (1996) Coagulation factor V levels as a prognostic indicator in fulminant hepatic failure. *Hepatology* Vol. 23, No. 6, (June 1996), pp. 1507-1511.

Jackson, EW, Zacks, S., Zinn, S., Ryan, J., Johnson, MW., et al. (2002) Delayed neuropsychological dysfunction after liver transplantation for acute liver failure: a matched, case-controlled study. *Liver Transpl* Vol. 8, No. 10, (October 2002), pp. 932-936.

Jalan, R., Olde Damink, SW., Deutz, NE, Davies, NA., Garden, OJ., et al. (2003) Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. *Transplantation* Vol. 75, No. 12, (June 2003), pp. 2034-2039.

Karvellas, CJ., Safinia, N., Auzinger, G., Heaton, N., Muiesan, P., et al. (2010) Medical and psychiatric outcomes for patients transplanted for acetaminophen-induced acute liver failure: a case-control study. *Liver Int* Vol. 30, No. 6, (July 2010), pp. 826-33.

Kawasaki, S., Makuuchi, M., Matsunami, H., Hashikura, Y., Ikegami, T., et al. (1998) Living related liver transplantation in adults. Ann Surg Vol. 227, No. 2, (February 1998), pp. 269-274.

Khuroo, MS., Faharat, KL. (2004) Molecular adsorbent recirculating system for acute and acute-on-chronic liver failure: a meta-analysis. *Liver Transpl* Vol. 10, No. 9, (September 2004), pp. 1099-1106.Kim-Schluger, L., Florman, SS., Schiano, T., O'Rourke, M., Gagliardi, R., et al. (2002) Quality of life after lobectomy for adult liver transplantation. *Transplantation* Vol. 73, No. 10, (May 2002), pp. 1593-1597.

Kitzberger, R., Funk, GCs. Holzinger, U., Miehsler, W., Kramer, L., et al. (2009) Severity of organ failure is an independent predictor of intracranial hypertension in acute liver failure. *Clin Gastroenterol Hepatol* Vol. 7, No. 9, (September 2009), pp. 1000-1006.

Kiuchi, T., Kasahara, M., Uryuhara, K., Inomata, Y., Uemoto, S., et al. (1999) Impact of graft size mismatching on graft

Koukoutsis, I., Bellagamba, R., Tamijmarane, A., Gunson, B., Muralidharan,V., et al. (2007) Outcomes after identical and compatible orthotopic liver transplantation for

fulminant hepatic failure: a single center experience in UK. *Transplant Int* Vol. 20, No. 8, (August 2007), pp. 659-665.

Larson, AM., Polson, J., Fontana, RJ., Davern, TJ., Lalani, E., et al. (2005) Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology* Vol. 42, No. 6, (December 2005), pp. 1364-1372

Liu, CL., Fan, ST., Lo, CM, Yong, BH., Fung, AS, et al. (2002) Right-lobe live donor liver transplantation improves survival of patients with acute liver failure. *Br J Surg* Vol. 89, No. 3, (March 2002), pp. 317-322.

Lee, SG., Ahn, CS., Kim, KH. (2007) Which types of graft to use in patients with acute liver failure? (A) Auxiliary liver transplant (B) Living donor liver transplantation (C) The whole liver. (B) I prefer living donor liver transplantation. *J Hepatol* Vol. 46, No. 4, (April 2007), pp. 574-578.

Lee, WM. (2003) Acute liver failure in the United States. *Semin Liver Dis* Vol. 23, No. 3, (August 2003), pp. 217-226.

MacQuillan, GC., Seyam, MS., Nightingale, P., Neuberger, JM., Murphy, N. (2005) Blood lactate but not serum phosphate levels can predict patient outcome in fulminant hepatic failure. *Liver Transpl* Vol. 11, No. 9, (September 2005), pp. 1073-1079.

Man, K., Fan, ST., Lo, CM, Liu, CL., Fung, PC., et al. (2003) Graft injury in relation to graft size in right lobe live donor liver transplantation: a study of hepatic sinusoidal injury in correlation with portal hemodynamics and intragraft gene expression. *Ann Surg* Vol. 237, No. 2, (February 2003), pp. 256-264.

Marcos, A., Fisher, RA., Ham, JM, Shiffman, ML, Sanyal, AJ., et al. (2000) Liver regeneration and function in donor and recipient after right lobe adult to adult living donor liver transplantation. *Transplantation* Vol. 69, No. 7, (April 2000), pp. 1375-1379.

Mas, A., Escorsell, A., Fernandez, J. (2010) Liver transplantation for acute liver failure: a Spanish perspective. *Transplant Proc* Vol. 42, No. 2, (March 2010), pp. 619-8621.

McPhail, MJ., Wendon, JA., Bernal, W. (2010) Meta-analysis of performance of Kings's College Hospital Criteria in prediction of outcome in non-paracetamol-induced acute liver failure. *J Hepatol* Vol. 53, No. 3, (September 2010), pp. :492-499.

Miraglia, R., Luca, A., Gruttadauria, S., Minervini, MI., Vizzini, G., et al. (2006) Contribution of transjugular liver biopsy in patients with the clinical presentation of acute liver failure. *Cardiovasc Intervent Radiol* Vol. 29, No. 6, (November-December 2006), pp. 1008-1010.

Mitchell, I., Bihari, D., Chang, R., Wendon, J., Williams, R. (1998) Earlier identification of patients at risk from acetaminophen-induced acute liver failure. *Crit Care Med* Vol. 26, No. 2, (February 1998), pp. 279-284.

O'Grady, JG., Alexander, GJ., Thick, M., Potter, D., Calne, RY., et al. (1988) Outcome of orthotopc liver transplantation in the aetiological and clinical variants of acute liver failure. *Q J Med* Vol. 68, No. 258, (October 1988), pp. 817-824.

Miwa, S. Hashikura, Y., Mita, A., Kubota, T., Chisuwa, H., et al. (1999) Living-related liver transplantation for patients with fulminant and subfulminant hepatic failure. *Hepatology* Vol. 30, No. 6, (December 1999), pp. 1521-1526.

Miyake, Y., Sakaguchi, K., Iwasaki, Y., Ideda, H., Makino, Y., et al. (2005) New prognostic scoring model for liver transplantation in patients with non-acetaminophen-related fulminant hepatic failure. *Transplantation* Vol. 80, No. 7, (October 2005), pp. 930-936.

Montalti, R., Nardo, B., Beltempo, P., Bertelli, R., Puviani, L., eta l. (2005) Liver transplantation in fulminant hepatic failure: experience with 40 adult patients over a 17-year period. *Transplant Proc* Vol. 37, No. 2, (March 2005), pp. 1085-1087.

Moore, K., Taylor, G., Ward, P., Williams. R. (1991) Aetiology and management of renal failure in acute liver failure. In: *Acute Liver Failure: Improved Understanding and Better Therapy*. Williams, R., Hughes, R. (Eds), pp. 47-53, Smith Kline Beecham Pharmaceuticals; Welwyn Garden City, UK.

Nishizaki, T., Hiroshige, S., Ikegami, T., Uchiyama, H., Hashimoto, K., et al. (2002) Living-donor liver transplantation for fulminant hepatic failure in adult patients with a left-lobe graft. *Surgery* Vol. 131, No. 1 (Suppl), (January 2002), pp. S182-S189.

O'Grady, JG., Alexander, GJ., Hayllar, KM., Williams, R. (1989) Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* Vol. 97, No. 2, (August 1989), 439-445.

O'Grady, JG., Schalm, SW., Williams, R. (1993) Acute liver failure: redefining the syndromes. *Lancet* Vol. 342, No. 8877, (October 1993), pp. 273-275.

O'Grady JG. (2007) Prognostication in acute liver failure: a tool or an anchor? *Liver Transpl* Vol. 13, No. 6, (June 2007), pp. 786-787.

O'Mahony, C., Patel, S., Suarez, J., et al. (2007) Have US orthotopic liver transplant (OLT) outcomes for acute liver failure (ALF) improved in the last decade? [abstract]. *Hepatology* Vol. 46, No. xx, (October 2007), pp. 492A.

Ostapowicz, G., Fontana, R., Schiødt, F., Larson, A., Davern, TJ., et al. (2002) Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* Vol. 137, No. 12, (December 2002), pp. 947-954.

Pauwels, A., Mostefa-Kara, N., Florent, C., Lévy, VG. (1993) Emergency liver transplantation for acute liver failure. Evaluation of London and Clichy criteria. *J Hepatol* Vol. 17, No. 1, (January 1993), pp. 124-127.

Pereira, LM., Langley, PG., Hayllar, KM., Tredger, JM, Williams, R. (1992) Coagulation factor V and VIII/V ratio as predictors of outcome in paracetamol induced fulminant hepatic failure: relation to other prognostic indicators. *Gut* Vol. 33, No. 1, (January 1992), pp. 98-102.

Pereira, SP., McCarthy, M., Ellis, AJ., Wendon, J., Portmann, B., et al. (1997) Auxiliary partial orthotopic liver transplantation for acute liver failure. *J Hepatol* Vol. 26, No. 5, (May 1997), pp. 1010-1017.

Philips, BJ., Armstrong, IR., Pollock, A., Lee, A. (1998) Cerebral blood flow and metabolism in patients with chronic liver disease undergoing orthotopic liver transplantation. *Hepatology* Vol. 27, No. 2 (February 1998) pp. 369-376.

Lee, WM., Larson, AM., Stravitz, RT. (2011) AASLD position paper: The management of acute liver failure: Update 2011. September 2011. Available at: http://www.aasld.org/practiceguidelines/Documents/AcuteLiverFailureUpdate2011.pdf

Reding, R. (2005) Is it right to promote living donor liver transplantation for fulminant hepatic failure in pediatric recipients? *Am J Transpl* Vol. 5, No. 7, (July 2005), pp. 1587-1591.

Reuben, A., Koch, DG., Lee, WM. (2010). Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology* Vol. 52, No. 6, (December 2010), pp. 2065-2076.

Ringe, B., Lubbe, N., Kuse, E., Frei, U., Pichlmayr, R. (1993) Total hepatectomy and liver transplantation as a two-stage procedure. *Ann Surg* Vol. 218, No. 1, (July 1993), pp. 3-9.

Riorden, SM., Williams, R. (2003) Mechanisms of hepatocyte injury, multiorgan failure, and prognostic criteria in acute liver failure. *Semin Liver Dis* Vol. 23, No. 3, (August 2003), pp. 203-215

Rolando, N., Harvey, F., Brahm, H., Philpott-Howard, J., Alexander, G., et al. (1990) Prospective study of bacterial infection in acute liver failure: an analysis of fifty patients. *Hepatology* Vol. 11, No. 1, (January 1990), pp. 49-53.

Rolando, N., Harvey, F., Brahm, H., Philpott-Howard, J., Alexander, G., et al. (1991) Fungal infection: a common, unrecognised complication of acute liver failure. *J Hepatol* Vol. 12, No. 1, (January 1991), pp. 1-9.

Rolando, N., Philpott-Howard, J., Williams, R. (1996) Bacterial and fungal infection in acute liver failure. *Semin Liver Dis* Vol. 16, No. 4, (November 1996), pp. 389-402.

Rolando, N., Wade, J., Davalos, M., Wendon, J., Philpott-Howard, J., et al. (2000) The systemic inflammatory response syndrome in acute liver failure. *Hepatology* Vol. 32, No. 4(Pt 1), (October 2000), pp. 734-739.

Russo, MW., Galanko, JA., Shrestha, R., Fried, MW., Watkins, P. (2004) Liver transplantation for acute liver failure from drug induced liver injury in the United States *Liver Transpl* Vol. 10, No. 8, (August 2004), pp. 1018-1023.

Samuel, D., Bismuth, H.(2001) Transplantation in patients with fulminant hepatitis. In: *Transplantation of the Liver. 3rd Edition.* Maddrey W, Schiff E, Sorrell M (Eds). pp. 361-370, Lippincott Williams & Williams; Philadelphia.

Sargent, S., Wainwright, SP. (2006) Quality of life following emergency liver transplantation for acute liver failure. *Nurs Crit Care* Vol. 11, No., 4, (July-August 2006), pp. 168-176.

Sargent, S., Wainwright. SP. (2007) A qualitative study exploring patients perceived quality of life following an emergency liver transplant for acute liver failure. *Intensive Crit Care Nurs* Vol. 23, No. 5, (October 2007), pp. 272-280.

Schiødt, FV., Atillasoy, E., Shakil A., Schiff, ER., Caldwell, C., et al. Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transpl Surg* Vol. 5, No. 1, (January 1999), pp. 29-34.

Schiødt, FV., Davern, TJ., Shakil, AO., McGuire, B., Samuel, G., et al. (2003) Viral hepatitis-related acute liver failure. *Am J Gastroenterol* Vol. 98, No. 2, (February 2003), pp. 448-453.

Schiødt, FV., Rossaro, L., Stravitz, RT., Shakil AO., Chung, RT., *et al.* (2005) Gc-globulin and prognosis in acute liver failure. *Liver Transpl* Vol. 11, No. 10, (October 2005), pp.1223-1227.

Schiødt, FV., Ostapowicz, G., Murray, N., Satyanarana, R., Zaman, A., et al. (2006) Alpha-fetoprotein and prognosis in acute liver failure. *Liver Transpl* Vol. 12, No. 12, (December 2006), pp. 1776-1781.

Schiødt, FV., Bangert, K., Shakil, AO., McCashland, T., Murray, N., et al. (2007) Predictive value of actin-free Gc-globulin in acute liver failure. *Liver Transpl* Vol. 13, No. 9, (September 2007), pp. 1324-1329.

Schmidt, LE., Dalhoff, K. (2002) Serum phosphate is an early predictor of outcome in severe acetaminophen-induced hepatotoxicity. *Hepatology* Vol 36, No. 3, (September 2002); pp. 659-665.

Schmidt LE., Dalhoff, K. (2005) Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. *Hepatology* Vol. 41, No. 1, (January 2005), pp. 26-31.

Schmidt, LE., Larsen, FS. MELD score as a predictor of liver failure and death in patients with acetaminophen-induced liver injury. *Hepatology* Vol. 45, No. 3, (March 2007), pp. 789-796.

Scotto, J., Opolon, P. Etévé, J., Vergoz, D., Thomas, M., et al. (1973) Liver biopsy and prognosis in acute liver failure. *Gut* Vol. 14, No. 12, (December 1973), pp. 927-933.

Shakil, AO., Jones, BC., Lee, RG., Federle, MP., Fung, JJ., et al. (2000)Prognostic value of abdominal CT scanning and hepatic histopathology in patients with acute liver failure. *Dig Dis Sci* Vol. 45, No. 2, (February 2000), pp. 334-339.

Simpson, KJ., Bates, CM., Henderson, NC., Wigmore, SJ., Garden, OJ., et al. (2009) The utilization of liver transplantation in the management of acute liver failure: comparison between acetaminophen and non-acetaminophen etiologies. *Liver Transpl* Vol. 15, No. 6, (June 2009) pp. 600-609

Smith, CM., Davies, DB., McBride, MA. (2000) Liver transplantation in the United States: a report from the Organ Procurement and Transplantation Network. *Clin Transpl* (2000), pp. 19-30.

Spital A. (2005) More on parental living liver donation for children with fulminant hepatic failure: addressing concerns about competing interests, coercion, consent and balancing acts. *Am J Transpl* Vol. 5, No. 11, (November 2005), pp. 2619-2622.

Stravitz, RT., Kramer, AH., Davern, T., Shaikh, AO., Caldwell, SH., et al. (2007) Intensive care of patients with acute liver failure: Recommendations of the US Acute Liver Failure Study Group. *Crit Care Med* No. 35, Vol. 11, (November 2007), pp. 2498-2508.

Strom, SC., Fisher, RA., Thompson, MT., Sanyal, AJ., Cole, PE., et al. (1997) Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* Vol. 63, No. 4, (February 1997), pp. 559-569.

Sugawara, Y., Makuuchi, M. (2006) Adult liver transplantation using live ABO-incompatible grafts in Western countries. *Liver Transpl* Vol. 12, No. 9, (September 2006), pp. 1324-1325.

Tofteng, F., Hauerberg, J., Hansen, BA., Pedersen, CB., Jorgensen, L., et al. (2006) Persistent arterial hyperammonemia increases the concentration of glutamine and alanine in the brain and correlates with intracranial pressure in patients with fulminant hepatic failure. *J Cereb Blood Flow Metab* Vol. 26, No. 1, (January 2006), pp. 21-27.

Toso, C., Al-Qahtani, M., Alsaif, F., Bigam, DL., Meeberg, GA., et al. (2007) ABO-incompatible liver transplantation for critically ill adult patients. *Transplant Int* Vol. 20, No. 8, (August 2007), pp. 675-681.

Trotter, JF., Hill-Callahan, MM., Gillespie, BW., Nielsen, CA., Saab, S., et al. (2007) Severe psychiatric problems in right hepatic lobe donors for living donor liver transplantation. *Transplantation* Vol. 83, No. 11, (June 2007), pp. 1506-1508.

Uemoto, S., Inomata, Y., Sukarai, T., Egawa, H., Fujita, S., (2000) et al. Living donor liver transplantation for fulminant hepatic failure. *Transplantation* Vol. 70, No. 1, (July 2000), pp. 152-157.

Van Hoek, B., De Boer, J., Boudjema, K., Williams, R., Corsmit, O., et al. (1999) Auxiliary versus orthotopic liver transplantation for acute liver failure: EURALT Study Group, European Auxiliary Liver Transplant Registry. *J Hepatol* Vol. 30, No. 4, (April 1999), pp. 699-705.

Van Thiel DH. (1993) When should a decision to proceed with transplantation actually be made in cases of fulminant or subfulminant hepatic failure: at admission to hospital or when a donor organ is made available? *J Hepatol* Vol. 17, No. 1, (January 1993), pp. 1-2.

Vaquero, J., Polson, J., Chung, C., Helenowski, I., Schiødt, FV, et al. (2003) Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology* Vol. 125, No. 3, (September 2003), pp. 755-764.

Vaquero, J., Fontana, R., Larson, AM., Bass, NM., Davern, TJ., et al. (2005) Complications and use of intracranial pressure monitoring in patients with acute liver failure and severe encephalopathy. *Liver Transpl* Vol. 11, No. 12, (December 2005), pp. 1581-1589.

Voigt M, Onwuameze O, LaBrecque D, et al. Liver biopsy to predict mortality in fulminant hepatic failure [abstract]. *Hepatology* 2007; 46:499A.

Wiesner RH. (2004) MELD/PELD and the allocation of deceased donor livers for status 1 recipients with acute fulminant hepatic failure, primary nonfunction, hepatic artery thrombosis and acute Wilson's disease. *Liver Transpl* Vol. 10, No. 10 (Suppl 2), (October 2004), ppS17-S22.

Wigg, AJ., Gunson, BK., Mutimer, DJ. (2005) Outcomes following liver transplantation for seronegative acute liver failure: experience during a 12-year period with more than 100 patients. Liver Transpl Vol. 11, No. 1, (January 2005), pp. 27-34.

Williams, R. (1996) Classification, etiology, and considerations of outcome in acute liver failure. *Semin Liver Dis* Vol. 16, No. 4, (November 1996), pp. 343-348.

Wu, J., Ye, S., Xu, X., Xie, H., Zhou, L., et al. (2011) Recipient outcomes after ABO-incompatible liver transplantation: a systematic review and meta-analysis. *PLoS One* Vol. 6, No. 1 (January 2011), pp. e16521.

Yamagishi, Y., Saito, H., Ebinuma, H., Kikuchi, M., Ojiro, K., et al. (2009) A new prognostic formula for adult acute liver failure using computer tomography-derived hepatic volumetric analysis. *J Gastroenterol* Vol. 44, No. 6, (April 2009), pp. 615-623.

Yang, SS., Cheng, KS., Lai, YC, Wu, CH., Chen, TK., et al. (2002) Decreasing serum alpha-fetoprotein levels in predicting poor prognosis of acute hepatic failure in patients with chronic hepatitis B. *J Gastroenterol* Vol. 37, No. 8, (2002); pp. 626-632.

Yantorno, SE., Trentadue, J., Ruf, A. (2004) The model for end-stage liver disease (MELD): a useful tool to access prognosos in fulminant hepatic failure. *Liver Transpl* Vol. 10, (2004), pp. C36.

Yantorno, SE., Kremers, WK., Ruf, AE., Trentadue, JJ., Podestá, LG., et al. (2007) MELD is superior to King's College and Clichy criteria to assess prognosis in fulminant hepatic failure. *Liver Transpl* Vol. 13, No. 6, (June 2007), pp. 822-828.

Yasutomi, M., Uemoto, S., Inomata, Y., Tanaka, K. (2000) Liver failure following living donor liver transplantation for fulminant hepatic failure. *Transplant Proc* Vol. 32, No. 7, (November 2000), pp. 2133.

Zaman, MD., Hoti, E., Qasim, A., Maguire, D., McCormick, PA., (2006) MELD score as a prognostic model for listing acute liver failure patients for liver transplantation. *Transplant Proc* Vol. 38, No. 7, (September 2006), pp. 2097-2098.

13

Liver Transplantation for Hepatocellular Carcinoma (HCC)

Alejandro Mejia, Hector Nazario and Parvez Mantry
The Liver Institute at Methodist Dallas, Texas
USA

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality worldwide and accounts for 20% of all liver transplants (Wigg, 2010). Its incidence has increased two fold in the last decade and it is the fifth leading cause of cancer in males. The availability of liver transplantation as a cure for chronic liver disease and the demonstration of outcomes exceeding 70% at 5 years after transplantation for HCC have pushed the field to refine this therapy in order to utilize this precious resource in the most effective, fair and safe manner. The combination of a rising incidence of HCC and a flat donor procurement rates has resulted in longer waiting times in many areas. The complex decision making and management issues of patients with HCC, cirrhosis and possibly undergoing oncological therapies while waiting for an organ transplant poses challenges to the management team, not encountered in any other clinical or surgical field.

2. Epidemiology

Hepatocellular carcinoma (HCC) accounts for 85% to 90% of the primary liver cancers (El-Serag & Rudolph, 2007). The alarming results of epidemiologic studies performed over the last 2 decades have raised awareness, and shed light on its magnitude as a public health problem. Hepatoma is the fifth most common cancer worldwide, accounting for roughly 4% of all the new cancers diagnosed. It is currently the third most common cause of cancer-related death in the world (Altekruse, 2009; Parkin, 2005). Recent data presented by the Center for Disease Control and Prevention has liver cancer listed as the ninth leading cause of cancer-related deaths in the United States. In addition, hepatocellular carcinoma has become one of the fastest growing causes of malignancy-related death in this North American country, and its overall age-adjusted incidence has also significantly increased over the last 20 years (El-Serag & Rudolph, 2007).

The incidence of hepatocellular carcinoma differs depending on aspects such as geographic location, sex, age, race and ethnicity, environmental exposure to certain agents, as well as presence of other risk factors. In general, it has been clearly established that the vast majority of the cases of hepatocellular carcinoma occur in the setting of cirrhosis arising from chronic liver disease with approximately 80% of the cases due to chronic hepatitis B and hepatitis C infection (Perz, 2006). Sub-Saharan Africa and Eastern Asia, which are

endemic areas for hepatitis B, are considered among the regions with highest rate of hepatoma. The incidence for men is as high as 35.5/100,000 in China, where more than 50% of all hepatocellular carcinomas occur (El Serag & Rudolph, 2007, as cited in Parkin, 2002). Interestingly, a downtrend in the rate of hepatocellular carcinoma has been seen in several high risk Asian countries. The success of vaccination programs against hepatitis B may be one of the main factors contributing to this decrease (Chang et al., 2009). In contrast to most other Asian countries, Japan's incidence of primary liver cancer is associated to chronic hepatitis C infection which came about after the rampant spread of this virus during the post-World War II years (Yoshizawa, 2002). Recent studies suggest that this incidence is also now decreasing (Tanaka et al., 2008).

The rates of primary hepatic cancer in areas that are considered low risk are well below 10 per 100,000. These include most of Europe, North and South America, Australia and New Zealand (Bosch, 2005). The risk factors for hepatoma in these geographic locations are somewhat different to Sub-Saharan and Asian countries with hepatitis C, alcohol and nonalcoholic fatty liver disease playing a pivotal role. In the United States, for example, the aging large population of chronic hepatitis C infected patients that progress to cirrhosis has caused a disturbing increase in the incidence of hepatocellular carcinoma over the last two decades (Davila, 2004, Kanwal, 2011). This trend has also been seen in other developed nations. In addition to the effect of chronic hepatitis C-related cirrhosis, these countries are experiencing growing problems with heavy alcohol consumption, as well as diabetes and obesity which are associated to nonalcoholic fatty liver disease, and could all lead to cirrhosis and liver cancer (Nordenstedt, 2010).

2.1 Age and sex

The rate of hepatocellular carcinoma is higher across the board in males than females. This has been well documented in multiple registries that looked at different populations affected by this cancer (Bosch et al., 2005). In general, the male to female ratios range between 2:1 and 4:1, with the larger variation seen in regions with higher and intermediate incidence of hepatoma. Interestingly, the discrepancy in rates is up to 5:1 in France (El-Serag & Rudolph, 2007). The grounds for this global disparity between men and women are not well understood, but several theories exist linking this phenomenon to differences in sex-specific exposure to risk factors such as viral hepatitis, alcohol, and tobacco (Donato, 2002). The trophic effects of androgens have also been implicated (Yu et al., 2001).

Hepatocellular carcinoma most commonly occurs in the presence of cirrhosis as a result of long standing chronic liver disease. In general, the process of inflammation and fibrosis that leads to cirrhosis usually takes many years, although it could be accelerated when more than one risk factor is affecting an individual. As a result, most cases of hepatoma are seen in older patients. The age at which the incidence of primary liver cancer peaks in high risk areas is typically lower than in areas of lesser risk, 50 to 60 years old and 70 to 75 years old, respectively. However, it is not uncommon to see hepatocellular carcinoma affecting people ages 20 to 35 in geographic locations of high incidence, and where factors such as chronic hepatitis B and aflatoxins, an environmental toxin and carcinogen, are endemic. Vertical transmission of hepatitis B with over 90% chronicity of infected persons, and early constant exposure to aflatoxins in these areas contribute to the earlier incidence of hepatoma (Bosch, 2005).

2.2 Race and ethnicity

The racial and ethnic variations seen in the incidence of hepatocellular carcinoma are influenced by the geographic distribution of this malignancy. Accordingly, the higher incidence is seen in individuals from Africa and Asia. The migratory patterns of populations moving from intermediate and high risk areas into developed countries, has contributed to change the frequency of primary liver cancer not only globally, but within people living in the same region. In the United States, for example, the highest age-adjusted rates of hepatocellular carcinoma are seen in Asians for both sexes (El-Serag & Rudolph, 2007). This group is followed in occurrence by Hispanics, African Americans and Caucasians. The marked growth in the Hispanic population over the last decade, making it the largest minority in the United States has also had an impact in the liver cancer demographics in this North American nation (El-Serag, 2007).

2.3 Risk factors

Several factors have been associated to hepatocellular carcinoma, being the most relevant viral hepatitis, alcohol, exposure to toxins and nonalcoholic fatty liver disease. In general, any etiology that causes chronic liver disease and that could lead to cirrhosis is a potential risk factor for primary liver cancer. It must be noted that hepatoma can very infrequently occur in the absence of cirrhosis as is the case of some individuals with chronic hepatitis B.

2.3.1 Viral hepatitis

Viral hepatitis, in particular chronic hepatitis B and C, account for over 80% of the cases of primary hepatic cancer. The degree of connection of these two viruses to hepatocellular carcinoma varies depending on the region being evaluated. Most of the hepatitis B-related hepatoma cases are seen in Africa and Asia, whereas most of the hepatitis C-related cases are seen in Europe, the United States and Japan (Bosch et al., 2004). Donato et al. and Shi et al. showed in their respective meta-analysis that chronic hepatitis B and C infection carry a significant risk for hepatoma. This risk was higher in co-infected patients with an odds ratio of 165 and 35.7 in their respective studies (Donato, 1998; Shi, 2005). Individuals with hepatitis B e antigen positivity have also shown to have an increased risk for primary liver cancer with a relative risk of 60.2 compared to a relative risk of 9.6 in those that are hepatitis B e antigen negative (Yang, 2002). Similar observations apply to those who have high hepatitis B DNA levels. In recent times, the improved antiviral therapies against hepatitis B, and the successful vaccination programs have helped lower the incidence of hepatocellular carcinoma particularly in what are considered endemic areas for hepatitis B (Chang et al., 1997; Sung, 2008).

The incidence of hepatitis C-related hepatocellular carcinoma has not seen the same encouraging trend that is been observed in hepatitis B-related cases. This is probably due to the interplay of numerous factors that include the natural history of the hepatitis C virus infection, host responses, age at the time of infection, co-morbidities, and alcohol consumption, to name a few. Approximately 15 to 20% of the chronic hepatitis C-infected individuals will develop cirrhosis over a period of 25 to 30 years from the time of infection (Thein, 2008). In developed countries such as the United States, the majority of the infections occurred from the late 1960s to the 1980s, thus in part explaining the rising number of

persons that are being diagnosed with hepatitis C-related cirrhosis over the last couple of decades (Davis, 2010). Hepatocellular carcinoma develops in this population at a rate of 1 to 4% per year, a worrisome statistic considering the escalating numbers of persons diagnosed with hepatitis C-related cirrhosis.

2.3.2 Alcohol

Alcohol consumption has also been linked to primary liver cancer. Mechanisms for direct toxic or carcinogenic effect have not been well-recognized, but it is clear that extended periods of heavy ingestion of more than 60g per day increment the risk for hepatoma. The synergistic effects of alcohol in the presence of hepatitis B or C or both have been established, and seem to have a greater impact in the risk to develop hepatocellular carcinoma (Donato et al., 2002).

2.3.3 Aflatoxin

A connection between the environmental exposure to aflatoxin, a mycotoxin produced by *Aspergillus* fungus, and primary liver cancer has also been documented. The chronic dietary exposure to high levels of this toxin is seen predominantly in developing countries, where hepatitis B is also prevalent. It has been postulated that the overall contribution of aflatoxin exposure to hepatocellular carcinoma cases worldwide is between 5 and 28% (Y. Liu 2010). However, taking into account that hepatitis B infection seems to play a much greater role in the risk of development of hepatoma in these developing countries, the true contribution of aflatoxins is considered minimal. It appears that the mechanism by which this toxin has carcinogenic effects is by causing mutations in the p53 tumor suppressor gene (Gursoy-Yuzugullu, 2011).

2.3.4 Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease is the most common form of chronic liver disease in developed countries. When aggressive, this disease can progress to cirrhosis and hepatocellular carcinoma. The alarming obesity epidemic affecting many of these nations, and components of the metabolic syndrome in particular insulin resistance and diabetes, are factors associated with the development of this chronic disease. These factors by themselves have also been related to an increased risk of primary liver cancer (Calle, 2003; El-Serag, 2004). Considering our current understanding of its rising prevalence, nonalcoholic fatty liver disease could also be responsible for a significant number of the idiopathic or cryptogenic cirrhosis cases as well as the cryptogenic cirrhosis-related hepatoma cases that are seen in industrialized countries (Bungianesi, 2002). Recent studies have shed some light of the molecular mechanisms by which fatty liver and obesity could eventually lead to hepatic cancer (Beyazit, 2010; Park et al., 2010; Wree, 2011).

3. Staging systems for HCC

One of the most fundamental steps in the field of Oncology once the diagnosis of cancer has been established is to determine the stage of this malignant process. This step is very important as it provides information about overall survival and prognosis, and could help

guide treatment strategies. The ideal staging system for hepatocellular carcinoma is one that has high discriminatory ability while remaining simple (Dohmen, 2004).

Many staging systems for hepatoma have been developed and presented in reputable medical journals and conferences. Nonetheless, more studies are constantly being published comparing these models, showing results of modifications made to some of these systems or simply introducing new staging classifications into this already complex field that tries to find the most accurate measure of prognosis and survival. The difficulty of coming up with a globally accepted staging system is related to multiple factors. One of the main causes for the current discrepancies is that the risk factors and their contribution to the development of primary liver cancer vary from one geographic area to another (Marrero, 2010). Another key issue is that overall survival of individuals that are diagnosed with hepatocellular carcinoma is not only determined by the extent and characteristics of this malignancy, but also by the liver function of that individual. Considering that over 80% of the cases of hepatoma occur in the presence of chronic liver disease and cirrhosis, the role of liver function in a prognosis model cannot be ignored. However, there is disagreement as to which markers of liver function should be used or included in such models. The use of the available staging classifications differ from one center or institution to another. It depends on their experience, available resources, and the predominant manifestations and characteristics of hepatocellular carcinoma in their region. Some of the main and most studied staging systems will be described below.

3.1 Tumor-node metastasis (TNM) system

The tumor-node-metastasis (TNM) staging system is one of the earliest models developed, and has been widely used for different solid tumors. This system describes the anatomic extent of cancer by evaluating size of the tumor at the primary site as well as the presence or absence of tumor in regional lymph nodes or beyond (Greene & Sobin, 2008). This classification has been studied in populations with hepatocellular carcinoma to assess prognosis. In a recent Chinese study looking at 243 patients with hepatoma undergoing curative resection, TNM classification was better at prognostic stratification and prognostic prediction than other three models (Lu, 2008). Similar conclusions were drawn from another smaller study comparing the prognostic value of TNM to 6 other staging systems (S.B. Choi, 2008). However, additional studies have shown no superiority to other staging classifications (Chen, 2007). There are some drawbacks to the use of this system in primary liver cancer. The main flaw is that liver function is not taken into consideration in this prognostic model. Another problem is that grading and pathologic staging cannot be assessed in the majority of the cases because very few patients with hepatoma undergo surgical therapies (Dohmen, 2004).

3.2 Cancer of the Liver Italian Program (CLIP)

The CLIP scoring system is another option available to evaluate overall survival in patients with hepatocellular carcinoma. This model combines Child-Pugh score, tumor morphology, alpha feto-protein (AFP) and portal vein thrombosis which were the four independent predictive factors of survival recognized in the multivariate analysis of the original retrospective study (Cancer of the Liver Italian Program [CLIP] Investigators, 1998). By tumor morphology the investigators meant percentage of parenchymal involvement (more

than or less than 50%) and if these were uninodular, multinodular or extensive tumors. Several subsequent studies with large patient populations have demonstrated its prognostic usefulness, including a recent study from Taiwan that compared this system to four other models in 1713 patients with primary liver cancer (Hsu, 2010; Ueno, 2001). A downside to this system is that its tumor morphology classification is too broad for the current practices of aggressive hepatoma screening in high risk populations. Nowadays, more patients are being diagnosed with very small tumors which could limit the use the CLIP score. This model has also been criticized for not discriminating well the cases of advanced stage (CLIP score 4-6), and for classifying most of the patients as early stage (CLIP 0-2), which hampers its stratification capacity (Dohmen 2004; Marrero et al., 2010).

3.3 Japanese Integrated Staging (JIS) and biomarker-combined JIS

In an attempt to also combine liver function and tumor characteristics in order to provide a more precise prognostic appraisal to patients with hepatocellular carcinoma, researchers in Osaka, Japan integrated the Child-Pugh score and the Japanese TNM staging to create the JIS system. In their evaluation of 722 cases with primary liver cancer, they concluded that compared to the CLIP score, their system was better stratifying patients and superior discriminating those cases that were in the early hepatoma phase. Statistically significant differences were seen for the lower JIS scores (Kudo et al., 2003). These findings suggest that this staging system could be more useful in regions where early detection of hepatocellular carcinoma has become more common. Other studies have found encouraging results of this model's ability to predict survival (Chen, 2007; Kudo, 2004; Nanashima, 2005). The main disadvantage of the JIS classification is the inability to discriminate well the cases of advanced stage (JIS score >4).

Biomarkers have been combined to the JIS system to determine if this modification enhances its prognostic value. The biomarker-combined JIS includes the assessment of the following three tumor markers for hepatoma: AFP, *lens culinaris* agglutinin-reactive AFP, and des-gamma-carboxyprothrombin. This modified system was studied in 1,924 patients with primary liver cancer, and proved to be more effective predicting prognosis and stratifying patients than the conventional JIS model (Kitai, 2008). Although this was a large provocative study, this combined system needs to be tested in populations outside of the Asian region.

3.4 Barcelona Clinic Liver Cancer (BCLC) staging system

The Barcelona Clinic Liver Cancer staging system has one peculiarity that is not present in any of the other available prognosis models: it incorporates treatment recommendations in its staging algorithm. The independent predictors of mortality in the original studies were constitutional syndrome, performance status, vascular invasion, and extrahepatic spread (Llovet, 1999a, Llovet, 1999b). By combining in a simple format the evaluation of liver function, tumor stage, performance status, and cancer-related symptoms, and providing suggestions to the best available therapeutic modalities for any particular stage, the BCLC system has demonstrated better predictive value of prognosis and survival stratification when compared to several other staging systems (Cillo, 2004; Guglielmi, 2008). This classification has been gaining wide acceptance as the main staging model used in multiple countries, and supported by several well-respected liver societies. This is related in part to the fact that the BCLC has been externally validated in Asia, Europe and the United States

(Cillo, 2006; Marrero, 2005; J.H. Wang, 2008; Xu, 2010), which has not been accomplished by many of the other staging models. It is also the principal system used in major drug company trials. Some of the criticism to this model is that it includes the subjective factor of performance status, and portal hypertension measurement, which in clinical practice is not routinely done. It also does not provide a classification for patients with single tumors greater than 5 centimeters in diameter or for those with recurrent disease after treatment, situations not infrequently encountered by clinicians treating hepatocellular carcinoma (Sherman, 2011).

There are many other classifications that have been developed to evaluate overall survival and prognosis. Some of these include the Okuda classification, the Chinese University Prognostic Index, the Advanced Liver Cancer Prognostic system, the Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire score, to name a few. Initial studies for several of these models have shown some prognostic value, but they all need further validation in larger more diverse populations.

4. Workup of the patient with diagnosis of HCC

4.1 Imaging studies

The key radiographic feature of hepatocellular carcinoma is the presence of arterial enhancement and venous washout in contrast enhanced imaging studies (Bruix & Sherman, 2010). The clinical staging of this cancer requires radiologic tests that would provide the finest of details about its gross morphology and extent of involvement. This information will be crucial to direct therapeutic decisions and to provide a better estimate of prognosis to the patient.

Liver transplantation is one of the curative treatment options for early stage hepatocellular carcinomas that meet specific criteria. Careful assessment with imaging studies helps select the most suitable candidates for this treatment option. Particular attention is paid to tumor size, the number of lesions, any presence of vascular invasion, as well as evidence of distant metastasis. The most common sites of extrahepatic metastasis are the lungs, abdominal lymph nodes and bones (Katyal, 2000).

Groundbreaking technological advances in computer tomography (CT), magnetic resonance (MR), ultrasonography, and other imaging modalities have been studied to determine their significance in the workup patients with primary liver cancer. Ultrasound has its merit in screening for hepatomas as it is noninvasive, offers no radiation exposure, is readily available in most centers, and is inexpensive compared to other imaging techniques. Unfortunately its sensitivity is not satisfactory in cirrhotic patients, and lesions can be missed (Kim, 2011). Nonetheless, the corner stones and only accepted techniques for staging hepatocellular carcinoma before liver transplantation are contrast-enhanced CT or MR (J.M. Lee, 2011).

4.1.1 Computer tomography (CT)

Computed tomography has proven to be a great tool for the evaluation of hepatocellular carcinoma in affected patients. It is also commonly the test of choice when looking for extrahepatic metastatic disease. The improvements in the scanners, their resolution as well

as the intravenous contrast material used in these studies, has improved the detection of this hepatic malignancy (B.I. Choi, 2010). The use of multidetector CT scans, for example has markedly highlighted the hypervascular characteristics of hepatomas which were not as evident in earlier CT scan technology (K.H. Lee, 2004). The sensitivity of CT scans has increased due to these advances. However, some studies have shown that the improved accuracy of this imaging technique is more apparent in the detection of larger tumors with classic radiographic features, and when the studies are interpreted by more experienced radiologists (Addley, 2011). It seems that in the setting of cirrhosis and when the lesions are smaller than 1 centimeter in diameter, CT has difficulties over and underestimating the diagnosis of hepatocellular carcinoma (Luca, 2010; Ronzoni, 2007). Similar observations have been shown in large and experienced liver transplant centers were the false positive diagnosis rate of primary liver cancer has been reported as high as 8%, and predominantly seen in lesions that were between 0.75 and 1.5 centimeters in diameter (Brancatelli, 2003). Perhaps the continued progress in the field of computed tomography might some day better characterize these small lesions.

4.1.2 Magnetic resonance (MR)

Magnetic resonance imaging provides another suitable option in the staging of hepatocellular carcinoma during the liver transplantation workup. There is ample evidence that attests the important role of MR in the diagnosis and description of hepatomas. The development of faster MR techniques has contributed to obtaining multiphase intravenous contrast-enhanced images that capture with more detail the hypervascular characteristics of this cancer. One of its most important attributes is its superior ability for the detection of small liver tumors, particularly those less than 2 centimeters in diameter (Burrel, 2003; Colli, 2006; Golfieri,2009, D.H. Lee, 2009,). Given the reported higher sensitivities and accuracy detecting these malignant lesions with the newer MR techniques and contrast agents, many transplant centers have adopted MR as their study of choice when evaluating potential transplant recipients with cirrhosis. One cannot forget that as in the case of CT scans and other imaging modalities, the detection and characterization of hepatocellular carcinoma could also be in part influenced by the experience of the radiologists interpreting these studies. MR use is limited in the setting of patients that have certain types of metallic medical implants or other devices, and on those who are claustrophobic or cannot hold their breath. Recently, gadolinium contrast has also been linked to nephrogenic systemic sclerosis which also limits the use contrast-enhanced MR in patients with significant renal failure, a not uncommon situation in the cirrhotic patient population (Idee, 2009). Nonetheless, the current available evidence has MR as the clear frontrunner in the search for the best imaging modality for diagnosing and characterizing hepatocellular carcinoma.

4.1.3 Contrast enhanced ultrasound (CEUS)

Contrast-enhanced ultrasonography's (CEUS) capability of detecting vascular liver lesions, particularly hepatomas, has been studied and shown some promise (Forner, 2008; Jang, 2007). This modality has even demonstrated remarkable sensitivities and accuracy of 87 and 93% respectively, for diagnosing liver cancer in lesions less than 2 centimeters in diameter (Jang, 2009). Other studies have looked at its utility characterizing portal vein thrombosis as malignant or benign. This differentiation is critical as it has major implications for those

patients being evaluated for liver transplantation. When compared to spiral CT for the detection and characterization of portal vein thrombosis in 50 patients with hepatocellular carcinoma and biopsy-proven portal vein thrombosis, CEUS outperformed CT by detecting 100% of the thrombi and correctly characterizing 49/50 (98%) of them. CT modality detected and correctly characterized 68% of these portal vein thrombi, respectively (Rossi, 2008). CEUS has also demonstrated to be as valuable when compared to biopsy for the assessment of the benign or malignant nature of these thrombi (Sorrentino, 2009). Major drawbacks of this modality are that CEUS is still not available in many countries, and that it is operator dependent.

4.1.4 Other imaging studies

Extrahepatic metastasis is not common in early stage hepatocellular carcinoma (Si, 2003). Nevertheless, physicians working at transplant centers fear to miss its presence in a liver cancer patient who is potentially going to be listed for liver transplantation. The United Network for Organ Sharing (UNOS) implemented a policy in which all centers need to include a chest CT to their protocol to evaluate for lung and lymph node metastasis of hepatoma patients being considered for transplantation (United Network for Organ Sharing [UNOS], 2010). Bone is the other preferred site of spread, and although UNOS had included bones scans in the policies implemented in the 1990s, in light recent evidence provided by several studies, this decision was amended in their most recent liver allocation statements. The significant number of false positive or indeterminate results obtained with this modality is a major disadvantage, as well as the costs incurred for a study that has little impact in the selection of patients given its negligible true-positive yield (Koneru, 2005; Sheth, 2005).

The role of positron emission tomography (PET) has also been studied in patients with hepatocellular carcinoma. In general, the sensitivity of this modality for detection of hepatoma that is less than 5 centimeters in diameter has been low (Trojan, 1999; Wolfort, 2010). PET may have some utility identifying extrahepatic metastasis, but the data available is not strong and sufficient enough to widely recommend this practice (Yoon et al., 2007).

4.2 Biopsy

The use of liver biopsies in the setting of hepatocellular carcinoma is controversial. The radiological advances that have taken place over the past decade have markedly improved detection and characterization of this malignancy, thus reducing the need for liver biopsies to confirm the diagnosis. The United Network for Organ Sharing has stated in its liver allocation policy that biopsy is not mandatory in cirrhotic liver transplant candidates with hepatoma as long as the lesion meets imaging criteria (UNOS, 2010). As a result, liver biopsies are now reserved to situations in which the lesion's radiographic studies are not showing the typical features of enhancement in arterial phase and washout in portal venous phase. This rule applies to tumors greater than 2 centimeters in diameter lacking classic features in one imaging modality, and to lesions between 1 and 2 centimeters in diameter with atypical radiographic characteristics in two different imaging modalities (Bruix & Sherman, 2010).

Pathologic staging of hepatocellular carcinoma is established the majority of the time after surgery (resection or transplantation), whereas clinical staging predominantly relies on

imaging studies and is done before the treatment is decided. Some groups advocate obtaining a pretransplant liver biopsy to exclude candidates with poorly differentiated tumors and to identify patients that might need more aggressive bridging therapy. Dubay and colleagues evaluated this and found that in patients exceeding Milan criteria (single tumor not greater than 5 centimeters in diameter or up to 3 tumors none larger than 3 centimeters in diameter) there was a significant increase in overall 5-year survival (61% versus 79%, p=0.03) after the introduction of pretransplant liver biopsies and the use of aggressive bridging therapies (Dubay, 2011). The authors of this study conclude that tumor differentiation might be a more important predictor of biologic behavior than other factors such as size, total tumor diameter, multifocality, and microvascular invasion.

Performing liver biopsies in any patient population carries risks that although small, are not negligible. While pain is the most common complication, bleeding is the most feared and important complication. Severe bleeding events requiring hospitalization and other interventions occur anywhere from 1 in 2,500 to 1 in 10,000 in patients with diffuse, nonfocal liver disease (Rockey, 2009). However, the bleeding risk may be higher in cirrhotic patients, who usually have some degree of coagulopathy and thrombocytopenia. Similar increased risk could be expected in cirrhotic individuals in whom a highly vascular lesion such as hepatocellular carcinoma is being percutaneously biopsied (Huang, 1996). Tumor seeding along the needle track after biopsy has also been reported as a complication in this population (Dubay, 2011; Huang, 1996; Schotman, 1999; Sood, 2002). A recent systematic review and meta-analysis showed that the overall incidence of needle track tumor seeding is 2.7%, or 0.9% per year (M.A. Silva, 2008). These statistics are probably not too high because biopsy of primary hepatic cancer is not a common practice nowadays. However, the risks and benefits of biopsying a liver lesion must always be carefully weighed. This procedure should be reserved to instances when there is reasonable doubt about the diagnosis of hepatoma.

4.3 Criteria for liver transplantation

The field of liver transplantation has gone through many transformations since its early days. Over time this surgical procedure evolved from being a treatment option mostly for individuals with irreversible severe liver dysfunction from any acute or chronic illness to also a providing a proven curative alternative for patients with early stage hepatocellular carcinoma. Currently, the success rate and outcomes seen in this subset of liver transplant recipients is similar to that of recipients who underwent transplantation for indications other than hepatoma. This is in great part due to the knowledge and understanding obtained from outstanding clinical studies published over the last 20 years (Bismuth, 1993; Figueras, 1997; Iwatsuki, 1991; Mazzaferro, 1996; Tan, 1995). These studies have paved the way to the creation of the criteria for liver transplantation and guidelines that are used today.

4.3.1 Milan criteria

The Milan criteria are at the present time the accepted and recommended measure to determine the liver transplantation candidacy of patients with hepatocellular carcinoma. This criteria described in a landmark paper by Mazzaferro and colleagues, demonstrated that in patients with cirrhosis and a single tumor up to 5 centimeters, or up to three lesions none larger than 3 centimeters, and with no evidence of extrahepatic spread or

macrovascular invasion the 4-year post-transplant survival was similar to that of recipients transplanted for reasons other than liver cancer (Mazzaferro, 1996). These findings of greater than 70% survival rate gave a second chance to the previously aborted efforts of transplanting cirrhotic patients with primary liver cancer. The United Network for Organ Sharing endorses these criteria, and adopted them for their liver allocation policies of patients with hepatocellular carcinoma (UNOS, 2010). Despite being validated by many studies, some believe that the Milan criteria are too restrictive and exclude a subset of patients with hepatoma that could have excellent outcomes if were transplanted (M.F. Silva & Sherman, 2011).

4.3.2 University of California, San Francisco (UCSF) criteria

Several groups have carried out studies in an effort to determine if patients with hepatocellular carcinoma exceeding Milan criteria could have similar survival and recurrence-free rates than those within the criteria. Yao and his colleagues from the University of California, San Francisco (UCSF) were the first to challenge the parameters set by the Milan criteria. In their retrospective study, cirrhotic patients with a single tumor up to 6.5 centimeters, or up to three lesions none larger than 4.5 centimeters, and total tumor diameter of no more than 8 centimeters had a 1-year and 5-year overall survival of 90% and 75.2%, respectively (Yao, 2001). The UCSF criteria were validated by the same group when they prospectively demonstrated comparable recurrence-free survival between the patients meeting Milan criteria and those meeting UCSF criteria (Yao, 2007). Unfortunately, data about overall 5-year survival was not included in that study.

4.3.3 Other criteria

Following the thought provoking findings of UCSF's study, other groups have also ventured into pushing the limits set by the Milan criteria without compromising overall patient survival and hepatoma recurrence rate. However, several shortcomings affect the validity of these studies. Most of them are retrospective studies, many do not clearly define the size of the tumors, in particular the upper limits, and others have small number of patients to provide convincing and significant findings. In contrast, the results of a recent well-designed, prospective, multi-center study from UNOS region 4 support the belief that liver transplantation could benefit patients whose hepatocellular carcinoma exceed Milan criteria. UNOS region 4 criteria showed patient, allograft and recurrence-free survival to be similar between patients meeting Milan criteria and those meeting their suggested criteria which consists of one lesion up to 6 centimeters, up to three nodules none larger than 5 centimeters, and a total tumor diameter less than 9 centimeters. It will be interesting to see if the data from their 5-year follow up still shows comparable survival rates (Guiteau, 2010). In general, the encouraging results seen from many of the extended criteria studies have stimulated the discussion about amending the current selection criteria to include this subgroup of liver cancer patients that could also benefit from transplantation.

5. Bridging therapies

Due to the aggressive nature of HCC, liver transplantation is of paramount importance in the eradication of HCC and cirrhosis, as it results in a 5-year overall survival of 70% and a recurrence rate of less than 15% among patients whose HCC falls within established criteria

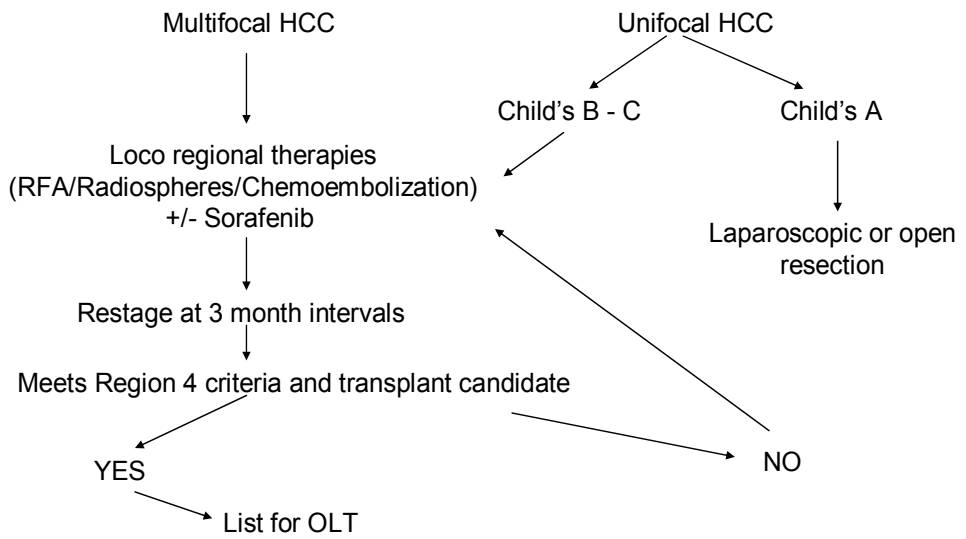
(Decans, 2005; Wigg, 2010). However, fewer than 25% of all patients with HCC qualify for liver transplants. Due to an organ shortage, the average wait period for a liver transplant is 6-9 months. Increased waiting time for HCC patients increases the risk for tumor progression, even with priority listing, and waiting list drop-out, which is defined as withdrawal from the transplant waiting list due to death; tumor progression; or reasons such as becoming too ill for transplantation (Majno, 2010). The dropout rate for HCC patients on the waiting list is 8.7%, 16.9%, and 31.8% at 90, 180, and 365 days respectively. As the doubling time for HCC is approximately 6 months without treatment (Lee, 2007), increased waiting time for transplantation is problematic, and Model for End-Stage Liver Disease (MELD) exception points have been implemented to combat this issue. Since the institution of the MELD system in 2002, transplant candidates with HCC are applicable for listing prioritization via MELD exception points. Patients with HCC within Milan criteria (MC) and in some regions, San Francisco criteria, are granted a MELD exception of 22, followed by upgrades to 25 and 28 every 3 months provided that their tumor burden remains within range. MC is defined as 1 lesion ≤ 5 cm or up to 3 lesions ≤ 3 cm. Exception points result in shorter time to transplant – in 2007, approximately 62% of patients nationwide underwent transplantation within 3 months of receiving exception points for HCC (Yao, 2008). However, this system can be controversial, as some believe that tumors do not adequately degenerate between the time of ablative therapy and transplantation and that it is safer to wait 6 months after bridging before the patient is transplanted (Roberts, 2010). Others argue that tumor size limits are excessively exclusive and that Milan criteria should be expanded to include a larger tumor burden (Yao, 2008). While patients wait for transplant, loco regional ablative therapy, or bridging therapy, in the form of modalites such as Transarterial Chemoembolization (TACE) and Radiofrequency Ablation (RFA) is often employed with the goals of decreasing the waiting list dropout rate; decreasing the rate of tumor recurrence post transplant; and increasing long term survival after transplant (Lee, 2007). The BCLC staging and treatment strategy incorporates the different ablative therapies in patients with early stage (0); early stage (A); and intermittent stage (B) HCC.

5.1 Goal of ablative therapies

Bridging therapy for HCC serves the following roles: 1) to downstage HCC in patients whose tumor burden is outside MC to make the patient transplantable; 2) to prevent disease progression in patients within MC as they await transplant; and 3) to provide palliative and curative therapy in patients who are not OLTx candidates (Table 1). Bridging therapy can also improve the results of transplantation by excluding patients with recurrent disease or unfavorable tumor biology (Majno, 2010). An example of an integral management of HCC is shown in Graph 1.

Downstaging to Milan/ UCSF criteria
Prevent disease progression on the wait list
Prevent dissemination during liver manipulation at transplant surgery
Tumor therapy in non transplant/ non surgical candidates
Palliative therapy

Table 1. Goal of bridging therapies



Graph 1. HCC Management algorithm at The Liver Institute at Methodist Dallas

5.2 Transarterial chemoembolization (TACE)

Of all bridging therapies, TACE is the most validated and widely studied. TACE consists of occluding blood supply to the tumor or tumors and delivering a chemotherapy agent to the tumor via a branch of the hepatic artery which specifically supplies the tumor. During TACE, a chemotherapy agent such as doxorubicin or cisplatin in combination with lipiodol is injected, followed by an agent such as Gelfoam to occlude the tract. An advantage of TACE is that its use results in a high concentration of chemotherapy to the tumor or tumors; also it is useful in downstaging tumors larger than 3 cm (Pompili, 2005). A disadvantage of TACE is that it is less tolerated among patients with severe hepatic decompensation, such as in patients with ascites and impaired coagulopathy. Also, it is contraindicated in patients with portal vein thrombosis. A meta-analysis of several studies showed a median survival of 20 months with arterial embolization. In one study of 61 patients, the survival rate among those who received TACE at 1 and 4 years post transplant was 87.5% and 69.3%, respectively (Yao, 2008).

5.3 Radiation ablation (RFA)

In RFA, a radiofrequency (RF) probe containing an alternating current of approximately 500 kHz and 131 degrees Fahrenheit, is inserted in or around a hepatic tumor via ultrasound guidance for approximately 4-6 minutes (Yao, 2008). Its mechanism of action is inducing thermal energy to the tissue via electromagnetic energy (Lee, 2007). Many centers use microwave ablation in contrast to RFA with similar or better results.In a study of 40 patients who underwent RFA, the rate of complete necrosis was 51.3% for nodules smaller than 3 cm and 14.3% for larger lesions (Pompili, 2005). In accordance with this study, TACE is perceived as more effective than RFA in treating lesions larger than 3 cm. The limitations of RFA are the anatomical location of the tumor, presence of large ascites, and multifocal HCC, in which cases it cannot be used.

5.4 Percutaneous Ethanol Injection (PEI)

In Percutaneous Ethanol Injection (PEI), ultrasound guidance is used to deliver ethanol over 4-8 sessions performed 1-2 times per week. Its mechanism of action is inducing local tumor necrosis as a result of cellular dehydration, protein denaturalization, and chemical occlusion of tumor vessels (Pompili, 2005). PEI is most effective in treating nodules <3 cm and is overall better tolerated than TACE. However, its major limitation is a high local recurrence rate, which can reach up to 43%. Other limitations include a long treatment time. Overall, RFA has better outcomes than PEI and is better tolerated. In one study, the overall 1-2 year survival rates were higher among patients treated with RFA versus PEI were 86% and 64% versus 77% and 43% respectively (Lencioni, 2010). PEI has similar limitations but less efficacy than RFA and is infrequently used in North America for treatment of HCC.

5.5 Yttrium microspheres

Treatment with Yttrium glass microspheres occurs when a catheter is placed in the hepatic artery and the Therasphere vial, which is comprised of silica containing Yttrium, is rapidly injected. The intent is to deliver 125–150 Gy (12,500 –15,000 rads) of radiation to the tumor or tumors. In a study of 65 patients treated from August 2000 to August 2003, 42 patients (64.6 %) had a significant decrease in tumor size within 4 months. The median survival among Okuda stage I patients was 649 days in historical comparison to a median of 244 days. The median survival among Okuda stage II patients was 302 days in historical comparison to a median of 64 days (Carr, 2004). A benefit of this procedure is that it is generally better tolerated than TACE. Drawbacks are potential radiation to other organ systems and elevated cost. Also, it is also contraindicated in patients with severe liver synthetic dysfunction. To be eligible for treatment, a patient must be relatively well-compensated, with a bilirubin < 2.0 mg/dL, creatinine < 2.0 mg/dL, platelets > 60 K/L, a lung shunt < 16%, and ECOG performance < 2 (Carr, 2004).

5.6 External Beam Radiation Therapy

External Beam Radiation Therapy (EBRT) occurs when radiation is delivered to a tumor after the placement of fiducial markers, which are markers are implanted via sterilized needles under ultrasound or CT guidance. Some clinical reports have demonstrated response rates to EBRT ranging from 80-87.5 for small HCC. EBRT may achieve a 10-12 log decrease in tumor, compared to up to 6 logs associated with chemotherapy. An advantage is that EBRT can be delivered to multiple lesions regardless of the proximity of the tumor or tumors to major hepatic vessels or bile ducts. Another advantage is that it is less costly than procedures such as treatment with Yttrium glass Microspheres: an estimation of EBRT is $4,047 for treatment and consultation (Wigg, 2010).

6. Surgical therapies for HCC

6.1 Surgical resection for Hepatocellular carcinoma

Surgical resection is contraindicated in patients with decompensated liver disease or Child's B-C classifications. In patients with Child's A cirrhosis and lack of portal hypertension, resection can be offered as an alternative to transplantation. The advantages of resectional therapy over liver transplantation include: no waiting time, no need for long-term

immunosuppression, can be offered to older patients, cost and transplant can be reserved as a salvage therapy. However, liver transplantation can cure not only HCC but also cures the cirrhosis in the remnant liver. Therefore, cancer recurrence in the remnant liver is a significant disadvantage for the resected patients. Poor prognostic factors identified after liver resection for HCC are microvascular invasion, positive margins and older age groups (>65 years old).When resection was offered to patients within Milan criteria, patients with solitary tumors (5 cm or less) had a significantly better 5-year survival rate of 70% versus 46% in patients with 2-3 tumors less than 3 cm (Fan, 2011).In a meta-analysis of the medline database, the 1, 3 and 5 year survivals for liver resection of HCC were 80%, 55% and 37%, respectively. In the same study, liver transplantation for HCC carried a 1, 3 and 5 year survivals of 80%, 70% and 62%,respectively (Morris-Stiff, 2009).

6.2 Salvage liver transplantation after liver resection

The use of resection as a bridge to transplantation has been advocated as a way of solving the organ donor shortage, whereby transplantation is offered only to patients who develop an intrahepatic recurrence. In the United States, a national Consensus Conference on Liver Allocation for HCC, recommended that : 1- a recurrence more than 2 years after resection for HCC of any stage should be considered de novo, and to considered priority score for HCC if the lesion meets Milan criteria; and 2- a recurrence that presents less than 2 years after resection for a T2 lesion should be elegible for HCC priority score if the lesion meets Milan criteria (Pomfret, 2009). The largest series of salvage transplantation come from French centers with conflicting results: Adam et al (Adam, 2003) report a 5 year survival for salvage transplantation of 29% versus 58% for primary liver transplantation for HCC. In their series, the recurrence rate for liver resection was 77% but only 17% of patients were elegible for salvage transplantation.By utilizing a strategy that offers salvage transplantation for decompensated liver function and positive margins after resection, Belghiti et al (Belghiti, 2003) have shown a comparable 5 year survival for both salvage and primary liver transplantation of 59% and 61%, respectively. The use of resection as a bridge to transplantation may offer important histopathological information that can help identify and subselect patients best suited for transplantation but its application is limited by the fact that only a relatively small number of patients with hepatocellular cancer are candidates for liver resection.

6.3 Laparoscopic resection for HCC

In cirrhotics with preserved liver function and the absence of significant portal hypertension, the laparoscopic approach carries several benefits. Due to the smaller incisions, the collateral abdominal wall circulation is better preserved and an increase in the portal pressure is avoided. Intra-abdominal adhesions are decreased and successful salvage transplantation after laparoscopic resections have been reported (Laurent, 2009). The accepted criteria for laparoscopic approach include: single lesions in peripheral segments of the liver and less than 6 cm in size (Buell, 2008).Outcomes after laparoscopic resection for HCC range from 68 to 74 % 3-year survival (Chen, 2008).In Europe, the 1 and 3 year disease-free survival after laparoscopic resection is 77.5% and 47.1%, respectively (Dagher, 2010); Pittsburgh reported an 88% and 82% disease-free survival at 1 and 3 years, respectively (Nguyen,2011). The option of laparoscopic or open liver resection for HCC should be

considered in the context of a multidisciplinary approach to the individual patient's tumor, liver reserve and potential transplant candidacy.

7. Liver transplantation for HCC

7.1 Technical considerations of liver transplantation for HCC

Patients undergoing liver transplantation for HCC do not usually have the same degree of liver dysfunction than their counterparts. This difference can be explained for example by the use of allocations systems such as the UNOS (United Network for Organ Sharing), where patients with HCC are prioritized based upon tumor criteria rather than the MELD score. As a result, patients undergoing liver transplantation for HCC can have a lesser degree of portal hypertension and the *hepatectomy* phase of the operation is usually less demanding (Table 2). For this reason, cell savers are usually not needed and should be avoided in case any tumor cells are present in the blood. From the technical standpoint, a few aspects should be considered. Every patient should have a recent staging no older than 3 months when brought in for the transplant. A thorough evaluation of the abdomen and hilum should be performed prior to dividing vital structures and if needed, lymph node biopsies should be obtained to rule out the possibility of metastatic disease. The patients should be informed of the possibility of incidental metastatic disease and no patient should be transplanted with known metastatic disease as this represents a contraindication. The possibility of a back up recipient should be considered in cases where patients are transplanted with criteria exceeding Milan or when the imaging is suggestive of possible metastatic or multifocal disease. Because the hepatectomy involves significant manipulation of the liver, pre-transplant tumor therapies are considered to be 'preventive' in releasing cancer cells in the circulation and are encouraged as a mean to prevent early recurrence of HCC. The survival benefit of pretransplant locoregional therapies has been demonstrated for trans-arterial chemoembolization (Maddala, 2004) and radiofrequency ablation (Pompili, 2005).

Recent Staging imaging (within 3 months)
Back-up recipient available
No cell saver
Explore abdomen and hilar nodes before dividing vital structures
No caval preservation if tumor close to cava
Dissect artery carefully in cases with prior embolization procedures and be prepared for vascular grafts

Table 2. Basic technical principles in Liver Transplantation for HCC

Several techniques are available for the *implantation* of the liver: veno-veno bypass, standard technique with clamp and sew, piggy-back technique and caval preservation with or without temporary portocaval shunt. Different techniques have been compared but no study has proven superiority of any particular technique over the others (Sakai, 2010; Gurusamy, 2011; Viera de Melo, 2011). The caval preservation technique (piggy-back) is the preferred technique at many centers but should be avoided in cases where tumors are close to the retrohepatic inferior vena cava or adjacent to the hepatic vein-caval confluence. In cases where pre-transplant therapies have been performed such as chemo- or radio-

embolization, the gastroduodenal artery has usually been embolized and significant arterial inflammation and fragility can be encountered for the arterial anastomosis. For this reason, arterial grafts should always be available as an alternative mean for reconstruction. The portal vein should be inspected for the presence of thrombus and frozen sections of any large or suspicious clot should be obtained intraoperatively as tumor thrombus is an ominous finding that portends a poor prognosis and is a contraindication for transplantation.

7.2 Immunosuppressive strategies after liver transplantation for HCC

The optimal immunosuppressive regimen for post-transplant HCC patients remains a subject of debate and research. In the patients transplanted for HCC within Milan criteria and without high risk features on explants' pathology, i.e. poor differentiation, vascular invasion or tumor viability, most centers would use the individual program's routine regimen. There is data favoring the use of mammalian target of rapamycin inhibitors (mTORi) as part of the immunosuppression of any patient transplanted for HCC, however this is not widely accepted practice. In a large study based upon the Scientific Registry of Transplant Recipients, 2,491 patients transplanted for HCC were compared to 12,167 patients transplanted for non-HCC diagnoses. In this study, a multivariate analysis demonstrated improved survivals for patients transplanted for HCC and induced with anti-CD25 antibodies or maintained with a sirolimus-based regimen (Toso, 2010). A more challenging clinical scenario comes up when patients present with high risk tumor features, when the liver explants' pathology reveals a larger tumor burden than anticipated and when the recipient presents with tumor recurrence. The use mTORi and sorafenib has been studied as a potential combination against Ras pathway activation in the genesis of hepatocellular carcinoma (Newell, 2009).The use of Everolimus or Sirolimus in combination with Sorafenib has anecdotally been proven to control HCC recurrence (Wang, 2010; Kim,2011). A close coordination between the transplant and oncology teams should be exercised in this scenario in order to avoid life threatening side effects of the immunosuppressive therapy chosen.

8. Outcomes of liver transplantation for HCC

The outcomes of Liver Transplantation for HCC have improved dramatically due to the introduction of tumor size criteria. In the 1990's, inferior outcomes after transplant were experienced due to recurrence of HCC leading to patient death. An influential observation was made by the Milan group in 1996 and resulted in the *Milan criteria* (Mazzaferro, 1996).This study showed that patients with pretransplant radiological evidence of 2 to 3 tumors ≤ 3 cm in diameter or a single tumor ≤ 5 cm in diameter had a 4 year recurrence free survival of 92% when the explant confirmed the presence of these criteria. The United Network for Organ Sharing in the United States adopted the Milan criteria for transplantation and many publications have confirmed the validity of restricting the tumor size and number. Increasing the number and/or size of transplantable tumors has raised the concern of transplanting a higher incidence of tumors with microvascular invasion, microsatellitosis and poor differentiation (Table 3). In 2001, The UCSF reported outcomes for 70 consecutive patients transplanted at their center and followed for 12 years (Yao et al, 2001). They identified poor survival in patients with pT4 tumors, total tumor diameter ≥ 8

cm, age > 55 years, poorly differentiated histological grade and an α- fetoprotein level > 1000 ng/ml. The survival rates after liver transplantation of patients meeting *the UCSF criteria* (solitary tumor < 6.5 cm, or < 3 nodules with the largest lesion < 4.5 cm and total tumor diameter < 8 cm) on pretransplant imaging was 90% and 75.2%, at 1 and 5 years, respectively. There has been some criticism to the original Milan criteria due to its restrictive nature and for disallowing other potential candidates with HCC who may benefit from transplant. In addition to the UCSF report, another study has demonstrated reasonable outcomes after liver transplantation for HCC. The Metroticket Study group has described the *Up-to Seven criteria*: seven is the result of the sum of size (in cm) and number of tumors for any given HCC (Mazzaferro, 2009). This complex statistical analysis demonstrated that patients who fulfilled the up-to seven criteria but had no microvascular invasion, achieved overall 5-year survivals of 71%. At the present time, the expected survival of Liver Transplantation for HCC at 5 years is 70% and most current allocation systems work under this goal (which equals transplantation for non HCC indications). The future may bring less restrictive allocation rules, as long as the long term benefit is not inferior to the current standards and as long as patients awaiting transplantation for other indications are not disadvantaged.

Macro and Microvascular invasion
Poor differentiation
Microsatellitosis
α- fetoprotein level > 1000 ng/ml
Tumors exceeding standard criteria (Milan, UCSF, Up-to-seven)

Table 3. Poor prognostic factors in Transplantation for HCC

9. Future therapies and challenges

Primary prevention and detection via sophisticated imaging studies, such as MRI and CT, are imperative for the elimination and minimization of HCC. Further research is also necessary in regards to bridging therapy. The development of molecular signatures which predict the natural behavior of HCCs is being explored, and this includes gene arrays which have already shown some promise. We can predict somewhat more accurately now than ever the propensity of tumors to metastasize and recur after transplant with certain markers such as micro vascular invasion or microsatellitosis on the explant speciments. The grade of HCCs, as characterized by an experienced pathologist on biopsy or explant, has gained importance over the years in prognosticating the natural history of the cancer and its risk of recurrence after resection or transplant. Immunohistochemical markers are being constantly developed- and some have already been tested– regarding the presence of positive CK7 and CK 17 staining in HCCs, which portends a more aggressive cancer with higher recurrence rates. The use of systemic chemotherapy in the form of multikinase inhibitors such as sorafenib in combination with ablative procedures such as TACE, RFA or radioembolization has been used with success and is likely to gain momentum in the future. Furthermore, systemic chemotherapy is more frequently employed after surgery or even after transplantation in patients with high risk tumors. An important consideration for bridging therapy is that it is difficult to completely eradicate tumors >3 cm in size or multifocal HCC, and this area especially merits further exploration. Finally, in the era of organ shortage,

alternative curative modalities to transplant are certainly a need of the hour. With advances in laparoscopic surgical techniques, use of portal vein embolization, and adequate and aggressive ablative techniques pre-operatively, more patients than in the previous years can be made suitable for surgical resection safely and effectively. On the vanguard of medicine is the phenomenon of stem cell research and a discussion on HCC is incomplete without its mention- like other solid tumors- it is believed though not completely elucidated that cancer stem cells may have an important role to play in the natural history and response to treatment of HCCs. Although these stem cells in the liver have not been accurately identified, there is intensive investigation in this area (Wen Xu, 2009). The further development of these stem cells has boundless potential in the prognostication and treatment of this cancer.

10. References

Adam R,Azoulay D, Castaing D et al. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy ? Ann Surg 2003; 238(4):508-518

Addley HC, Griffin N, Shaw AS et al. Accuracy of hepatocellular carcinoma detection on multidetector CT in a transplant liver population with explants liver correlation. Clin Radiol 2011;66(4):349-56

Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009;27(9):1485-91

Belghiti J, Cortes A, Abdalla EK, et al. Resection prior to liver transplantation for hepatocellular carcinoma. Ann Surg 2003;238(6):885-892

Beyazit Y, Kekilli M, Kurt M et al. Role of IL-6 activated STAT3 and TNF expression in obesity-associated hepatocellular carcinoma. Aliment Pharmacol Ther 2010;32:304-5

Bismuth H, Chiche L, Adam R et al. Liver resection versus transplantation in cirrhotic patients with hepatocellular carcinoma in cirrhosis. Ann Surg 1993;218:145-51

Bosch FX, Ribes J, Cleries R et al. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9(2):191-211

Bosch FX, Ribes J, Diaz M, et al. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127(suppl 1):s5-16

Brancatelli G, Baron RL, Peterson MS et al. Helical CT screening for hepatocellular carcinoma in patients with cirrhosis: frequency and causes of false-positive interpretation. Am J Roentgenol 2003;180(4):1007-14

Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. American Association for the Study of Liver Diseases, July 2010

Buell JF, Cherqui D, Geller DA, et al. World Consensus Conference on Laparoscopic Surgery. The international position on laparoscopic liver surgery: the Louisville Statement, 2008. *Ann Surg.* 2009;250(5):825-830.

Bungianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134-40

Burrel M, Llovet JM, Ayuso C et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explants correlation. Hepatology 2003;38(4):1034-42

Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625-38

Carr, B. Hepatic Arterial Yttrium Glass Microspheres (Therasphere) for Unresectable Hepatocellular Carcinoma: Interim Safety and Survival Data on 65 Patients. Liver Transplantation 10 (2004): 107-110.

Chang MH, Chen CJ, Lai MS et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336(26):1855-9

Chang MH, You SL, Chen CJ et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vacinees: a 20 year follow up study. J Natl Cancer Inst 2009;101(19):1348-55

Chen HY, Juan CC, Ker CG. Laparoscopic liver surgery for patients with hepatocellular carcinoma. *Ann Surg Oncol*. 2008;15(3):800-806.

Chen TW, Chu CM, Yu JC et al. Comparison of clinical staging systems in predicting survival of hepatocellular carcinoma patients receiving major or minor hepatectomy. Eur J Surg Oncol 2007;33(4):480-7

Choi BI. Advances of imaging for hepatocellular carcinoma. Oncology 2010;78(suppl 1):46-52

Choi SB, Lee JG, Kim KS et al. The prognosis and survival analysis according to seven staging systems of hepatocellular carcinoma following curative resection. Hepatogastroenterology 2008;55:2140-5

Cillo U, Bassanello M, Vitale A et al. The critical issue of hepatocellular carcinoma prognostic classification: Which is the best available? J Hepatol 2004;40:124-31

Cillo U, Vitale A, Grigoletto F et al. Prospective validation of the Barcelona Clinic Liver cancer staging system. J Hepatol 2006;44:723-31

Colli A, Fraquelli M, Casazza G et al. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha feto-protein in the diagnosis of hepatocellular carcinoma: a systematic review. Am J Gastroenterol 2006;101(3):513-23

Dagher I, Belli G, Fantini C, et al. Laparoscopic hepatectomy for hepatocellular carcinoma: a European experience. *J Am Coll Surg*. 2010;211:16-23

Davila JA, Morgan RO, Shaib Y et al. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372-80

Davis GL, Alter MJ, El-Serag HB, et al. Aging of hepatitis C virus-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. Gastroenterology 2010;138(2):513-21

Decans T, Roudot-Thoraval F, Bresson-Hadi, C, et al. Impact of Pretransplantation Transartierial Chemoembolization on Survival and Recurrence After Liver Transplantation for Hepatocellular Carcinoma. Liver Transpl 11 (2005): 767-775.

Dohmen K. Many staging systems for hepatocellular carcinoma: Evolution from Child-Pugh, Okuda to SLiDe. J Gastroenterol Hepatol 2004;19(11):1227-32

Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75(3):347-54

Donato F, Tagger A, Gelatti U, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002;155(4):323-31

Dubay D, Sandroussi C, Sandhu L et al. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criteria. Ann Surg 2011;253:166-72

El-Serag HB, Lau M, Eschbach K, et al. Epidemiology of hepatocellular carcinoma in Hispanics in the United States. Arch Intern Med 2007;167(18):1983-9

El-Serag HB, Rudolph L. Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology 2007;132(7):2557-76

El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460-8

Fan ST, Poon RT, Yeung C et al. Outcome after partial hepatectomy for hepatocellular cancer within the Milan criteria. Br J Surg 2011 Sep; 98 (9):1292-1300

Figueras J, Jaurrieta E, Valls C et al. Survival after liver transplantation in cirrhotic patients with and without hepatocellular carcinoma: a comparative study. Hepatology 1997;25(6):1485-9

Forner A, Vilana R, Ayuso C et al. Diagnosis of hepatic nodules 20mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology 2008;47(1):97-104

Freeman AJ, Dore GJ, Law MG, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. Hepatology 2001;34(4):809-16

Golfieri R, Marini E, Bazzocchi A et al. Small (<or=3cm) hepatocellular carcinoma in cirrhosis: the role of double contrast agents in MR imaging vs. multidetector-row CT. Radiol Med 2009;114(8):1239-66

Greene FL, Sobin LH. The staging of cancer: a retrospective and prospective appraisal. CA Cancer J Clin 2008;58(3):180-90

Guglielmi A, Ruzzenente A, Pachera S et al. Comparison of seven staging systems in cirrhrotic patients with hepatocellular carcinoma in a cohort of patients who underwent radiofrequency ablation with complete response. Am J Gastroenterol 2008;103:597-604

Guiteau JJ, Cotton RT, Washburn WK et al. An early regional experience with expansion of Milan criteria for liver recipients. Am J Transpl 2010;10(9):2092-8

Gursoy-Yuzugullu O, Yuzugullu H, Yilmaz M, et al. Aflatoxin genotoxicity is associated with a defective DNA damage response bypassing p53 activation. Liver Int 2011;31(4):561-71

Gurusamy KS, Pamecha V, Davidson BR. Piggy-back graft for liver transplantation. Cochrane Database Syst Rev 2011 Jan 19;(1) CD008258

Hsu CY, Hsia CY, Huang YH, et al. Selecting an optimal staging system for hepatocellular carcinoma: comparison of 5 currently used prognostic models. Cancer 2010;116(12):3006-14

Huang GT, Sheu JC, Yang PM et al. Ultrasound-guided cutting biopsy for the diagnosis of hepatocellular carcinoma: a study based on 420 patients. J Hepatol 1996;25:334-8

Idee JM, Port M, Dencausse A et al. Involvement of gadolinium chelates in the mechanism of nephrogenic systemic fibrosis: an update. Radiol Clin North Am 2009;47(5):855-69

Iwatsuki S, Starzl TE, Sheahan DG et al. Hepatic resection versus transplantation for hepatocellular carcinoma. Ann Surg 1991;14(3):221-8

Jang HJ, Kim TK, Burns PN et al. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. Radiology 2007;244(3):898-906

Jang HJ, Kim TK, Wilson SR. Small nodules (1-2cm) in liver cirrhosis: characterization with contrast-enhanced ultrasound. Eur J Radiol 2009;72(3):418-24

Kanwal F, Hoang T, Kramer JT et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterology 2011;40(4):1182-88

Katyal S, Oliver JH, Peterson MS et al. Extrahepatic metastases of hepatocellular carcinoma. Radiology 2000;216(3):698-703

Kim KA, Kim MJ, Choi JY et al. Development of hepatocellular carcinoma in patients with absence of tumors on a prior ultrasound examination. Eur J Radiol 2011, doi:10.1016/j.ejrad.2011.03.053

Kim R, Aucejo F. Radiologic complete response with sirolimus and sorafenib in a hepatocellular carcinoma patient who relapsed after orthotopic liver transplantation.J Gastrointest cancer 2011 Mar ;42 (1):50-3

Kitai S, Kudo M, Minami Y et al. A new prognostic staging system for hepatocellular carcinoma: value of the biomarker combined Japan Integrated Staging score. Intervirology 2008;51(suppl 1):86-94

Koneru B, Teperman LW, Manzarbeitia C et al. A multicenter evaluation of utility of chest computer tomography and bone scan in liver transplant candidates with stage I and II hepatoma. Ann Surg 2005;241:622-8

Kudo M, Chung H, Haji S et al. Validation of a new prognostic staging system for hepatocellular carcinoma: The JIS score compared with the CLIP scope. Hepatology 2004;40:1396-1405

Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellualr carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging score (JIS score). J Gastroenterol 2003;38:207-15.

Laurent A, Tayar C, Andréoletti M, et al. Laparoscopic liver resection facilitates salvage liver transplantation for hepatocellular carcinoma. J Hepatobiliary Pancreat Surg. 2009;16(3):310-4.

Lee DH, Kim SH, Lee JM et al. Diagnostic performance of multidetector row computed tomography, superparamagnetic iron oxide-enhanced magnetic resonance imaging, and dual-contrast magnetic resonance imaging in predicting the appropriateness of a transplant recipient based on Milan criteria: correlation with histopathological findings. Invest Radiol 2009;44(6):311-21

Lee JM, Trevisani F, Vilgrain V et al. Imaging diagnosis and staging of hepatocellular carcinoma. Liver Transpl 2011, doi:10.1002/lt.22369

Lee KH, O'Malley ME, Haider MA et al. Triple-phase MDCT of hepatocellular carcinoma. Am J Roentgenol 2004;182(3):643-9

Lee, F. Treatment of Hepatocellular Carcinoma in Cirrhosis: Locoregional Therapies for Bridging to Liver Transplant. Liver Transpl 13 (2007). 24-26.

Lencioni, R., Cioni, D., Crocetti, L., and Bartolozzi, C. "Percutaneous Ablation of Hepatocellular Carcinoma: State-of-the-Art." Liver Transpl 10 (2004): 91-96.

Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ Health Perspect 2010;118:818-24

Llovet JM, Bru C, Briux J. Prognosis of hepatocellular carcinoma: the BCLC stating classification. Semin Liver Dis 1999;19(3):329-38

Llovet JM, Bustamante J, Castells A et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology 1999;29(1):62-7

Lu W, Dong J, Huang Z, et al. Comparison of four staging systems for Chinese patients with hepatocellular carcinoma undergoing curative resection: Okuda, CLIP, TNM, and CUPI. J Gastroenterol Hepatol 2008;23(12):1874-8

Luca A, Caruso S, Milazzo M et al. Multidetector-row computer tomography (MDCT) for the diagnosis of hepatocellular carcinoma in cirrhotic candidates for liver transplantation: prevalence of radiological vascular patterns and histological correlation with liver explants. Eur Radiol 2010;20(4):898-907

Maddala YK, Stadheim L, Andrews JC et al. Drop-out rates of patients with hepatocellular cancer listed for liver transplantation: outcome with chemoembolization. Liver Transpl 2004:10:449-455

Majno, P., Mentha, G., and Toso, C. "Transplantation for Hepatocellular Carcinoma: Management of Patients on the Waiting List." Liver Transpl 16 (2010): 2-11.

Marrero JA, Fontana RJ, Barrat A et al. Prognosis of hepatocellular carcinoma: Comparison of 7 staging systems in an American cohort. Hepatology 2005;41:707-16

Marrero JA, Kudo M, Bronowicki JP. The challenge of prognosis and staging for hepatocellualr carcinoma. Oncologist 2010;15(suppl 4):23-33

Mazzaferro V, LLovet JM, Miceli R, et al.Predicting survival alter liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet oncol 2009 Jan 10(1):35-43

Mazzaferro V, Regalia E, Doci R et al. Liver transplantation for the treatment of small hepatocellular carcinoma in patients with cirrhosis. N Eng J Med 1996;334(11)693-9

Morris-Stiff G, Gomez D, de Liguori Carino N et al. Surgical management of hepatocellular carcinoma: is the jury still out ?. Surg Oncol 2009 Dec;18(4):298-321

Nanashima A, Omagari K, Tobinaga S et al. Comparative study of survival of patients with hepatocellular carcinoma predicted by different staging systems using multivariate analysis. Eur J Surg Onc 2005;31(8):882-90

Newell P, Toffanin S, Villanueva A, et al. Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo.J Hepatol 2009, Oct ; 51 (4): 725-33

Nguyen KT, Marsh JW, Tsung A, et al. Comparative benefits of laparoscopic vs open hepatic resection: A critical appraisal. *Arch Surg*. 2011; 146(3):348-356.

Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. Dig Liver Dis 2010;42(suppl 3):s206-14

Park EJ, Lee JH, Yu GY et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 2010;140:197-08

Parkin DM, Bray F, Ferlay J et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74-108

Perz JF, Armstrong GL, Farrington LA et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006;45(4):529-38

Pomfret EA, Washburn K, Wald C et al.Report of a national Conference on Liver Allocation in patients with hepatocellular carcinoma in the United States. Liver Transpl 2010;16:262-278

Pompili M, Mirante VG, Rondinara G et al. Percutaneous ablation procedures in cirrhotic patients with hepatocellular carcinoma submitted to liver transplantation: assessment of efficacy at explant analysis and of safety for tumor recurrence. Liver Transpl 2005; 41:1130-1137

Roberts, J., Vennok, A., Kerlan, R. et al. "Hepatocellular Carcinoma: Ablate and Wait Versus Rapid Transplantation." Liver Transplantation 16 (2010): 925-929.

Rockey DC, Caldwell SH, Goodman Z et al. Liver biopsy. Hepatology 2009;49(3):1017-44

Ronzoni A, Artioli D, Scardina R et al. Role of MDCT in the diagnosis of hepatocellular carcinoma in patients with cirrhosis undergoing orthotopic liver transplantation. Am J Roentgenol 2007;189(4):792-8

Rossi S, Ghittoni G, Ravetta V et al. Contrast-enhanced ultrasonography and spiral CT in detection and characterization of portal vein thrombosis complicating hepatocellular carcinoma. Eur Radiol 2008;18:1749-56

Sakai T, Matsusaki T, Marsh JW et al. Comparison of surgical methods in liver transplantation: retrohepatic caval resection with veno-venous bypass (VVB) versus piggyback (PB) with VVB versus PB without VVB. Transpl Int 2010 Dec;23(12):1247-58

Schotman SN, De Man RA, Stoker J et al. Subcutaneous seeding of hepatocellular carcinoma after percutaneous liver biopsy. Gut 1999;45(4)625-6

Sherman M. Hepatocellular carcinoma: screening and staging. Clin Liver Dis 2011;15(2):323-34

Sheth H, Javed SS, Hilson AJW et al. Radioisotope bone scan in preoperative staging of hepatopancreatobiliary cancer. Br J Surg 2005;92(2):203-7

Shi J, Zhu L, Liu S, et al. A meta-analysis of case-control studies on the combined effects of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. Br J Cancer 2005;92(3):607-12

Si MS, Amersi F, Golish SR et al. Prevalence of metastases in hepatocellular carcinoma: risk factors and impact on survival. Am Surg 2003;69(10):879-85

Silva MA, Hegab B, Hyde C et al. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. Gut 2008;57(11):1592-6

Silva MF, Sherman M. Criteria for liver transplantation for HCC: what should the limits be? J Hepatol 2011, doi:10.1016/jhep.2011.05.012

Sood A, Midha V, Sood N. Needle track metastasis of primary liver carcinoma after percutaneous needle aspiration. Am J Gastroenterol 2002;97(3):765-6

Sorrentino P, D'Angelo S, Tarantino L et al. Contrast-enhanced sonography versus biopsy for the differential diagnosis of thrombosis in hepatocellular carcinoma patients. World J Gastroenterol 2009;15(18):2245-51

Sun JJ, Tsoi KK, Wong VW, et al. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther 2008;28(9):1067-77

Tan KC, Rela M, Ryder SD et al. Experience of orthotopic liver transplantation and hepatic resection for hepatocellular carcinoma of less than 8 cm in patients with cirrhosis. Br J Surg 1995;82:253-6

Tanaka H, Imai Y, Hiramatsu N et al. Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. Ann Intern Med 2008;148(11):820-6

The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: A retrospective study of 435 patients. Hepatology 1998;28:751-5

Thein HH, Yi Q, Dore GJ, et al. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology 2008;48(2):418-31

Toso C, Merani S, Bigam DL et al. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. Hepatology, 2010 Apr; 51 (4):1237-43

Trojan J, Schroeder O, Raedle J et al. Flourine-18 FDG positron emission tomography for imaging of hepatocellular carcinoma. Am J Gastroenterol 1999;94(11):3314-9

Ueno S, Tanabe G, Sako K, et al. Discrimination value of the new western prognostic system (CLIP score) for hepatocellular carcinoma in 662 Japanese patients. Cancer of the Liver Italian Program. Hepatology 2001;34:529-34

United Network for Organ Sharing. Policies 3.6:organ distribution: allocation of livers. Available at http://www.unos.org

Viera de Melo PS, Miranda LE, Batista LL et al. Orthotopic liver transplantation without venovenous bypass using the conventional and piggyback techniques. Transplant Proc 2011 May; 43(4): 1327-33

Wang JH, Changchien CS, Hu TH et al. The efficacy of treatment schedules according to Barcelona Clinic Liver Cancer staging for hepatocellular carcinoma: Survival analysis of 3892 patients. Eur J Cancer 2008;44:1000-6

Wang Y, Speeg KV, Washburn WK et al. Sirolimus plus sorafenib in treating HCC recurrence after liver transplantation: a case report. World J Gastroenterol 2010 Nov 21;16(43):5518-22

Wen Xu et al. Progress and Prospects in cancer stem cell research in hepatocellular carcinoma. Chinese Journal of Cancer 28 (2009): 43

Wigg, A., Palumbo, K., and Wigg, D. Radiotherapy for Hepatocellular carcinoma: Systemic review of radiobiology and modeling projects indicate reconsideration of its use. Journal of Gastroenterology and Hepatology 25 (2010): 664-617.

Wolfort RM, Papillion PW, Turnage RH et al. Role of FDG-PET in the evaluation and staging of hepatocellular carcinoma with comparison to tumor size, AFP level and histologic grade. Int Surg 2010;95(1):67-75

Wree A, Kahraman A, Gerken G, et al. Obesity affects the liver- the link between adipocytes and hepatocytes. Digestion 2011;83:124-33

Xu LB, Wang J, Liu C et al. Staging systems for predicting survival of patients with hepatocellular carcinoma after surgery. World J Gastroenterol 2010;16(41)5257-62

Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk for hepatocellular carcinoma. N Engl J Med 2002;347(3):168-74

Yao FY, Ferrel L, Bass NB et al. Liver Transplantation for Hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival.Hepatology 2001; 33:1394-1403

Yao FY, Ferrell L, Bass NM et al. Liver transplantation for hepatocellular carcinoma: expansion fo the tumor size limits does not adversely impact survival. Hepatology 2001;33:1394-1403

Yao FY, Xiao L, Bass NM et al. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. Am J Transpl 2007;7(11):2587-96

Yao F, Kerlan R, Hirose R, et al. Excellent Outcome Following Down-staging of Hepatocellular Carcinoma prior to Liver Transplantation: An Intention-to-treat Analysis. Hepatology (2008): 819-827.

Yoon KT, Kim JK, Kim do Y et al. Role of 18-fluorodeoxyglucose positron emission tomography in detecting extrahepatic metastasis in pretreatment staging of hepatocellular carcinoma. Oncology 2007;(suppl 1):104-10

Yoshizawa H. Hepatocellular carcinoma associated wth hepatitis C virus infection in Japan: projection to the other countries in the foreseeable future. Oncology 2002;62(suppl 1):8-17

Yu MW, Yang YC, Yang SY. Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men. J Natl Cancer Inst 2001;93(21):1644-51

14

Liver Transplantation Due to Abdominal Trauma

Matthias Heuer[1], Sven Lendemans[2],
Gernot M. Kaiser[1] and Andreas Paul[1]
[1]*Department of General, Visceral and Transplantation Surgery,*
[2]*Department of Trauma Surgery,*
University Hospital of Essen,
Germany

1. Introduction

1.1 Organ shortage situation in Eurotransplant regions/Germany

An organ transplant is currently the treatment method of choice for a large number of patients with chronic or acute organ failure. However, the shortage of suitable donor organs poses a considerable problem for transplantation medicine not only in Germany (1, 2). The figure of 3,897 available postmortal donated organs in 2009 currently contrasts with the needs of approximately 12,000 patients waiting for a suitable donor organ (3). At the same time, the entries on transplant waiting lists have increased by about 45% in the last 17 years; this upward trend is expected to continue (4, 5). As a result, the shortage of suitable donor organs means that in Germany more than 1,000 patients on the transplant waiting list die every year. According to the German Organ transplant Foundation (DSO), three times as many people are waiting for a kidney transplant than the number of organs that can be procured (6-10). As a result, on average 3 people on the waiting list die every day because no suitable donor organ is available in time (11, 12).

1.2 Liver transplantation as a valuable option due to trauma

The isolated trauma of the liver are a rare event in blunt injuries of severely injured patients; yet liver injuries probably lead to a clear increase in post-trauma mortality due to the complex functioning of this organ. The immunological changes caused by blunt liver trauma are just as difficult to classify as the specific mortality. As the liver injury increases in severity, other organ systems become involved, so that total mortality results from the cumulation of all damaged organs. However, there are definitive indications leading to speculation that liver involvement superproportionally increases total mortality (13-16). The mortality rate after liver trauma documented in the literature has a wide spread and ranges between 7 and 36% (17, 18). This is differentiated between early mortality, mainly due to blood loss, and late mortality. Late mortality is frequently based on secondary complications from intensive medical treatment in connection with immunological failure after a trauma which can cause sepsis/SIRS and multi-organ failure. The actual specific significance of liver injury for the emergency of such complications in this event is to date not yet fully understood.

The liver is crucial to the post-traumatic recovery of a severely injured patient. This is where proteins are formed, which constitute among other things components for coagulation and non-specific defense. It has a decisive effect on inflammatory processes and represents the center of the energy metabolism. Moreover, the Kupffer cells represent the largest macrophage pool in humans. The knowledge that liver damage alone negatively affects both early and late mortality may be an initial approach leading to organ-specific post-traumatic treatment.

In this context, it must be kept clearly in mind that the last two decades have seen a clear paradigm change concerning surgical treatment for liver injuries (19). With the introduction of computer tomography and the availability of clotting factors, conservative treatment of the liver injury became the method of choice for hemodynamically stable patients after blunt liver trauma (20). Different studies have shown that 71-89% of all patients with blunt liver trauma can be successfully conservatively treated. As a result, the survival rate is 85 to 94% (21). There is also agreement that despite all the opportunities for intensive fluid, blood and coagulation substitution, hemodynamically unstable patients must still be operated on (22).

Here, the management of a liver injury aims to control hemorrhage, preserve sufficient hepatic function and prevent secondary complications. If an adequate control of the bleeding cannot be achieved despite exhausting the current therapy options, the indication for liver transplant (LT) needs to be assessed critically in individual cases. These cases are extremely scarce in the clinical daily routine (23).

Nonetheless, LT are carried out due to acutely uncontrollable liver injuries in exceptional cases only. For this, indication is judged critically and discussed controversially due to usually existing secondary injuries, early septic complications, and poor general condition. Due to poor results, LT in these patients is occasionally described as “waste of organs”, however based on insufficient data (24, 25).

Patients with subacute and chronic results of a liver injury need to be considered differently from the acute and due to their initial position very special group of surgically uncontrollable patients with liver trauma. However, they share the fact that also the indication for transplantation for instance in patients with “shock liver” in the context of polytrauma or with induced liver failure after a longer intensive therapy need to be measured (26, 27).

2. Investigate the significance of liver trauma and prognostic factors in severely injured patients

Based on an analysis of the trauma registry data from the German Society of Trauma Surgery (DGU) [Deutsche Gesellschaft für Unfallchirurgie] from 1993 to 2005 (n=24,711), the present study examined whether the participating liver injury in a polytraumatized patient superproportionally increases the incidence of sepsis and multi-organ failure, and whether survival after polytrauma is definitively decreased when the liver is involved.

2.1 Investigate the indication of liver transplantation for uncontrollable liver trauma

Our study was aimed to critically question the indication of LT on the basis of blunt and uncontrollable liver trauma; we therefore report our experience with 4 patients who all

underwent LT due to accident-caused uncontrollable acute liver trauma at our center along with a comparison and discussion of our results based on the current literature.

2.2 Try to answer the question – Is transplantation a valuable option or just a "waste of organs" in polytraumatized patients with liver injury –

2.2.1 Find new approaches of organ donation improvement

First of all, with regard to the methodology of this work it should be pointed out that in order to respond to the self-declared question posed by this work, various databases and registers, which are listed in detail in the following, were used for analysis.

3. Prognostic factors of liver injury in polytraumatic patients

Based on an analysis of the trauma registry data from the DGU from 1993 to 2005 (n=24,711), the present analyses examined whether the participating liver injury in a polytraumatized patient superproportionally increases the incidence of sepsis and multi-organ failure, and whether survival after polytrauma is definitively decreased when the liver is involved.

It is a standardized and anonymized documentation of severely injured patients at defined phases from time point of accident to hospitals discharge (28). In this analysis the following eligibility criteria were used:

1. Injury Severity Score (ISS) ≥16
2. direct admission from scene to a trauma center
3. no isolated head injury

Injury severity score (ISS) and the severity of individual injuries were determined with the 1998 revision of the Abbreviated Injury Scale (AIS), table 1.

The existence of sepsis was defined based on the criteria of Bone et al. (29). The definition of organ failure followed the SOFA score (Sequential Organ Failure Assessment) (30). An individual organ failure was defined by at least 3 SOFA score points; a multi-organ failure (MOF) was defined as simultaneous failure of at least two organs.

All those patients with a documented liver injury (AIS abdomen <3 and AIS liver 2-5) were assigned to the "liver trauma" group. Patients with abdominal injuries (AIS abdomen 2-5 or AIS liver <3) were placed in the "abdominal non-liver injury" group. All remaining patients who had an AIS abdomen or liver <3 were placed in the third "non-abdominal trauma" group (control group). The restriction to cases with ISS ≥16 guaranteed a minimum injury severity of AIS 3 for the primary region in the respective study groups.

3.1 Statistics (I)

From 1993 until 2001, data were collected and entered on paper sheets. Since 2002, data collection was done with internet-based data entry software with integrated plausibility checks. The anonymized data were analyzed with the statistical program SPSS (Version 14, Chicago, USA). Incidences are presented with counts and percentages, continuous values with mean and standard deviation (SD). Analysis was mainly restricted to descriptive

statistics. Statistical tests were avoided due to the multiple comparisons (several groups and outcome parameters), as well as the high sample size which could lead to irrelevant significances. In selected situations only, data from the group with liver trauma were compared statistically against the remaining groups (χ^2 test for incidence rates and U-test for continuous values).

AAST Grade	Injury	Injury Description	AIS-98* Grade
I	hematoma	subcapsular, <10% surface	2
	laceration	capsular tear, <1cm parenchymal depth	2
II	hematoma	subcapsular, 10–50% surface; intraparenchymal hematoma, <10cm in diameter	2
	laceration	capsular tear, 1–3cm parenchymal depth, <10cm length	2
III	hematoma	subcapsular, >50% surface; intraparenchymal hematoma, >10cm in diameter	3
	laceration	>3cm parenchymal depth	3
IV	laceration	parenchymal disruption involving 25–75% of hepatic lobe or 1–3 segments	4
V	laceration	parenchymal disruption involving >75% of hepatic lobe or >3 segments within a single lobe	5
	vascular	hepatic venous injuries	5
VI	vascular	hepatic avulsion	6

*Note–AIS-98 = Abbreviated Injury Scale, 1998 version.

Table 1. American Association for the Surgery of Trauma (AAST) -scale and modified scale for classification of liver injuries

3.2 Transplantation after blunt trauma to the liver

Our study was aimed to critically question the indication of LT on the basis of blunt and uncontrollable liver trauma; we therefore report our experience with 4 patients who all underwent LT due to accident-caused uncontrollable acute liver trauma at our center along with a comparison and discussion of our results based on the current literature.

From September 1987 to December 2008, our center performed 1,529 LT (6 traumatic and 1,523 others in 4 and 1,475 patients, respectively). Apart from transplant surgery, the clinic's

second major focus is on hepatobiliary surgery. In this analysis the following eligibility criteria were used:

1. patients ≥ 18 years;
2. trauma-caused blunt liver injury;
3. uncontrollable clinically situation without transplantation.

The transplantations conformed to the local ethical guidelines and followed the ethical guidelines of the 1975 Declaration of Helsinki. LT was indicated in cases of uncontrollable liver injuries. It was considered contraindicated in cases of irreversible cerebral damage (i.e. slight cerebral edema is not considered a contraindication), absence of uncontrolled extrahepatic infection (i.e. no SIRS), absence of uncontrolled multiple organ failure (MOF) (less than 3 organs including the liver).

In order to offer the best sized organ in a timely fashion, the following surgical procedures were considered for all recipients when available: deceased donor liver transplantation (DDLT) (full size and split-left lateral, left, right, extended right) and living donor liver transplantation (LDLT) (left lateral, left, right).

The conservative management of our patients consisted of: a) causal therapy, b) intense monitoring of hemodynamic, respiratory, renal, neurological, infectious, hepatic and metabolic parameters, c) minimal handling and no sedation whenever possible, d) fluid restriction but enough fluid to assure cerebral perfusion, e) hypercaloric protein-free nutrition, f) intestinal sterilization with Neomycine and Lactulose, g) fresh frozen plasma in cases of coagulation disorder. All patients received immunosuppressive induction with Prednisolone. Maintenance immunosuppression consisted of a dual therapy with calcineurin inhibitors and Prednisolone post-transplant.

We monitored the peri-operative course of each patient and noted short-term and long-term outcomes. The end of follow-up for this study was the end of July 2009.

3.2.1 Statistics (II)

Continuous variables are expressed as mean (±SD) or median (range).

4. Prognostic factors of liver injury in polytraumatic patients

The average age was 39.6±19.5 years, and 72.8% were male. The average ISS was 31.9±12.1 points. Patients with liver trauma were found to be younger (liver 34.9±15.6; abdomen 37.7±18.2) and more frequently female (66.0% vs. 73.5%). The number of blunt traumas was only slightly less in the liver group (91.8%) than in the non-liver abdominal trauma group (93.5%). The incidence of a primary liver injury according to the criteria mentioned was rather small, with 3.1% in the total group studied (abdomen 5.5%).

4.1 Mortality

Mortality in the liver trauma group was significantly increased (34.9%) compared to patients in the abdominal trauma group (12.0%) and patients with no primary liver or abdominal injury (control group 12.0%).

Further analysis of these differences between abdominal trauma group and the control group showed that the higher mortality in the control group is explained by the high mortality of the accompanying head injuries. Thus, a subgroup analysis shows that of the 9,574 trauma patients in the control group, 2,160 patients had suffered a relevant head injury (AIS >3). In this subgroup, mortality even reached 32.8%. The investigation of early mortality showed that 27.3% of patients in the liver trauma group died within the first 24 hours, while this rate was only 6.6% in the non-liver abdominal group.

4.2 Blood transfusion

Compared to patients with non-liver abdominal injuries, patients with severe liver trauma clearly had a greater need for blood transfusions (67.0% vs. 48.0%). The high blood loss in the liver group is correlated with the blood pressure pattern in both the preclinical and emergency room (ER) phases. Initial blood pressure was ≤90mmHg preclinically in 36.4% of the liver group and 30.0% of the abdomen group. Both groups are clearly above the rate in the control group (22.0%). Blood pressure in the liver group could not be raised in any definitive way during initial clinical care (ER phase in contrast to the abdomen group (RR <90mmHg, liver: 32.2% with delta RR 4.2 mmHG; abdomen: 18.2% with delta RR: 11.2mmHG). In the ER, an initial hemoglobin content of less than 8g/dl was much more frequent in the liver group with 38.1% than in the abdomen group with 16.9% and the control group with 13.9%. Analogous to this, the average amount of transfused erythrocyte concentrate (EC) until admission to the intensive care unit was much higher in the group of patients with liver injury (8.6 units) compared to the abdomen group (4.5 units) and the control group (2.1 units).

Patients who fulfilled the criteria of a massive transfusion (number of transfused EC >10 were filtered out of the liver and abdomen groups.

Given that the average number of ECs and the average ISS in both groups of liver and abdominal trauma were almost the same (liver: 20.9 EC, ISS 39.2; abdomen 19.9 EC, ISS 38.5), the possible measured variable of an unequal EC quantity was leveled out. Thus, the high total mortality in the liver group (55.8%) compared to the abdomen group (36.5%) cannot be explained by the number of ECs. The same applies to the increased MOF (96.0% vs. 60.0%) and sepsis rate (72.0% vs. 36.0%) of the survivors.

4.3 Sepsis, organ failure

Compared to the other groups, increased early mortality in the liver group did not lead to a simultaneous reduction in late mortality. Patients with a liver injury showed - apart from the patients with head injuries – an average late mortality of 7.8%. One cause for the increased late mortality in comparison with patients with no liver injury is possibly the high sepsis rate (19.9%), if the first 24 hours were survived.

The increased sepsis rate in the liver group is also reflected in the frequency of organ failure (OF 48.6%) and multi-organ failure (MOF 33.3%). Compared to patients with abdominal injuries with no severe liver trauma, all three characteristics are significantly more fully developed (sepsis 11.0%, OF 33.2%, MOF 16.6%). Patients from the control group also showed a significantly decreased incidence for sepsis and multi-organ failure.

The frequency of a laparotomy is reduced from 71.6% (before 2001) to 60.4% (from 2001). Remarkably, mortality is reduced in the same period from 35.5 to 33.1%. The ISS is almost identical with 39.7 vs. 38.8.

4.4 Severity adjustment

Adjusting for severity with the RISC Score shows that patients with liver trauma die significantly more frequently than expected. The 33.0% mortality observed (95.0% confidence interval 27.6 – 38.4) offsets a prognostic mortality rate of only 23.4%. In the other two groups of injuries, prognosticated mortality hardly deviates at all from the observed mortality. These results could imply that the resuscitation and/or operative management was suboptimal. However, this is not true. Liver trauma is rather underestimated regarding the expected prognostically impact and shows significantly worse mortality rates than in patients without liver injuries. Therefore, severe liver injury should be judged more critically with respect to mortality than the remaining abdominal injuries, with which the RISC prognosis illustrates actual mortality very well.

4.5 Transplantation after blunt trauma to the liver

Six LT were performed in 4 patients with acute liver injury (2 patients were re-transplanted). The demographics and the clinical presentation of these patients are reported individual. There were 3 men and 1 woman, ranging in age from 36 to 50 years (mean and median, 42 years and 41 years, respectively). All patients had uncontrollable liver injuries caused by motor vehicle accidents. After a median (range) follow-up of 32.95 months (10.3-55.6), 2 out of 4 patients are still alive. Half- and 4-year patient survival rates are 50% and 25% with a corresponding graft survival of 25%, respectively.

5. “Liver transplantation due to abdominal trauma” (Discussion)

The aim of this retrospective investigation was to evaluate possible differences in the characteristics early and late mortality, sepsis and multi-organ failure as a function of the area of organ injury. Consideration of purely isolated organ injuries would not do justice to the complexity of a polytrauma, and may possibly lead to conclusions of no clinical relevance. The selection criteria “great severity of injury” of a specific organ system, with no attention paid to the average frequency and severity of additional injuries, would inaccurately illustrate the information value regarding organ-specific characteristics. It is well-known that liver injuries almost always accompany injuries to other organ systems. To consider only isolated liver injuries would lead to the description of a group that does not occur in this form in reality. The present study illustrates a patient group with a most severely injured organ system and the approach chosen was meant to investigate the impact on an organ system, in view of additional injuries, on the development of early mortality, transfusion requirement, sepsis, organ failure and late mortality.

To date, the effects of an isolated or primary liver injury on immunological function parameters has not to date been examined in either humans or animals. Only a retrospective evaluation weighted according to organ system can contribute to a more precise understanding of their significance for outcome, sepsis and MOF.

The results presented here show a clear increase in the incidence of sepsis from an MOF and early and late mortality with a severe liver injury. This increase seems to be liver-specific and stands out from the other organ systems investigated. Publications by Strong and Turnkey, which reported a mortality of over 11% of in isolated liver injuries, show a significantly lower mortality after liver trauma. However, these were not assessed in a comparably severely injured collective (31, 32). This stresses the significantly higher survival rates in patients with isolated liver injuries in comparison to poly-traumatized patients.

A review of the literature shows that the classification of more specific e.g. immunological consequences to different organ systems subsequent to polytrauma has not yet been examined. This applies both to experimental and clinical investigations and therefore the results presented here seem debatable, since they are only limited, given the low amount of literature in this regard. Despite the small amount of data, it seems beyond question that the participation of the liver in a traumatic event leads to an increase in mortality. However, there are some indirect references that characterize the liver as being a key organ after a trauma. At the beginning of the 1990s once Tinkhoff et al. had pointed out for the first time a connection between cirrhosis and outcome after trauma, this hypothesis was confirmed by numerous authors. In a matched pairs study, Dangleben et al. proved that cirrhosis of the liver is an independent prognosis marker of mortality, and with this they were able to demonstrate a correlation between mortality and the degree of the cirrhosis (definition according to Child-Turcotte-Pugh). These results were also verified by Christmas et al.: in addition to an increase in mortality and length of hospital stay, they showed a significant increase in the sepsis rate after trauma. Altogether 55% of the patients with cirrhosis of the liver in their study population died from sepsis. 33% of the patients with cirrhosis died compared to only 1% in the non-cirrhosis control group. These studies on cirrhosis of the liver and polytrauma show a close association between liver function and outcome after trauma.

In animal experiments, depending upon the quantity of the liver tissue removed, a liver resection leads to a clear restriction of synthesis efficiency, particularly for coagulation products (33). Furthermore, the clearance function for bacterial endotoxins is drastically reduced. The consequences can be expressed in a decompensated coagulation system, through to a Disseminated Intravascular Coagulation (DIC) in a spontaneous multi-organ failure after sepsis or in refractory shock to the extent that the effects of a liver resection resemble those of traumatic liver destruction (34-36).

However, traumatic liver damage is not necessarily associated with a measurable reduction in liver function. This is why, for example, Perdrizet et al. were able to demonstrate a clear increase in early mortality after reperfusion using a pig model, in which a blunt liver trauma was combined with a hemorrhagic shock. The increase in mortality resulted from continuous post-ischemic shock (37).

The significance of the liver in early trauma events was also demonstrated for example by Perl et al. after a thorax trauma in a mouse model. They showed for the first time a response to thorax trauma by Kupffer cells within 30 minutes. In so doing, the liver formed IL-6, TNF-alpha and IL-10 in high concentrations, without the liver itself being traumatized (38).

It has been proven that a tissue trauma leads to a significant reduction in immunological strength. The liver is a central organ of the reticuloendothelial system (RES) and its significance to the defense against infection has been described several times.

The results shown here from the trauma registry indicate that in the group with severe liver trauma, there is a clear increase in the number of ECs in the early and late phases after trauma. This observation after liver trauma is also supported by other research groups. Thus, for example, the number of transfused ECs constitutes an independent prognosis factor in the post-traumatic period after liver trauma. The authors argue that the blood products possibly lead to an increase in the incidence of sepsis due to their antigenicity (39). Both Moore et al. and Malone et al. showed a clear connection between the number of transfused ECs and the occurrence of post-traumatic organ failure; Malone et al. even showed this correlation within the first 24 hours after trauma (40, 41). Critical in this respect, however, it should be fair to pose the question whether and to what extent the administration of erythrocytes causes immunoparalysis, particularly since trauma patients can develop sepsis and MOF without erythrocytes being administered. Hence, it should be discussed whether the correlation between ECs and mortality must possibly be considered as only an epiphenomenon, e.g. an extended tissue ischemia period. So the number of transfused blood products is also always a marker for injury severity, incidence of shock and length of ischemia time. This cannot be obviously separated by a multivariance analysis. In order to examine this question more closely, two subgroups were formed in the present analysis. Here it shows up remarkably that despite a similar ISS and number of transfused ECs, the patients with severe liver participation continue to predominate, with regard to mortality, sepsis and MOF. In this context, immune modulating substances contribute to a considerable reduction in infectious complications. After polytrauma, proteins such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon gamma can contribute to an improvement in post-traumatic immunoparalysis (42, 43). Patients with immune insufficiency, e.g. also due to liver damage, could benefit from the early use of immune modulating substances.

The evaluation of the data from the trauma registry concerning liver trauma (AIS>2) and treatment before and after 2000 shows the paradigm shift starting in 2000 mentioned in the introduction. The reduction in the rate of laparotomies from 2000 to 11.2% in hospitals affiliated with the trauma registry proves a rethink in care after abdominal injury. This resulted in a reduction in mortality of 2.4% in similar patients (ISS: 39.7 vs. 39.8). In order to better support this advantage of conservative treatment, however, more detailed study is necessary given that both preclinical and clinical care have made progress in the same time period. While in former times an exploratory laparotomy was nearly always performed, now conservative therapy under hemodynamically stable conditions is increasingly being recommended (44). Therefore, the portion in an American (multicenter) study was 47%. With 404 patients, a success rate of 98.5% was reported, where hemorrhaging appeared in only 3.5% of other complications (45).

In another series of 495 conservatively treated patients, the success rate was 94% and the average hospital treatment was 13 days, where only 1.9 EC/patient had to be transfused. The complication rate was 6.2%, whereby there was only 2.8% with hemorrhages. Liver-related deaths or overlooked intestinal injuries were not observed.

Both groups predominantly involved not so serious liver traumas, whereas Moore type IV and V injuries (14%) were rather rare. In a study from Germany up to 2004, only 14% of all patients were treated conservatively. Moreover, the not so serious Type I-III injuries were operated in 2/3 of the cases (31/44), where no liver-related mortality was observed. The authors came to the conclusion that in view of the convincing data from the multicenter

studies mentioned and numerous other, at times large patient groups, laparotomy is probably an overtreatment in most patients with Type I-III injuries and seems to be of no real advantage regarding survival, morbidity and duration of treatment (46). Data from this study corroborates this statement.

The matter of the urgent criteria for operating on abdominal and liver trauma is not clearly answered in the literature. The criteria are not uniform and often refer to the term "unstable". It has been shown however by Clarke et al. that mortality increases by 1% every 3 minutes after a trauma involving hematogenic shock, so the time from arrival at the ER to the laparotomy has a crucial effect on the outcome (47).

In addition to acute trauma care following abdominal injury, the therapeutic option of transplant plays a role in chronic hepatic damage rather than in acute injuries. Persistent chronic hepatic damage is mostly seen in the form of "secondary sclerosing cholangitis". The option of transplantation for acute, inoperable hepatic damage also plays an admittedly minor role, but in times of scarce organ availability this should be exercised within reason.

Therefore, treatment of liver trauma has rapidly changed over the past decades. Thus, especially development of the intensive and emergency medicine as well as coagulation substitution reveal a more and more conservative therapy approach against the severity of the injury. To date, merely 10% of the liver trauma patients are surgically treated, 90% follow a conservative therapy regimen. In the process, the overall mortality of 60% could be reduced to about 6% over the past century (48-50).

However, in a few patients with liver injuries it may still occur that they cannot be treated adequately despite exploitation of all conventional surgical measures. Continuous non-controllable acute bleeding, non-reconstructible liver injuries, like e.g. injuries of the liver's veins or the bile duct system, and a liver insufficiency caused by trauma, e.g. shock liver, allow for the consideration of LT (51, 52).

LT then remains the only available life-saving procedure for these patients. However, not all patients are suitable candidates for LT. Pre-transplant neurological status, severe sepsis, MOF, and accompanying severe injuries may all be contraindications to LT. Furthermore, there is a fundamental difference whether a patient is transplanted due to acute non-controllable liver injury or due to subacute (e.g. shock liver) respectively chronic (e.g. secondary biliary cirrhosis) liver mutation after occurred trauma. Ultimately, only a fraction of patients with uncontrollable liver trauma are deemed to be candidates for transplantation. Like those patients who die before LT, mortality after LT is usually secondary to hemodynamically instability, infections and MOF (53, 54).

The underlying severity of the injury and the occasionally life-threatening other injuries are reflected by the results in our patients who received a LT due to trauma all from a motor-vehicle accident. These patients differ fundamentally from the majority of our other liver transplant patients in the peri-operative prognosis. Based on our clinical experience, the most relevant preoperative prognostic factors negatively influencing the post-transplant outcome have been the hemodynamic, secondary injuries and the recipient age. There are diverging opinions about the role of the MELD score as a prognostic factor for the postoperative outcome in such cases.

The results following LT for uncontrollable traumatic liver injuries are substantially worse than those of LT for sub-acute/chronic and elective indications. In fact, the general patient

survival rates are approximately 50-75%. Unfortunately, the few reported cases in the current literature are quite inhomogeneous, reflecting different transplant eras, clinical experience, LT techniques/procedures, and clinical conditions of the patients prior to undergoing LT. In addition these case reports mostly outline the clinical course of liver transplant patients following trauma. While accurate comparison of the clinical presentation of patients across various case reports is not always possible, we can say, based on the available data in 3 case series, that the clinical conditions of our patients appear to be similar to those reported (55-57).

Delis et al. also describe 4 patients with liver trauma in their work who were transplanted in the course of their disease. Non-uniform genesis of these patients are reflected in a range of relatively positive GCS scores. These may be explained by the fact that 3 of the above-mentioned patients had gun-shot liver injuries and hence no, as common in blunt liver injuries, large-area, complex liver injuries. Furthermore, one patient was transplanted after two years due to secondary biliary cirrhosis caused by trauma. This explains the fairly good results in this group with a patient survival rate of 75% after more than 9 years.

Altogether 3 patients with liver injuries due to car accidents, that were hepatectomized pre-operatively due to massive unsalvageable liver trauma, are described by Ringe et al. This quite more homogenous patient population is better comparable to our study and demonstrated a patient survival rate geared to our results. Thereby, Ringe postulates a bilateral approach in patients where no sufficient hemostasis after liver trauma is achievable. After an indication for total hepatectomy depending on hemodynamic parameters, a than obligatory liver transplantation is carried out as soon as possible. In his works, however, also patients are described that could not be allocated with an adequate organ in time due to the present lack of donor organs.

Also comparable with our results are those published in the 1980ies by Esquivel et al. on 2 traffic accident victims with nonreconstructable injuries to the portal vein and following nonfunctional hepatic remnants. In literature, these are the first published cases of liver transplantations after liver trauma.

The majority of our patients demonstrated one or more of negative prognostic factors. This study covers all recorded liver transplantations for otherwise uncontrollable liver trauma due to motor-vehicle accidents at our hospital. These cases often had poor general prognoses. Despite the acute condition of our patients, our results, patient survival rate is 50% with a corresponding graft survival of 25%, are among the first reports on survival rates in a homogenous series to date in the literature.

6. Conclusion

6.1 Investigate the significance of liver trauma and prognostic factors in severely injured patients

In our opinion, unstable patients should be identified by the following parameters: 1) location of the source of bleeding, i.e., free fluid in the abdomen in the initial ultrasound, if need be with an increase in the course of action; 2) volume loss, i.e., substitution is required for hemodynamic stability when systolic blood pressure falls below 80 - 90 mmHG; 3) signs of systemic hypoperfusion with negative base excess and pH and where applicable with an initial hemoglobin under 8 mg/dl with signs of consumptive coagulopathy.

Knowledge of the additional dangers documented here as they can arise from a liver injury and may possibly be positively affected by e.g. a specific coagulation treatment and an early substitution of ECs. The immunological changes to be expected from a liver injury in the meantime may possibly even reinforce the frequently described post-trauma immunosuppression.

6.2 Investigate the indication of liver transplantation for uncontrollable liver trauma

In conclusion, we largely agree with the aforementioned reports. The therapeutic option of liver transplantation also needs to be accessible for patients with liver injuries caused by trauma. However, not least due to the mentioned poor transplantation results in severely injured patients, indication for transplantation needs to be critically proposed by the attending surgeons.

6.3 Try to answer the question – Is transplantation a valuable option or just a "waste of organs" in polytraumatized patients with liver injury –

It is essential to sensibly and appropriately allocate the organs so that the shortage of donor organs is not further enlarged. In patients where no hemodynamic stabilization can be achieved despite exhaustion of all extensive care measures, transplantation should not be considered any further. Although, there is a fundamental difference regarding the timeframe after trauma during which patients are to be transplanted. It has shown, that especially patients with acute, non-controllable liver injuries as described by us have clinically changed for the worse rapidly after transplantation and have died in MOF. Therefore, we postulate that indication for transplantation in these patients may only be provided after critically reviewing every single case as not to "waste of organs".

6.4 Identifying new approaches to improving organ donation

It should be noted that the success of transplantation medicine with a simultaneously increasing shortage of donor organs will only be assured if all available resources are exploited. Increasing acceptance of "expanded criteria donor" organs appears more justified than ever under these circumstances, but also with sustained good results despite constantly deteriorating organ quality. Approaches to increase the transplant quality, not only of extended criteria donor (ECD) organs, offer further developed possibilities that support perfusion such as machine perfusion and optimized perfusion solutions. Moreover, shortened ischemic periods are achieved through further improved logistics and allocation processes, which together with individualized, medicinal immune suppression ultimately benefit the transplant and the organ recipient. In addition to this continuously improving and thus optimized use of postmortal organs, it must also be the common objective of the medical profession and politics to increase the overall number of donor organs and to improve their quality.

In order to achieve this, priority should be given to an improved exploitation of the existing organ donation potential through hospital-based advising, for example by a contact person for organ donation at each intensive care unit. Information and advising by Eurotransplant and the transplant centers for physicians, nursing staff and the population are of key importance here. Furthermore, to an ever great extent it will be the task of all parties

involved in the field of transplantation to present organ donation, the allocation and transplantation of organs, and all the decisive aspects relating to the readiness of organ donation such as trust, safety and equity in a transparent manner. Conducting advisory discussions on the topic of organ donation with the relatives of the deceased is a special task for physicians. One measure should include involving a physician with special communicative expertise. It remains to be hoped that in this way a higher acceptance rate for organ donation will be more successfully achieved throughout all population groups in the future. In tapping into all resources and approaches for the optimized exploitation of donated postmortal organs, it should be possible to assure the medical care mandate of transplantation medicine in Germany in the future as well.

Based on previous studies, the recording of organ donors with "expanded criteria" in a targeted analysis is useful and necessary for new ways of improving organ donation. Further local, national and international analyses are additionally necessary to identify the limits to expanding donor acceptance criteria.

7. References

[1] Abouna GM. Organ shortage crisis: problems and possible solutions. Transplant Proc 2008;40:34-38.

[2] Pomfret EA, Sung RS, Allan J, Kinkhabwala M, Melancon JK, Roberts JP. Solving the organ shortage crisis: the 7th annual American Society of Transplant Surgeons' State-of-the-Art Winter Symposium. Am J Transplant 2008;8:745-752.

[3] Deutsche Stiftung Organtransplantation (DSO). Organspende und Transplantation in Deutschland. Jahresbericht 2009.

[4] Heuer M, Zeiger A, Kaiser GM, et al. Use Of Marginal Organs In Kidney Transplantations For Marginal Recipients: Too Close To The Margin Of Safety? Eur J Med Res 2009;15:31-34.

[5] Schold JD, Meier-Kriesche HU. Which renal transplant candidates should accept marginal kidneys in exchange for a shorter waiting time on dialysis? Clin J Am Soc Nephrol 2006;1:532-538.

[6] Merion RM, Ashby VB, Wolfe RA, et al. Deceased-donor characteristics and the survival benefit of kidney transplantation. JAMA 2005;294:2726-2733.

[7] Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009;360:7-19.

[8] Ojo AO, Hanson JA, Meier-Kriesche H, et al. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplants candidates. J Am Soc Nephrol 2001;12:589-597.

[9] Ojo AO. Renal disease in recipients of non-renal solid organ transplantation. Semin Nephrol 2007;27498-507.

[10] Ponticelli C. Renal transplantation 2004: where do we stand today? Nephrol Dial Transplant 2004;19:2937-2947.

[11] Weber F, Napieralski BP. Public information about organ donation: what results can be achieved? Transpl Proc 1999;31:3403-3405.

[12] Wesslau C. Transplantationsmedizin: Organspender-Potenzial ist nicht ausgeschöpft. Dtsch Arztebl 2006;103:A-517/B-447/C-426.

[13] Tinkoff G, Rhodes M, Diamond D, et al. Cirrhosis in the trauma victim. Effect on mortality rates. Ann Surg 1990;211:172-177.

[14] Dangleben DA, Jazaeri O, Wasser T, et al. Impact of cirrhosis on outcomes in trauma. J Am Coll Surg 2006;203:908-913.
[15] Christmas AB, Wilson AK, Franklin GA, et al. Cirrhosis and trauma: a deadly duo. Am Surg 2005;71:996-1000.
[16] Blair SL, Karpoff HM, Hodyl C, et al. Pretreatment with IFN-gamma decreases infectious complications after partial hepatectomy in the rat. J Interferon Cytokine Res 1998;18:175-180.
[17] Pachter HL, Feliciano DV. Complex hepatic injuries. Surg Clin North 1996;76:763-782.
[18] Verney M, Becker H, Röher H. Prognose und Therapie der Leberverletzungen beim polytraumatisierten Patienten. Chirurg 1990;61:711-716.
[19] Malhotra AK, Fabian TC, Croce MA, et al. Blunt hepatic injury: a paradigm shift from operative to nonoperative management in the 1990s. Ann Surg 2000;231:804-813.
[20] Pachter HL, Feliciano DV. The current status of nonoperative management of adult blunt hepatic injuries. Am J Surg 1995;169:442-454.
[21] Matthes G, Stengel D, Seifert J, et al. Blunt liver injuries in polytrauma: results from a cohort study with the regular use of whole-body helical computed tomography. World J Surg 2003;27:1124-1130.
[22] Velmahos GC, Toutouzas KG, Radin R, et al. High success with nonoperative management of blunt hepatic trauma: the liver is a sturdy organ. Arch Surg 2003;138:475-481.
[23] Delis SG, Bakoyiannis A, Selvaggi G, Weppler D, Levi D, Tzakis AG. Liver transplantation for severe hepatic trauma: Experience from a single center. World J Gastroenterol 2009;15:1641-1644.
[24] O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 1989;97:439-445.
[25] Schnüriger B, Inderbitzin D, Schafer M, Kickuth R, Exadaktylos A, Candinas D. Concomitant injuries are an important determinant of outcome of high-grade blunt hepatic trauma. Br J Surg 2009;96:104-110.
[26] Schnitzbauer AA, Tsui TY, Kirchner G, et al. Liver transplantation for sclerosing cholangitis in a polytraumtized patient. Nat Clin Pract Gastroenterol Hepatol 2009;6:121-126.
[27] de Santibanes E, Ardiles V, Gadano A, Palavecino M, Pekolj J, Ciardullo M. Liver transplantation: the last measure in the treatment of bile duct injuries. Liver Transpl 2008;14:1534-1535.
[28] Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707-710.
[29] Ruchholtz S. The trauma registry of the German society of trauma surgery as a basis for interclinical quality management. A multicenter study of the German society of trauma surgery. Unfallchirurg 2000;103:30-37.
[30] Bone RC, Balk RA, Cerra FB. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-1655.
[31] Strong RW, Lynch SV, Wall DR, Liu CL. Anatomic resection for severe liver trauma. Surgery 1998;123:251-257.

[32] Trunkey DD. Hepatic trauma: contemporary management. Surg Clin North Am 2004;84:437-450.
[33] Meijer C, Wiezer MJ, Hack CE, et al. Coagulopathy following major liver resection: the effect of rBPI21 and the role of decreased synthesis of regulating proteins by the liver. Shock 2001;15:261-271.
[34] Schroder J, Gallati H, Kremer B. Increased serum levels of soluble tumor necrosis factor a-receptors in patients undergoing partial liver resection. Hepatogastroenterology 1998;45:1807-1812.
[35] Vo NM, Chi DS. Effect of hepatectomy on the reticuloendothelial system of septic rats. J Trauma 1988;28:852-854.
[36] Fukushima H, Ikeuchi J, Tohkin M, et al. Lethal shock in partially hepatectomized rats administered tumor necrosis serum. Circ Shock 1998;26:1-14.
[37] Perdrizet GA, Giles DL, Dring R, et al. Major hepatic trauma: warm ischemic tolerance of the liver after hemorrhagic shock. J Surg Res 2006;136:70-77.
[38] Perl M, Gebhard F, Braumuller S, et al. The pulmonary and hepatic immune microenvironment and its contribution to the early systemic inflammation following blunt chest trauma. Crit Care Med 2006;34:1152-1159.
[39] Robinson WP 3rd, Ahn J, Stiffler A, et al. Blood transfusion is an independent predictor of increased mortality in nonoperatively managed blunt hepatic and splenic injuries. J Trauma 2005;58:437-444.
[40] Moore FA, Moore EE, Sauaia A. Blood transfusion. An independent risk factor for postinjury multiple organ failure. Arch Surg 1997;132:620-624.
[41] Malone D, Napolitano LM, Genuit T, et al. Total cytokine immunoassay: a more accurate method of cytokine measurement? J Trauma 2001;50:821-825.
[42] Lendemans S, Kreuzfelder E, Waydhas C, et al. Differential immunostimulating effect of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interferon gamma (IFNgamma) after severe trauma. Inflamm Res 2007;56:38-44.
[43] Flohé S, Lendemans S, Selbach C, et al. Effect of granulocyte-macrophage colony-stimulating factor on the immune response of circulating monocytes after severe trauma. Crit Care Med 2003;31:2462-2469.
[44] Croce MA, Fabian TC, Menke PG, et al. Nonoperative management of blunt hepatic trauma is the treatment of choice for hemodynamically stable patients. Results of a prospective trial. Ann Surg 1995;221:744–753.
[45] Pachter HL, Knudson MM, Esrig B, et al. Status of nonoperative management of blunt hepatic injuries in 1995: a multicenter experience with 404 patients. J Trauma 1996;40:31–38.
[46] Ott R, Schön MR, Seidel S, et al. Surgical management, prognostic factors, and outcome in hepatic trauma. Unfallchirurg 2005;108:127-134.
[47] Clarke JR, Trooskin SZ, Doshi PJ, et al. Surgical management, prognostic factors, and outcome in hepatic trauma. J Trauma 2002;52:420-425.
[48] Veroux M, Cillo U, Brolese A, et al. Blunt liver injury: from non-operative management to liver transplantation. Injury 2003;34:181-186.
[49] Schroeppel TJ, Croce MA. Diagnosis and management of blunt abdominal solid organ injury. Curr Opin Crit Care 2007;13:399-404.
[50] Richardson JD. Changes in the management of injuries to the liver and spleen. J Am Coll Surg 2005;200:648-669.

[51] Chiumello D, Gatti S, Caspani L, Savioli M, Fassati R, Gattinoni L. A blunt complex abdominal trauma: total hepatectomy and liver transplantation. Intensive Care Med 2002;28:89-91.
[52] Angstadt J, Jarrell B, Moritz M, et al. Surgical management of severe liver trauma: a role for liver transplantation. J Trauma 1989;29:606-608.
[53] Polanco P, Leon S, Pineda J, et al. Hepatic resection in the management of complex injury to the liver. J Trauma 2008;65:1269-1270.
[54] Tucker ON, Marriott P, Rela M, Heaton N. Emergency liver transplantation following severe liver trauma. Liver Transpl 2008;14:1204-1210.
[55] Catalano G, De Simone P, Montin U, et al. Severe liver trauma: the transplant surgeon`s perspective. A case report. Hepatogastroenterology 2008;55:1458-1459.
[56] Ringe B, Pichelmayr R. Total hepatectomy and liver transplantation: a life-saving procedure in patients with severe hepatic trauma. Br J Surg 1995;82:837-839.
[57] Esquivel CO, Bernardos A, Makowka L, Iwatsuki S, Gordon RD, Starzl TE. Liver replacement after massive hepatic trauma. J Trauma 1987;27:800-802.

Part 8

Anesthesia and Periopertive Period

15

Intensive Care Management of Patients Prior to Liver Transplantation

Kathy M. Nilles[1] and Ram M. Subramanian[2]
[1]*Northwestern University Feinberg School of Medicine, Chicago IL,*
[2]*Emory University School of Medicine, Atlanta, GA,*
USA

1. Introduction

Liver failure is a devastating illness with extremely high morbidity and mortality. With the advent of life-saving orthotopic liver transplantation, the mortality and morbidity due to liver failure has been significantly reduced. Many patients awaiting transplant are critically ill. The intensive care management of patients before liver transplantation is aimed at optimizing hepatic and extrahepatic organ function before the transplant operation, with a goal to favorably influence the perioperative and postoperative graft and patient survival.

Critical illness due to liver disease may present in the context of acute liver failure (ALF) or acute on chronic liver failure (ACLF). The differing pathophysiologic processes underlying these two categories of liver failure necessitate specific approaches to their intensive care management. In their extreme presentations, both types of liver failure result in multi-organ system failure; therefore, the intensive care management of these conditions requires a systematic multi-organ system approach to address hepatic and extrahepatic organ dysfunction (*Ford et al., 2010*). This chapter will provide a multi-organ system–based description of critical care management of ALF and ACLF before liver transplantation.

2. Acute liver failure

2.1 Definition and etiology

Acute liver failure (ALF) represents a clinical syndrome of varying etiologies that ultimately manifests as hepatic encephalopathy and coagulopathy (International Normalized Ratio [INR] greater than 1.5) in the setting of acute liver dysfunction. By definition, coagulopathy and hepatic encephalopathy occur within 6 months following the initial symptoms of hepatic dysfunction in a patient without chronic liver disease. The timing of development of hepatic encephalopathy after an initial presentation of jaundice helps further subdivide ALF into categories of hyper-acute (hepatic encephalopathy developing within 7 days of onset of jaundice), acute (8-28 days), and sub-acute (29-84 days).

The etiologies of ALF are numerous, and reversible causes must be actively sought. The most common etiology of ALF in the United States and United Kingdom is drug-induced, with acetaminophen toxicity the leading responsible agent. Other common classes of

medications known to provoke ALF in susceptible patients include antimicrobials, antidepressants, antiepileptics, anti-hypertensives, HIV therapy, chemotherapeutic agents, lipid-lowering agents, and glucose-lowering agents. Analgesics and recreational drugs are also known causes of drug-induced ALF.

Additional etiologies of ALF include viral infections, including Hepatitis A, B, C, D, and E, as well as herpes simplex virus, varicella zoster virus, cytomegalovirus, and Epstein-Barr virus. Toxins responsible for ALF include *amanita phyloides* (mushrooms), herbal preparations, organic solvents, and bacterial toxins, such as *bacillus cereus*. Pregnancy-related conditions include the HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) and the acute fatty liver of pregnancy syndrome. Lymphoma, metastatic disease, hepatic ischemia, wilson disease, heat stroke, Budd-Chiari syndrome, autoimmune hepatitis, and extensive hepatic resection are other causes of ALF. Approximately 15-20% of patients with ALF have an undetermined cause.

2.2 Diagnosis and initial management considerations in ALF

Potential causes of ALF must be actively sought in the initial workup, as certain etiologies have specific life-saving treatments. Acetaminophen toxicity is treated with N-acetylcysteine, autoimmune hepatitis with corticosteroids, herpes simplex and varicella zoster viruses with intravenous acyclovir, acute fatty liver of pregnancy and HELLP syndrome by delivery of the fetus. Recent data suggests that the use of N-acetylcysteine (NAC) improves the outcome of patients with ALF, independent of the etiology (*Lee et al., 2009*). Many transplant centers advocate the use of NAC for all patients with ALF. NAC can be administered intravenously at a dose of 150 mg/kg over 15 minutes followed by 50 mg/kg over 4 hours, followed by 100 mg/kg over 16 hours. Adverse effects of NAC include bronchospasm and anaphylaxis and are managed by coadministration of antihistamines and corticosteroids as well as reduction of infusion rate. Oral preparations of NAC are also available. Discontinuation of NAC is appropriate following resolution of ALF or at the time of transplantation.

In addition to ruling out reversible causes of ALF, exclusion of chronic liver disease is crucial for appropriate management. Physical examination of patients presenting with liver failure should therefore focus on stigmata of chronic liver disease, including abdominal ascites, spider angiomata, or *caput medusae*. Hepatic imaging with ultrasound, CT or MRI is useful to evaluate for the presence of portal hypertension and chronic liver disease, as well as to evaluate hepatic size and vasculature, ascites, and hepatic masses. Recommended laboratory testing for potential etiologies of ALF include autoimmune markers, viral serologies, toxicology screen, and serum and urine testing for copper overload.

2.3 Patient stabilization

All patients with ALF should be monitored and treated in an intensive care unit. Many patients have progressed to multi-organ failure upon arrival, and immediate supportive measures should be undertaken. These may include interventions such as endotracheal intubation and mechanical ventilation, intravenous fluid resuscitation, placement of arterial and central venous lines, and vasoactive agent support.

2.4 Organ-specific management

2.4.1 Hepatic encephalopathy

Hepatic encephalopathy is defined by the presence of neuropsychiatric symptoms in the absence of other causes of altered mental status. Mental status changes range from subtle cognitive impairments to frank coma. The impaired hepatic clearance of ammonia and other toxins are poorly tolerated in patients with ALF, and result in astrocyte swelling and cytotoxic cerebral edema. Hepatic encephalopathy is divided into four grades based on the West Haven Criteria (*Atterbury et al., 1978*); grade I denotes mild cognitive changes and attention deficits, grade II lethargy and apathy, grade III confusion and semi-stupor, and grade IV a comatose state.

Hepatic encephalopathy in ALF can result in cerebral edema, herniation, and death. Any patient with ALF and altered mental status should undergo an emergent head CT to rule out cerebrovascular accident, intracranial hemorrhage, or mass effect prior to treatment of hepatic encephalopathy. Those with grade III or IV encephalopathy should undergo elective endotracheal intubation and mechanical ventilation for airway protection, with maintenance of adequate sedation and patient-ventilator synchrony to reduce sudden increases in intracranial pressure. Neurosurgical placement of an intraparenchymal pressure monitor should then be strongly considered in order to continuously monitor and treat elevated intracranial pressure.

Intracranial hypertension is managed primarily with pharmacologic osmotherapy, with the goal to reduce intracranial pressure to less than 25 mmHg. Mannitol and hypertonic saline can be used for this purpose; if these measures fail, neuromuscular paralysis and therapeutic hypothermia with a target core body temperature of 32-33 degrees Celsius can be co-administered. In the setting of intracranial hypertension refractory to the above interventions, salvage therapy with barbiturate coma can be considered. Elevation of the head of the bed to 30 degrees is recommended for all patients. Furthermore, cerebral perfusion pressure, defined as the difference between mean arterial pressure and intracranial pressure, should be maintained at 60 mmHg or greater. In the setting of intracranial hypertension, this may require the use of vasoactive agents to increase the mean arterial pressure. Hyperventilation, formerly used to reduce intracranial pressure, may induce cerebral hypoxemia, and is no longer recommended for this purpose. Patients with grade I or II encephalopathy do not require intracranial pressure monitoring or endotracheal intubation for airway protection. However, serial neurologic exams are critical in these patients, as they can rapidly deteriorate to stage III or IV hepatic encephalopathy.

2.4.2 Coagulopathy

In addition to hepatic encephalopathy, *coagulopathy*, represented by an increasing INR, is a defining characteristic of progressing ALF. The synthetic function of the failing liver is diminished, and levels of clotting factors are reduced, resulting in elevations in the INR. In fact, the INR is considered the most sensitive indicator of hepatic function and is commonly used as a prognostic tool to aid prediction of spontaneous recovery or need for liver transplantation. Therefore, correction of the INR in the absence of bleeding is discouraged.

For invasive procedures, the INR can be temporarily corrected with recombinant Factor VIIa (Novo-7) at a dose of 40 μg/kg. Such therapy reduces the INR to below 1.5 within 30 minutes of administration, and allows approximately 90-120 minutes for the performance of invasive procedures. Vitamin K may reduce the INR if malnutrition is contributing to coagulopathy.

Despite the coagulopathic state observed in ALF, anticoagulant proteins such as protein C and S are reduced, and patients are at risk of venous thrombotic complications. Therefore, venous thromboembolism prophylaxis with subcutaneous heparin or low-molecular weight heparin formulations should be considered despite the presence of coagulopathy.

2.4.3 Renal impairment

Acute kidney injury in ALF usually results from impaired renal perfusion or direct renal insults. Hepatorenal syndrome, which occurs in the setting of portal hypertension and chronic liver disease, does not occur in ALF. Management of acute kidney injury includes avoidance of additional nephrotoxic insults, as well as supportive measures. In order to avoid increases in intracranial pressure and significant fluid shifts, if renal replacement therapy is necessary, continuous renal replacement therapy (CRRT) is preferred over conventional hemodialysis.

2.4.4 Infections

Infections occur in ALF from functional immunosuppression. Patients are susceptible to overwhelming bacterial and fungal sepsis, although clinical signs of infection may be absent. Empiric antibacterial and antifungal therapy should be considered in the setting of advanced hepatic encephalopathy, shock, or for patients listed for transplantation. Associated sepsis and septic shock are managed with broad-spectrum antibiotics, vasoactive agent support and high-dose corticosteroids.

2.4.5 Pulmonary complications

Respiratory disturbances, including acute respiratory distress syndrome (ARDS) and acute lung injury (ALI), are frequent manifestations of ALF. In the setting of ALI and ARDS, lung-protective strategies with low tidal volume (6 cc/kg ideal body weight) ventilator settings and mild permissive hypercapnea are recommended. Hypoxemic respiratory failure portends a poor prognosis and is treated supportively. Severe hypercapnea, bronchoscopy, and patient-ventilator asynchrony can exacerbate intracranial hypertension; titration of the set respiratory rate to compensate for hypercapnea, as well as adequate sedation and analgesia to improve synchrony, are imperative. Neuromuscular-blocking paralytic agents may be necessary if patient-ventilator asynchrony persists despite adequate sedation.

2.4.6 Metabolic derangements

Metabolic derangements result both from impaired hepatic metabolic function and resulting multi-organ failure. Consequences include lactic acidosis and disturbances in arterial pH,

glucose, and electrolytes. Hypoglycemia results from impaired gluconeogenesis and glycogenolysis, and is managed via dextrose infusion and frequent glucose monitoring. Electrolyte disturbances include hyponatremia, hypokalemia, hypomagnesemia, and hypophosphatemia, and should be corrected when recognized. However, the appearance of hypophosphatemia may indicate a favorable prognosis due to intracellular phosphorus consumption and hepatic regeneration. Finally, the high metabolic demands in ALF create a generalized catabolic state with resultant high nutritional needs. Enteric nutrition is recommended over parental routes to reduce gastrointestinal bacterial translocation and bleeding from stress ulceration.

3. Acute on chronic liver failure

3.1 Definition and etiology

Acute on chronic liver failure (ACLF), or decompensated cirrhosis, occurs when cirrhosis of any etiology is complicated by the development and sequelae of portal hypertension. Longstanding portal hypertension results from intrahepatic resistance to portal flow, and increased portal inflow from inappropriate splanchnic vasodilation. These pathophysiologic changes result in splanchnic and systemic derangements, including the development of gastroesophageal varices, hepatic encephalopathy, pulmonary decompensation, and hepatorenal syndrome. Given the importance of splanchnic vasodilation in the pathophysiology of ACLF, pharmacologic therapy with splanchnic vasoconstrictors plays a central role in the therapy of ACLF.

Etiologies of cirrhosis are numerous, and include alcoholic steatosis, chronic viral hepatitis, metabolic diseases (e.g. non-alcoholic fatty liver disease, hemachromatosis, wilson disease), autoimmune hepatitis and cholestatic liver diseases (e.g. primary biliary cirrhosis and primary sclerosing cholangitis).

3.2 Diagnosis

The diagnostic workup of a patient with suspected cirrhosis is similar to that of a patient presenting with ALF. Physical examination may reveal stigmata of chronic liver disease, including ascites and splenomegaly. Abdominal imaging with CT or MRI provides radiographic confirmation of chronic portal hypertension. Non-hepatic causes of portal hypertension, such as cardiac cirrhosis from longstanding heart failure, should also be excluded.

3.3 Organ-specific management

3.3.1 Hepatic encephalopathy

Hepatic encephalopathy in decompensated cirrhosis shares several qualities with the encephalopathy observed in ALF, including the grades of severity. However, major differences exist in clinical presentation and management of hepatic encephalopathy in ACLF. The chronicity of portal hypertension allows time for the development of ammonia fixation mechanisms and neuronal adaptation to ammonia. Thus, the hepatic encephalopathy of ACLF is not typically associated with cerebral edema. Treatment is

supportive and focuses on patient safety and avoidance of complications. As in ALF, patients with grade III or IV encephalopathy warrant elective intubation for airway protection. Causative factors of hepatic encephalopathy include dehydration, over-diuresis, infection, use of benzodiazepines and narcotics, gastrointestinal bleeding, constipation, electrolyte or acid-base imbalances, or recent transjugular intrahepatic portosystemic shunt (TIPS) procedure. Progression of underlying liver disease may be the only identifiable precipitant; when reversible causes are identified, they should be treated.

Medical treatment of hepatic encephalopathy consists of oral agents to assist in toxin elimination. Lactulose and other nonabsorbable disaccharides improve intestinal excretion of nitrogen and reduce production of ammonia by enteric bacteria. Intestinal decontamination with oral antibiotics such as rifaximin or metronidazole reduces the burden of ammonia-producing bacteria.

3.3.2 Gastroesophageal varices

Hemorrhage due to gastroesophageal variceal bleeding can be imminently fatal, and requires emergent therapy. Airway protection with endotracheal intubation reduces the risk of aspiration during massive hematemesis. Intravenous volume resuscitation or blood products should be administered carefully, as excess volume can increase portal pressures and exacerbate bleeding. An appropriate post-transfusion hemoglobin goal is 8 g/dL.

Infusion of the somatostatin analogue octreotide reduces portal pressure and can induce splanchnic vasoconstriction and facilitate hemostasis during variceal bleeding. Terlipressin, a vasopressin analogue, has been used with success in Europe to help control variceal bleeding. All patients should receive prophylactic antibiotics with either ceftriaxone or a fluoroquinolone, which have been shown to reduce infections, reduce the risk of rebleeding, and improve survival (*Bernard et al., 1999*).

Following initiation of pharmacologic therapy, definitive therapy for gastroesophageal varices requires upper endoscopy with endoscopic band ligation or sclerotherapy. If these measures fail, or if gastric varices are detected, urgent TIPS can be used to achieve hemostasis and prevent the development of new varices. For severe uncontrolled bleeding, esophageal balloon tamponade may be necessary for temporary stabilization until definitive TIPS therapy is undertaken.

3.3.3 Cardiovascular impairments

Cardiovascular system derangements frequently complicate the hemodynamic picture of decompensated cirrhosis. Circulatory changes resemble those of septic shock, including increased cardiac output, reduced systemic vascular resistance, wide pulse pressure, and decreased mean arterial pressure. Patients are susceptible to sepsis-induced hypotension and septic shock. Norepinephrine is the preferred vasoactive agent in such patients, as it preserves cardiac output while increasing vascular resistance. Hypotension can also occur by decreased venous return if severe ascites produces compression of the inferior vena cava.

Finally, the phenomenon of cirrhotic cardiomyopathy with impaired systolic and diastolic dysfunction, and reduced response to inotropic therapy has been described (*Zardi et al., 2010*). Several mechanisms for cirrhotic cardiomyopathy have been proposed, including myocardial apoptosis, involvement of circulating carbon monoxide and nitric oxide, and cardiomyocyte receptor impairments. If overt heart failure develops, consultation with a cardiologist is advisable.

3.3.4 Pulmonary complications

The pulmonary system derangements in decompensated cirrhosis are characterized by distinct disorders of varying severity. In the *hepatopulmonary syndrome* (HPS), excess vasodilation of the pulmonary vasculature system limits oxygen diffusion across the alveolar-capillary membrane. Vasodilation may occur through nitric oxide and other circulating vasodilators, or through arteriovenous malformations resulting in intrapulmonary shunts; the difference in these two mechanisms distinguish type I and type II HPS, respectively. Presenting symptoms include dyspnea, platypnea, orthodeoxia, cyanosis, and hypoxemia. Arterial blood gas and transthoracic double bubble echocardiogram or lung perfusion scans can help establish the diagnosis. Supplemental oxygen improves hypoxemia in type I HPS, whereas embolization of arteriovenous malformations can be performed in type II syndrome. In both forms, liver transplantation is the definitive treatment, and the presence of either form facilitates priority listing for transplantation.

Portopulmonary hypertension (PPH) is a form of pulmonary arterial hypertension occurring in the presence of portal hypertension, and portends a poor prognosis. The mechanism of the adverse effects of portal hypertension on the pulmonary vasculature remains unclear. Proposed explanations include endothelial remodeling in response to a hyperdynamic circulation, as well as inflammatory cascades related to cytokines. Patients present with exertional dyspnea, fatigue, chest pain, and signs of volume overload. Diagnosis is confirmed by right heart catheterization, which demonstrates a pulmonary artery pressure (PAP) of 25 mmHg or greater with a normal pulmonary capillary wedge pressure. The degree of PAP elevation correlates with mortality during liver transplant, and patients with moderate or severely elevated pressures generally are not candidates for transplantation. Continuous infusion of vasodilatory prostaglandins such as epoprostenol may improve hemodynamics and reduce PAP to allow patients improved likelihood of tolerating transplantation.

Hepatic hydrothorax refers to pleural effusions that occur when diaphragmatic defects allow the transudation of ascitic fluid into the pleural space. Dyspnea, cough, chest discomfort, and respiratory collapse can occur. Diagnostic and therapeutic thoracentesis should be performed to exclude infection, malignancy, and cardiopulmonary etiologies of pleural effusions. Diuretics such as furosemide and spironolactone can be administered, but patients with respiratory compromise should undergo therapeutic thoracentesis. Chest tubes are contraindicated in hepatic hydrothorax, as re-expansion pulmonary edema and hypovolemic shock can occur and are poorly tolerated in the cirrhotic patient. If hepatic hydrothorax is refractory to diuresis and thoracentesis, TIPS is indicated to help prevent the formation of ascites and subsequent transudation.

3.3.5 Renal impairments

Hepatorenal syndrome (HRS) is defined as an increase in creatinine to greater than 1.5 g/dL or a decrease in creatinine clearance to below 40 mL/min. HRS is a dreaded complication of cirrhosis, and can develop unexpectedly at any point in the course of illness. Splanchnic vasodilation in the setting of cirrhosis results in reduced effective blood volume and pre-renal acute kidney injury. HRS is a diagnosis of exclusion. Two forms exist; type I progresses rapidly, whereas type II progresses over a longer time period; both are fatal without transplant. When diagnosing HRS, other causes of acute kidney injury in cirrhosis must be excluded, including dehydration and over-diuresis, medication effects, and intrinsic renal insults. Diagnostic criteria for HRS include: cirrhosis with ascites, creatinine level of at least 1.5 mg/dL, lack of response to diuretic withdrawal and volume expansion, absence of shock, and absence of nephrotoxic and parenchymal renal etiologies.

A definitive curative treatment for HRS is limited to liver transplantation, but supportive measures aimed at correcting the pathophysiology are in trial. Terlipressin has been studied for HRS in addition to its use in variceal hemorrhage, and has demonstrated the ability to reverse Type 1 HRS. Octreotide and the alpha agonist midodrine can induce splanchnic vasoconstriction, thereby potentially reversing the pathophysiology of HRS. Avoidance of additional renal insults, including diuretics and other nephrotoxic agents, is essential. The TIPS procedure can be used to improve overall circulatory function through portal venous decompression. Finally, renal replacement therapy is often required while patients await transplant.

3.3.6 Infectious complications

Further complications of decompensated cirrhosis include a predisposition to infections, owing to a chronic low-grade inflammatory state produced by excess cytokines and reduced clearance of toxins. Impaired function of macrophages and antigen presenting cells and decreased levels of complement are also implicated. In the setting of septic shock, early goal-directed therapy is warranted, but over-resuscitation of volume can increase portal pressures and lead to exacerbation of portal hypertension. As in acute liver failure, patients with decompensated cirrhosis may benefit from use of glucocorticoids to supplement vasoactive agents (*Fernandez et al., 2006*). Bacterial pathogens are most typical, but fungal infections occur frequently in cirrhosis and should be considered in the differential diagnosis.

3.3.7 Ascites

Abdominal ascites is another notable manifestation of decompensated cirrhosis. Ascites can cause significant morbidity, including abdominal pain and discomfort, dyspnea and orthopnea, hepatic hydrothorax, spontaneous bacterial peritonitis, and abdominal compartment syndrome. Abdominal compartment syndrome is characterized by restrictive lung mechanics, renal and mesenteric vascular compromise, and hypotension due to compression of the inferior vena cava.

Ascites develops in response to renal hypoperfusion, which results in upregulation of the renin-angiotensin-aldosterone system to increase sodium and water retention. Elevated

portal pressures produce a capillary hydrostatic pressure gradient, forcing fluid into the abdominal interstitium. Sodium restriction and use of diuretics can be used to manage ascites. Refractory ascites can be managed with serial large-volume paracentesis or placement of a TIPS shunt.

3.3.8 Spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is a frequent complication of ascites, and can precipitate hepatorenal syndrome. Common pathogens of SBP include *E coli*, *K pneumonia*, and *S pneumococcus*, although culture-negative SBP occurs as well. Recommended antibiotics include third-generation cephalosporins or fluoroquinolones; daily maintenance antibiotics are used for secondary prophylaxis. If SBP is suspected, treatment with antimicrobials while awaiting culture results is appropriate.

4. Indications for liver transplantation

Many patients with worsening ALF or ACLF are eligible for orthotopic liver transplant. Indications for transplant are numerous and include acquired or congenital etiologies, viral hepatitis, drug-induced ALF, cirrhosis, cholestatic diseases, metabolic disorders, vascular derangements, and hepatocellular carcinoma. Because the supply of donor grafts is exceeded by the demand for transplantation, organ allocation is critical. The process of organ allocation is defined by country-specific donor and recipient allocation schemes.

Several models for prognostic data in ALF have been proposed. Consensus exists in the belief that the degree and clinical trend of coagulopathy and hepatic encephalopathy remain the most important prognostic indicators, and are helpful in determining patient appropriateness for transplantation listing. ALF may resolve with supportive treatment, but frequently progresses to death in the absence of transplantation. Patients with ALF therefore have highest priority for liver transplantation.

Patients with decompensated cirrhosis are classified by the Model For End Stage Liver Disease (MELD) score (*Murray and Carithers, 2005*), which has largely replaced the Childs Pugh system, with higher MELD scores indicating higher mortality. Although exceptions are made, including for hepatocellular carcinoma, a MELD score of 15 or higher is generally required to list patients with end stage liver disease for transplantation.

Several contraindications for transplantation exist. Active alcohol or substance abuse, medical non-adherence, poor social support, extrahepatic malignancy, significant cardiopulmonary disease, uncontrolled sepsis, extrahepatic systemic infections, and uncontrolled psychiatric illness are examples of contraindications to liver transplantation.

5. Salvage mechanisms and bridges to transplant

Although liver transplantation is life-saving for acute and chronic liver failure, the immunosuppressant medications necessary to prevent rejection and allow optimal graft function following transplant are frequently accompanied by adverse metabolic effects, nephrotoxicity, and susceptibility to potentially fatal infections. In addition, the demand for

transplant greatly exceeds the supply of donor livers, and many patients either die while awaiting a donor or become too critically ill to qualify for transplant. The discrepancy in the supply and demand for organs, and the high pre-transplant mortality and post-transplant morbidity have generated interest in surgical techniques to avoid transplantation and chronic immunosuppression, and support systems to serve as a bridge to transplant or spontaneous recovery. Examples of these include auxiliary liver transplantation and liver assist devices.

5.1 Auxiliary liver transplantation

An alternative to traditional orthotopic liver transplantation for patients with ALF is that of auxiliary transplant, based on the well-established regenerative capacity of hepatocytes. Unlike traditional transplantation, where native hepatectomy is performed simultaneously with donor engraftment, in auxiliary transplants, the patient's native liver is left surgically intact, while a partial or smaller sized donor graft is transplanted. This procedure allows assumption of hepatic functions by the donor graft, resolution of multi-organ failure, and clinical stabilization of the patient. In turn, the native liver benefits both from additional time, as well as improved physiological conditions, thus maximizing the opportunity for hepatic regeneration. Younger patients (below 40 years of age) with ALF due to viral hepatitis or acetaminophen toxicity appear to have the best outcome with this strategy. After native hepatic function is demonstrated, the auxiliary graft can be removed, but is most frequently allowed to atrophy by withdrawal of immunosuppression. Complete cessation of immunosuppression can be achieved in many patients (*Boudjema et al., 1995*).

5.2 Artificial and bioartificial hepatic support systems

Mechanical hepatic support systems serve as a bridge to transplant in patients with ACLF, and as a bridge to transplant or spontaneous recovery in patients with ALF. These systems are designed to reproduce the detoxifying functions of the liver, and mimic the principles upon which renal replacement therapy is based. While conventional renal dialysis removes small toxins and water-soluble toxins, the liver detoxifies larger toxins and protein-bound toxins. Dialysis of these larger and protein-bound toxins through unbound human albumin solutions allows the removal from the patient's circulation.

Several artificial systems have been developed utilizing albumin dialysis. The Molecular Adsorbent Recirculating System (MARS), Single Pass Albumin Dialysis (SPAD), and Prometheus are examples; of these, MARS has been the most widely studied. MARS has been utilized for management of hepatic encephalopathy, cerebral edema, hepatorenal syndrome, treatment of drug overdoses, and as a bridge to transplantation (*Mitzner, 2011*). MARS has been shown to improve hemodynamic parameters and organ perfusion during circulatory collapse, and has been associated with improvement in hepatic synthetic function. For patients with ACLF, MARS can provide temporarily relief of intractable pruritis and fatigue.

Bioartificial systems work similarly to artificial systems to remove toxins by albumin dialysis, but additionally utilize human or porcine hepatocytes to mimic hepatic synthetic

function. Advances in the development of bioartificial systems have been limited by the challenges in maintaining hepatocyte viability.

6. Conclusion

Acute liver failure and acute on chronic liver failure are complex illnesses often culminating in multi-organ failure, and require meticulous care in the pre-transplant phase. Mortality in the absence of transplantation is high, but the advent of multi-disciplinary critical care has significantly improved the outcome in these disease processes. A protocolized approach to the intensive care management of patients prior to liver transplantation will favorably impact the pre-transplant and post-transplant status of these patients.

7. References

Atterbury, C. E., Maddrey, W. C., & Conn, H. O. (1978). Neomycin-sorbitol and lactulose in the treatment of acute portal-systemic encephalopathy. A controlled, double-blind clinical trial. *American Journal Digestive Diseases*, Vol. 23, No. 5, May 1978, pp. 398-406, 354373

Bernard, B., Grangé, J. D., Khac, E. N., Amiot, X., Opolon, P., & Poynard, T. (1999). Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology*, Vol. 29, No. 6, June 1999, pp. 1655-61, 10347104

Boudjema, K., Cherqui, D., Jaeck, D., Chernard-Neu, M.-P., Steib, A., Freis, G., Becmeur, F., Brunot, B., Simeoni U., Bellocq, J.-P., Tempe, J.-D., Wolf, P., & Cinqualbre, J. (1995). Auxiliary liver transplantation for fulminant and subfulminant hepatic failure. *Transplantation*, Vol 59, No. 2, Jan 1995, pp. 218-23, 7839443

Fernandez, J., Escorsell, A., Zabalza, M., Felipe, V., Navasa, M., Mas, A., Lacy, A. M., Ginès, P., & Arroyo, V. (2006). Adrenal insufficiency in patients with cirrhosis and septic shock: Effect of treatment with hydrocortisone on survival. *Hepatology*, Vol. 44, No. 5, Nov 2006, pp. 1288-95, 17058239

Ford, R. M., Sakaria, S. S., & Subramanian, R. M. (2010). Critical care management of patients before liver transplantation. *Transplant Reviews*, Vol. 24, No. 4, Oct 2010, pp. 190-206, 20688502

Lee, W. M., Hynan, L. S., Rossaro, L., Fontana, R. J., Stravitz, R. T., Larson, A. M., Davern, T. J. 2nd, Murray, N. G., McCashland, T., Reisch, J. S., & Robuck, P. R.; Acute Liver Failure Study Group. (2009). Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. *Gastroenterology*, Vol. 137, No. 3, June 2009, pp. 856-64, 864 e1, 19524577

Mitzner SR. (2011). Extracorporeal liver support—albumin dialysis with the Molecular Adsorbent Recirculating System (MARS). *Annals Hepatology,* Vol. 10, Suppl 1, 2011, pp. S21-28, 21566251

Murray, K. F., & Carithers Jr., R. L. (2005). AASLD practice guidelines: Evaluation of the patient for liver transplantation. *Hepatology*, Vol. 41, No. 6, Jun 2005, pp. 1407-32, 15880505

Zardi, E. M., Abbate, A., Zardi, D. M., Dobrina, A., Margiotta, D., Van Tassell, B. W., Afeltra, A., & Sanyal A. J. (2010). Cirrhotic cardiomyopathy. *Journal American College Cardiology*, Vol. 56, No. 7, Aug 2010, pp. 539-49, 20688208

16

Anesthesia in Liver Transplantation

Meral Kanbak[1], Ayse Heves Karagoz[1] and Filiz Üzümcügil[2]
[1]Hacettepe University School of Medicine,
Department of Anesthesiology and Reanimation, Ankara,
[2]TCSB Ankara Etlik Zübeyde Hanım Maternity and Women's Health Clinic
Teaching and Research Hospital, Department of Anesthesiology and Reanimation Ankara,
Turkey

1. Introduction

Liver transplantation, is the replacement of unhealthy liver with a new liver allograft. This surgical procedure is now widely common all over the world in various medical centers. The major limiting factors of this surgery is the lack of available donors and decreased chance of appropriate patient selection. Anesthetic management of organ donors includes intensive management of heart beating and brain dead donors; however the augmentation of waiting list for liver and inadequate cadaveric organs resulted in elevated living donor transplantation rates especially for the critically ill patients who will not survive waiting until a brain dead donor is provided, resulting in the growing experience in anesthetic techniques for the management of living donors (Pickett et al, 1994; Lutz et al, 2003).

The most common indications for orthotopic liver transplantation (OLT) in adults are alcoholic cirrhosis, chronic cirrhosis due to hepatitis C, primary biliary cirrhosis and primary sclerosing cholangitis. The most common indication for OLT in pediatric patients is biliary atresia, followed by metabolic disorders (alfa-1 antitrypsin deficiency, Wilson's disease, tyrosinemia, Crigler-Najjar type-1 syndrome), fulminant hepatic failure, cryptogenic cirrhosis, neonatal hepatitis, and malignancy.

The association of liver failure with pathologic status of all other organ systems requires a thorough examination of the liver host and a fastidiously scheduled anaesthesia. A detailed monitoring of the patient and a careful therapeutic concept is required to meet the extraordinary conditions during liver transplantation. This report sheds light on the anaesthesiological approach of the liver transplantation and summarizes suitable therapeutic options.

2. Anesthetic management of the living donors

In order to permit successful transplantation of the liver, it is necessary to provide excellent conditions for the donor while preserving optimal hemodynamic parameters (Pickett et al, 1994; Lutz et al, 2003). However anesthetists may face severe hemodynamic instability, which is frequently seen in donors especially during the harvesting period when organs are removed (Pickett et al, 1994). Hemodynamic stability can be achieved by maintaining

sufficient organ perfusion, adequate cardiac output, avoiding excessive bleeding, keeping hematocrit at about 30% and preventing coagulopathies (Lutz et al, 2003).

Invasive monitoring is obligatory to ensure sufficient organ perfusion and cardiac output, because of the major hemodynamic, hematologic and metabolic disturbances associated with hemorrhages and electrolyte imbalances that may be seen especially in right hepatectomy procedures (Lutz et al, 2003). During the operation, a central venous pressure (CVP) below 5 cm H_20 is suggested in many liver transplantation protocols to decrease blood loss and graft edema. Chen et al. found a correlation between CVP and blood loss during resection of liver; however, Chhibber et al. reported no significant decrease in blood loss in patients with low CVP (Chen et al, 2000; Chhibber et al, 2007). Balci et al. has recommended an acute intraoperative normovolemic hemodilution technique and fluid restriction in a group of live donors (Balci et al, 2008).

The type of the intravenous solutions are also important in liver transplantation as well as their amount. Although there is no definitive data about a detrimental effect, 0.9% saline is known to be associated with hypercloremic metabolic acidosis. The Ringer's lactate solution is contraindicated because lactate metabolism will be disrupted as the liver is resected, furthermore serum lactate levels increase after resection. Plasmalyte may be an alternative being devoid of lactate, however there is no clear data that it is superior to other crystalloids. Despite the absence of definitive data about adverse outcome, since hydroxyethyl starch solutions are known to effect the coagulation system, they should be used with caution. 5% albumin can be used after hepatectomy, but it has also no proven benefit (Hwang and McCluskey, 2010).

Preventing major life threatening bleeding during transsection of liver requires extraordinary attention by the surgical team. Preparation of autologous blood and hemodilution in operating room may prevent transfusion complications (Merritt et al, 2004).

Anesthetic management of living donors is maintained with general anesthesia. Commonly used anesthetic agents such as modern inhalational anesthetics, sufentanil, fentanyl, remifentanil, propofol, cis-atracurium and vecuronium have no adverse effects on liver functions. In live donor hepatectomies, Rabie et al. investigated the effects of propofol or isoflurane, both of which were similar in terms of perioperative hemodynamics, blood loss, duration of surgery and hospital stay (Rabie et al, 2006).

Appropriate antibiotic prophylaxis (at least 20 minutes before skin incision) including a third-generation cephalosporin and metronidazol covering anaerobic infections, in addition to venous thromboembolism prophylaxis including subcutaneous heparin or low-molecular weight heparin with pneumatic compression stockings should be administered. Prior to donor graft perfusion 1000-5000 IU intravenous heparin is administered and its reversal is often not needed (Hwang and McCluskey, 2010).

Considering the rapid recovery from anesthesia and difficulties in pain control, general anesthesia combined with epidural anesthesia seems to be effective; a mid thoracic epidural application may be the best form of pain relief during early postoperative period. However, despite the data that epidural analgesia seems to be safe in spite of postoperative coagulation disorders in hepatectomy operations (Choi et al. 2007), it has been discouraged because of postoperative unpredictable coagulation profile and epidural hematoma risk of

living donors (Stamenkovic et al, 2011). On the other hand, since the kinetics of intravenous analgesics and opioids after liver resection have not been clarified yet, extra attention is required for the usage of these agents. Hwang and McCluskey reported that intravenous patient-controlled analgesia or intrathecal morphine may become an alternative for pain control in living donors. Regional techniques such as paraspinal blocks, transversus abdominus plane blocks, incisional field blocks are still investigated for their effect and safety in these patients (Hwang and McCluskey, 2010).

3. Anesthetic management of the recipients

Once the patient is scheduled for liver transplantation, the transplantation team has to consider the deterioration in the recipient functional status. All members of team, especially anesthesiologists should do their best to reduce the morbidity and mortality of this procedure in such high risk patient population.

3.1 Preoperative evaluation and premedication

The selection of appropriate and perfectly prepared recipient is the gold standard for the success of liver transplantation. Besides the complexity of the operation, most patients have an already disturbed physiology because of the hepatic disease, challenging the anesthesiologist (Table 1) (Findlay, 2002). Liver diseases strike all major organ systems leading to an unexpectedly chaotic scenario for the anesthesiologist, including hepatic failure, multiorgan dysfunction, encephalopathy, and severe metabolic disorders, and revealing the preoperative evaluation and premedication an essential part of the preparation of the patient. Besides, sepsis, metastatic malignancy, severe congestive heart failure, pulmonary hypertension and unresolved alcoholism are the contraindications for liver transplantations.

Cardiovascular system: Since the criteria for transplantation is expanded, the age limits has been extended to the older ages, thus bringing ischemic heart diseases as a major problem to be evaluated in the preoperative period (Steadman, 2004). Although coronary angiography is the gold standart for this assessment, considering the usage of radiographic contrast in a patient group with a high-risk of renal dysfunction precludes the usage of this technique; leading to other screening methods such as transthoracic echocardiography in combination with a stress test. Exercise tests are not suitable for end-stage liver disease patients, because they cannot complete the test adequately. Thus, pharmacologic stress tests; stress echocardiography or myocardial perfusion scan have to be used instead (Niemann, 2010). Dobutamine stress echocardiography (DSE) is the screening method in many centers; including the advantage of diagnosing pulmonary hypertension and valvular heart disease (Niemann, 2010; Steadman, 2004). Preoperative assessment should include echocardiography in order to determine the baseline cardiac function and pulmonary artery pressures (Findlay, 2002). In alcoholic liver disease, amyloidosis, hemochromatosis and Wilson's disease nonischemic cardiomyopathy may be seen. However hypertrophic cardiomyopathy is rarely seen, it may cause dynamic left ventricule outflow tract obstruction during liver transplantation (Aniskevich et al, 2007). Most of the end-stage liver disease patients have a hyperdynamic state characterised by an increased cardiac output and arteriolar vasodilatation (Glauser et al, 1990). Portopulmonary hypertension may be found in these patients. Severe

System	Patophysiologic changes related to liver failure	
Cardiovascular	• Hyperdynamic Circulation • Portopulmonary Hypertension	• High cardiac output, • Low resistance, • Increased cardiac index and left atrial size, • Mild left ventricular hypertrophy and ischemic heart disease; clinical cardiomyopathy (especially alcohol, amyloid, Wilson's hemochromatosis); • Autonomic neuropathy (mild in cirrhosis, moderate in amyloid)
Respiratory	• Hypoxia	• Restrictive pattern (ascites), • Pleural effusion; • Flow-related or anatomical intrapulmonary shunting (hepatopulmonary syndrome); • Non-cardiogenic pulmonary edema (fulminant hepatic failure); • Obstructive airways disease (especially cystic fibrosis, alpha-1 anti-trypsin deficiency); • Interstitial lung disease (primary biliary cirrhosis)
Hematologic	• Coagulopathy • Anemia	• Decreased and defective synthesis of Vit K dependent clotting factors, • Trombocytopenia (hypersplenism or marrow depression), • Platelet dysfunction; • Low grade DIC and/or hyperfibrinolysis
Central Nervous System	• Hepatic encephalopathy • Cerebral edema (fulminant failure)	
Renal System, Electrolyte and Metabolic disorders	• Hepatorenal Syndrome (prerenal failure from splanchnic 'steal'); • Acute tubular necrosis from sepsis; • Tacrolimus/cyclosporin-related renal impairment; renal tubular acidosis • Hyponatremia • Hypomagnesemia, • Hyperkalemia, • Hypokalemia, and/or metabolic acidosis and hypoglycemia	

Table 1. Patophysiologic changes related to liver failure (Findlay, 2002; Ozier & Klinck, 2008)

portopulmonary hypertension is associated with increased perioperative mortality and right heart failure (Krowka et al, 2000; Ramsay et al, 2000). For detecting preoperative portopulmonary hypertension, transthoracic echocardiography may be reliable enough in experienced hands, but echocardiography should be reperformed.

Respiratory system: Respiratory complications seen in liver disease include restrictive lung disease, intrapulmonary shunts, pulmonary hypertension and ventilation-perfusion abnormalities. Hypoxemia is often related to the restrictive lung disease caused by ascites and/or pleural effusions, frequently responding to fluid removal. However, hypoxia may occur in the absence of ascites or intrinsic pulmonary disease, then this is called hepatopulmonary syndrome; contributing to shunting, ventilation-perfusion mismatch and/or diffusion defects. The presence of pulmonary hypertension responding to vasodilators is not a contraindication for transplantation. Pulmonary hypertension may improve, persist or develop following transplantation (Steadman, 2004).

Hematological system: In addition to the routine blood tests; coagulation tests and arterial blood gases should also be obtained (Findlay, 2002). In the normal hemostasis, liver is important for the production of prothrombin, fibrinogen, factors V, VII, IX and X (except von Williebrand factor-synthesized in endothelial cells), sythesis of antithrombotic modulating factors (protein S, protein C and antithrombin III) and components of fibrinolytic system (plaminogen and α2-antiplasmin); also the clearance of activated coagulation factors. As the liver function is impaired, this natural balance between coagulation and its inhibition is impaired, whereas the balance between the fibrin polymerization and fibrinolysis is also disturbed; which occurs due to the decreased production of antiplasmin and inadequate clearance of tissue plasminogen activators, (Hannaman&Hevesi, 2011). All the coagulation factors are decreased, except fibrinogen and factor VIII. Besides, Fitzgerald factor, alpha-1 antitrypsin, alpha-2 macroglobulin, antithrombin-III and plasminogen levels are all decreased. Fibrin degradation products are positive in one third of the patients. Thrombocytopenia occurs in 70% of the patients, often complicated with the functional derangement of the platelets. For the best approach to treat coagulopathic disorders, defects must be identified in laboratory screening tests to predict bleeding risk in recipients, but the liver diseases have complicated effects on the balance between prohemostatic and antihemostatic mechanisms. Prothrombin time (PT), the activated partial thromboplastin time (APTT) and platelet count shows the defect in procoagulant functions. Unfortunately, defects in inhibitory pathways are less clear. The net effect of instability of these systems is unpredictable and administration of fresh frozen plasma (FFP), platelets and other blood products before the surgery should be reconsidered. There is a correlation between the severity of preoperative coagulopathy and intraoperative requirement for blood and blood products; as the severity increases, requirements are increased. Chronic disease anemia, malnutrition and bleeding is also common in recipients. In patients with the coagulation disorders intramuscular injections should be avoided.

Central nervous system: In advanced liver diseases, hepatic encephalopathy within a range of mild stupor, deep coma and unresponsiveness, is often seen. Diuretic therapy, gastrointestinal bleeding, infections and advancement in liver disease worsen encephalopathy. In order to exclude a preexisting organic disease mimicing hepatic encephalopathy, EEG, stimulated potential tests and computed tomography are recommended to be performed.

Any organic disease, that contributes to the changes in cerebral functions, is a contraindication for liver transplantation. Cerebral edema occurs in 50% of the patients with acute fulminant hepatitis. Cortical atrophy and non-specific changes are also seen at variable degrees in patients with chronic liver disease. Chronic liver disease is rarely associated with cerebral edema, but hepatic clearance failure leads to accumulation of toxins and alterations in endogenous transmitters, messengers such as γ-amino butyric acid (GABA), glutamate and nitric oxide. In preencephalopathic patients benzodiazepines should be avoided. In fulminant liver failure with Grade III-IV encephalopathy intracranial pressure monitoring may be required in order to maintain cerebral perfusion pressure >60 mmHg (Ozier&Klink, 2008). A severe coagulopathy may result in intracranial hemorrhage.

Renal system: The most common cause of renal failure associated with hepatic failure is hepatorenal syndrome, which is characterized by the absence of primary renal disease, proteinuria, hypovolemia and hemodynamic cases of renal hypoperfusion. Treatment with vasoconstrictors improving splanchnic vasodilation, decreasing endogenous vasoconstrictors leading to an improvement in renal blood flow is often successful (Duvoux, 2002; Gines, 2004; Wong, 2004). Contrast for diagnostic procedures and nephrotoxic agents should be avoided in these patients. Even a less advanced renal disease deserves management because it may also worsen the posttransplant period (Davis, 2002).

Gastrointestinal system: Esophageal varices, portal hypertension, ascites are frequently seen in patients with end-stage liver-disease. This complex state also includes delayed gastric emptying which contributes to a major problem especially during the induction of the anesthesia, thus premedications should include "aspiration" prophylaxis with ranitidine, metoclopramide, and particulate-free antacid.

Endocrine system: In liver diseases it is widely known that the carbonhydrate and protein metabolism is impaired, and glucose intolarence and insulin resistance occur. Serum insulin level is increased both because of the hypersecretion and decreased clearance. In addition to this, in acute fulminant hepatitis, depletion of glycogen stores, decreased gluconeogenesis and other humoral changes may result in a severe hypoglycemia. In advanced liver diseases, severe reductions in albumin levels may also be seen (<2gr).

Drug metabolism: All the plasma proteins, especially albumin which mainly provides plasma oncotic pressure, are produced in liver, except gama-globulin. The decrease in albumin levels (<2gr) results in intra- and extravascular volume changes, leading to an increase in distribution volume of drugs (e.g. neuromuscular blocking agents). Also, the duration of action of some anesthetic agents (such as opioids) are prolonged because of the increased volume of distribution and decreased metabolism. End-stage liver disease patients may be resistant to some drugs due to increased binding to globulin. Thus, initial dosages of the drugs are increased; on the other hand, because of the decreased levels of albumin, the unbound fraction of the drugs is increased; which leads to increased effectivity and duration of action of these drugs.

Premedication is usually administered unless the patient has an advanced hepatic encephalopathy. Oral diazepam 5-10 mg, lorazepam 2-3 mg can be used for adult patients, whereas 0.1-0.2 mg/kg of diazepam can be used orally for pediatric group of patients. Intramuscular injections should be avoided in patients with coagulopathy. Low dose midazolam (1-2 mg/kg) has become a routine before induction in the anesthetic practice.

All patients should be considered to have full stomach; H2 receptor blockers and particulate-free antacids can be used preoperatively.

3.2 Monitoring

Routine monitoring consisting of electrocardiography, pulse oximetry, capnograph and temperature monitor should be in place (Findlay, 2002). Full invasive monitoring including direct arterial blood pressure (close monitoring of the systemic pressures, also facilitating frequent blood sampling) (Peterfreund and Allain, 2003), central venous, and pulmonary artery pressure measurements are obligatory for the management of severe coagulopathy, metabolic disorders, massive blood loss, temperature alterations, hemodynamic instability, and other organ dysfunctions (Table 2).

Monitoring
• ECG
• Pulse Oximetry
• Capnography
• Temperature
• Arterial Catheter
• Central Venous Catheter
• Pulmonary Artery Catheter
• Transesophageal Echocardiography

Table 2. Monitoring during liver transplantation (Findlay, 2002; Peterfreund and Allain, 2003)

ECG: ECG monitoring is a cornerstone showing clinical outcome of electrolyte changes. Due to the rapid and profound changes in electrolyte levels during the various phases of liver tranplantation, ECG may reflect the cardiac disturbances; which require prompt treatment. Fatal ventricular fibrillation due to the hyperkalemia during reperfusion period has also been reported (Ozier & Klink, 2008).

Arterial blood pressure: Invasive arterial blood pressure monitoring is essential for providing continuous monitoring to see frequent hemodynamic changes usually secondary to surgical manipulations such as caval clamping, sudden blood loss, hepatic reperfusion during liver transplantation and also providing access for blood sampling for coagulation tests. Radial artery pressure monitoring may underestimate aortic pressure in hypotensive scenarios and femoral arterial systolic pressure is higher than radial, and the use of vasoconstrictors increases these variances (Arnal et al, 2005).

Central venous cannulation and pulmonary artery catheterization: Pulmonary artery catheterization (PAC) and CVP monitoring are standards in many centers. Liver transplant recipients are hyperdynamic, characterized by increased cardiac output (CO) and decreased peripheral vascular resistance (SVR) and arterial pressure (Liu et al, 2006; Moller & Henriksen, 2008). The patients with cirrhosis have a reduced total blood volume index (Henriksen et al, 1989), because of this relative hypovolemia, adequate volume management during liver transplantation is a gold standard for improvement of tissue perfusion (Nasraway et al, 1995). The various phases of liver transplantation may limit the accuracy of CVP measurements, however considering the low CVP approach to reduce blood loss and

liver congestion, central venous cannulation is necessary (Niemann, 2010). Central venous cannulation can be applied before or after anesthetic induction and endotracheal intubation. However, the patients with encephalopathy, tense ascites, respiratory compromise caused by atelectasis or pleural effusions, may not tolerate the Trandelenburg position and draping of the face that are required for the central cannulation; thus it may be more suitable performing these procedures after the induction of anesthesia (Peterfreund and Allain, 2003). Although less invasive monitoring gets more popular nowadays, because of severe PAC-induced ventricular arrhythmias (Gwak et al, 2007); PAC may still be particularly useful in renal insufficiency, respiratory compromise or unstable hemodynamics that exist prior to surgery (Peterfreund and Allain, 2003).

Transesophageal echocardiography: Transoesophageal echocardiography (TEE) is increasingly used for cardiac monitoring. It is relatively noninvasive and provides visual information on valvular and ventricular function and gives a chance of diagnosis of embolization, but unfortunately it is not available in many centers (Burtenshaw et al, 2006). In a report of multi transplant centers the rate of PAC and TEE used was respectively 30% and 11.3% (Schumann et al, 2003). During the last decade, transpulmonary thermodilution has been becoming popular to measure circulating blood volumes (Shippy et al, 1984). In recent years, noninvasive techniques such as lithium thermodilution is compared with PAC (Costa et al, 2008). Vigileo monitor is a good choice at low and normal CO, but is not appropriate for the hyperdynamic cirrhotic patients (Della et al, 2008; Biancofiore et al, 2009; Biais et al, 2008) (see also Future Directions for Circulatory Monitoring).

Temperature: Large insicions and prolonged duration of the procedure result in a high risk of hypothermia; to prevent the heat loss warming devices, heated humidifier, warming blanket, and a forced-air warming device should be present (Findlay, 2002).

Venous access: Sufficient large-bore venous access should be in place in case of a sudden, massive hemorrhage. Since the procedure involves inferior vena cava (IVC) obstruction; the cannulas should be in the upper part of the body. This venous access is also needed for the return of blood if veno-venous bypass (VVB) is used. Peripheral or central often two 8F or larger cannulas are preferred. A rapid infusion pump should be ready to infuse blood or fluids warmed to 37 C at a rate of 1.5L/min (Findlay, 2002).

Other monitors: All patients have nasogastric tubes and bladder catheters (Peterfreund and Allain, 2003). Nasogastric tubes should be used with caution because of the possible esophageal varices and coagulopathies that may cause bleeding (Topal & Celik, 2009).

Future directions for circulatory monitoring: A perfect monitoring device for circulation has not been clearly defined yet. There are ongoing investigations for continuous hemodynamic monitoring such as the FloTrac/Vigileo (Edwards Lifesciences, Irvine, CA, USA), which is a self-calibrating arterial pulse contour cardiac output monitoring system; and LİDCO (LiDCO Cardiac Sensor System, London, UK) which is a pulse contour waveform analysis system. However, because of the various changes in hemodynamics during phases of liver transplantation, these monitors lose their accuracy. Similarly, thermodilution technique may also become inaccurate because of the rapid changes in temperature, rapid infusion of fluids and changes due to reperfusion of the graft. It is difficult to recommend one monitor as a superior to the other, so while planning monitoring, one of the devices including PAC, CVP and TEE is going to be chosen in addition to arterial line or lines

(sometimes two arterial lines are preferred) (Liu et al, 2011). As the correlation between the pathophysiology of hepatic microcirculation and ischemia/reperfusion injury has been demonstrated, intraoperative analysis if ischemia/reperfusion-induced impairment of hepatic microcirculation has gained interest. Orthogonal polarization spectral imaging has been used accurately for this purpose; quantifying the sinusoidal perfusion rate, vessel diameter and venular RBC velocity (Puhl et al, 2005).

3.3 Anesthetic induction and maintenance

Drug metabolism and clearance depend on the state of liver blood flow and P450 cytochrome system in hepatocytes. Due to the various disease states with different patterns of liver dysfunction, there is not a standard protocol for any drug. Drug biotransformation is related to the anesthetic practice in two ways. One of them is the sensitivity to the microsomal enzyme induction accelarate the biotransformation; the inhalational anesthetic agents and barbiturates cause microsomal enzyme induction. The second one is the impairment of hepatic blood flow in hepatic diseases leading to an increase in half-lives of drugs, because of the decrease in their biotransformation rates. In advanced liver diseases, the half-lives of meperidine, lidocaine and diazepam have been shown to be increased, similar to the duration of action of thiopenthal. In healthy patients thiopenthal binds to protein at a rate of 75%, while 50% in liver diseases. Thus, considering the decrease in metabolism and protein binding of the drugs in these patients, it should be kept in mind that intermittent dosing and usage of different drugs as combinations may result in accumulation of these drugs, which is very important in anesthetic practice.

Careful monitoring of drug effects with titrating the drug, considering the coagulation profile, volume status, and general hemodynamic state of patients, is important for clinicians to be current in their understanding of how transplant patients should be managed.

After preoxygenation, the induction of anesthesia may be performed by using either one of the hypnotic agents, thiopental or ketamine using invasive monitoring, and also midazolam can be used for its amnestic properties and minimal effects on hemodynamics. A routine rapid sequence induction with cricoid pressure should be performed because of the risk of aspiration. A semi-upright position can be applied until the abdomen is open; in order to prevent rapid oxygen saturation and to facillitate ventilation. Liver failure associated with renal dysfunction with increased potassium levels may prevent the usage of succinylcholine (Peterfreund and Allain, 2003). The pharmacokinetic changes, increase in extracellular volume, decrease in serum albumin and glycoprotein levels and elevated bilirubin and other metabolites in liver disease; result in an increase in the requirement of first dose of non-depolarizing muscle relaxants and prolong the duration of their action. Because of the organ-independent elimination and diminished histamin release cis-atracurium and atracurium are preferred; however, the others can also be used safely (Topal&Celik, 2009); vecuronium bromide and rocuronium as neuromuscular blocking agents provide optimal conditions. The new liver graft may be evaluated by vecuronium since the time for the return of a train-of-four (TOF) with a nerve stimulator correlates with the function of the new liver (Lukin et al, 1995). Similarly, the duration of action of rocuronium is also used for the assessment of allograft function; >150 minutes refers to allograft dysfunction (Marcel et al, 1997).

In the intraoperative period; using lower tidal volumes (6-8 ml/kg) and avoiding positive end-expiratory pressure may lower the preload and decrease the risk of bleeding (Hannaman&Hevesi, 2011). Different variations have been used for anesthesia maintenance and all have been informed to have less side effects on liver functions (Adachi et al, 2003). Maintenance of anesthesia can be provided by using isoflurane, desflurane or sevoflurane (Lukanovic et al, 2008) in an air-oxygen mixture with their minimal metabolism in the liver, supplemented with sufentanil or remifentanil infusions. Isoflurane has been the volatile agent to be preferred because of its vasodilation effect on hepatic circulation, which is advantageous for the reperfused graft, preserving splanchnic blood flow better than the others; especially when it is compared to the vasoconstrictor effects of halothane. Investigations addressing the effects of desflurane has conflicting results. In an animal study it has been shown to decrease hepatic blood flow in a dose dependent manner at concentrations up to 1 MAC; however in a human study although excluding patients with hepatic diseases and the results were not significant, it has been shown to provide better hepatic blood flow compared to that of isoflurane. In another study comparing the effects of desflurane and sevoflurane in terms of hepatic blood flow and hepatocellular integrity; both agents well preserved the hepatic functions, but decreased the splanchnic perfusion and oxygen delivery to the liver disturbing the hepatocellular integrity and gastric tonometry. The increased metabolism of sevoflurane which is a hundred times that of desflurane is not known to cause a detrimental effect on the liver (Steadman, 2004). In one of our study, we investigated the effects of sevoflurane in terms of metabolism and renal functions in liver transplantation, and sevoflurane seemed to have minimal effects on the kidney during liver transplantation (Kanbak et al, 2007). Erdem et al. reported a case of liver transplantation for the effects of sevoflurane in terms of extrahepatic metabolism and possible nephrotoxicity in liver disease; revealing a correlation between the levels of N-acetyl glucoseaminidase excretion and urine fluoride levels and no impairment in serum BUN and creatinin levels (Erdem, 2006). In fulminant hepatic failure, due to the elevated intracranial pressure, volatile anesthetic agents should be avoided or used with caution at lower dosages combined with ICP monitoring (Steadman, 2004). As an intravenous anesthetic agent propofol is another good alternative. Takizawa et al. found that during the anhepatic phase, compared with the dissection phase, the clearance of propofol was decreased and afterwards liver allografts immediately metabolized propofol (Takizawa et al, 2005). Morphine and propofol which rely on conjugation pathway may be more tolerated (Brown et al, 1993).

Rossaint et al. combined early postoperative extubation and restrictive intraoperative fluid management techniques in liver transplant recipients (Rossaint et al, 1990). This approach resulted in rapid recovery of new liver functions and favoured early extubation. Early extubation is a goal for the operating team, because early liver graft recovery is associated with early patient recovery. Postoperative mechanical ventilation following liver transplantation is not required nowadays in the majority of patients, since immediate postoperative extubation is usually safe and well tolerated. In poor clinical condition with severe preservation injury, special attention is required in extubation. These patients may not be appropriate for fast tracking protocols and may be at elevated risk of prolonged postoperative ventilation.

Although the progress in liver transplantation owing to improved techniques in medicine is excellent, maintaining the stability of the patient who has marginal liver functions is critically complicated.

3.4 Hemodynamic and haematological management: The three phases of liver transplantation

3.4.1 The preanhepatic phase

The **preanhepatic phase** involves dissection and mobilization of the liver. Hemodynamic instability may be seen as a result of drainage of liters of ascitic fluid, transection of large varices and surgical manipulation of the liver, temporarily obstructing venous return. However, in this period of liver transplantation, the primary issue is surgical bleeding. Several approaches have been applied to reduce bleeding: preoperative autologous blood donation, erythropoietin administration, intraoperative isovolemic hemodilution, blood salvage, maintenance of low intraoperative central venous pressure (CVP), and normothermia to prevent hypothermia-induced coagulation abnormalities (Chibber et al, 2007). The low CVP approach maintaining the pressure at or below 5 cm H2O has been shown to provide an 80% reduction in blood loss, although the usage of this strategy still remains controversial (Liu & Niemann 2011). Fluid resuscitation results in a decrease in coagulation factors and platelet count (Murray et al, 1995). Packed red cells and fresh frozen plasma should be prepared at this time. During this time period for the evaluation of the coagulation, thromboelastograph and standard laboratory tests (prothrombin time, fibrinogen and platelet count) can be used. Monitoring coagulation parameters differs, but the prothrombin time, INR, partial thromboplastin time, fibrinogen and platelet counts are monitored in most of the transplantation centers. While thromboelastography is used in approximately 33% of centers, the activated clotting time is used in approximately 18% of centers (Schumann et al, 2003). Excessive treatment of coagulopathy is not usually recommended at this phase unless bleeding is extreme. As a result of improved intraoperative techniques, some centers reported average RBC transfusion rates as low as 2 U (De Boer et al, 2005). The requirements for the blood products has been reduced for over the last decade, that several reports revealed the number of liver transplants without any administration of blood products has been increased. Because, blood tranfusion may have its own adverse events such as; subacute transfusion complications including fluid overload, hypothermia, hypocalcemia, hyperkalemia, acid-base disturbances; and more seriously, acute hemolytic transfusion reactions, infusion of a bacterially contaminated unit, tranfusion associated lung injury, severe allergic reactions and anaphylaxis; moreover immune modulation associated with worse outcome (Niemann, 2010; Hannaman & Hevesi, 2011). Diuretics can be used to maintain euvolemic conditions and reduce transfusion requirements. Mannitol, with its potential for free radical scavenging and antioxidant properties, can help to remove free water in the abdominal organs, which is due to the congestion of blood flow through the fibrosed liver; particularly being useful in hepatorenal syndrome. Mannitol can be used before clamping or during the anhepatic phase (Vater et al, 2004) (Table 3).

Calcium chloride administration during the absence of hepatic functions avoids citrate intoxication related with the infusion of citrate rich blood products (Scott et al, 1996).

The risk of an air embolism during the manipulation of vena cava should be considered in case of sudden decrease in expired carbon dioxide associated with hemodynamic instability.

3.4.2 The anhepatic phase

The **anhepatic phase** starts with the occlusion of blood inflow to the liver and ends with graft reperfusion. The procedure of clamping the inferior vena cava and portal vein, and

dividing the hepatic vasculature (including IVC) results in the loss of venous return leading to a high risk of cardiovascular collapse with a marked decrease in cardiac output and hypotension. The resulting increase in distal venous pressure may increase bleeding, impair renal perfusion and often promotes edema and ischemia of intestines. This may be overcome by veno-venous bypass which involves cannulation of portal and femoral veins, diverting the blood flow from IVC and portal veins to the axillary vein; improving renal perfusion pressure, lessening splanchnic congestion and delaying the development of metabolic acidosis (Findlay, 2002; Steadman, 2004). VVB improves hemodynamic stability, reduces blood loss, and allows extra time during the anhepatic phase. The first successful use of venovenous bypass (VVB) is reported in 1984 (Shaw et al, 1984). Venovenous bypass is used only in some centers. Although there are many benefits of venovenous bypass such as reducing hemodynamic instability and blood loss during anhepatic phase, maintaining intraoperative renal function and cerebral perfusion pressure in patients with acute fulminant failure by avoiding rapid swings in blood pressure (Shaw et al, 1984; Chari et al, 1998; Grande et al, 1996; Veroli et al, 1992), the use of it is not without risk such as; air embolism, thromboembolism, brachial plexus injuries and incorrect cannulation which may be mortal and may increase the risk of morbidity. The decision of using VVB depends on medical team experiences, preferences and judgement. The volume restriction brings the usage of vasopressors, as an alternative to veno-venous bypass. Transient inotropic support in addition to blood and fluid replacement is often required until effective veno-venous bypass is established. Norepinephrine and vasopressin improve circulatory stability and renal perfusion without impairing the mesenteric blood flow (Wadei et al, 2006; Alessandra et al, 2007). In order to preserve renal functions, prophylactic mannitol administration prior to and during venous clamping may be beneficial.

Transfusion of blood leads to a large citrate load that can no longer be metabolised when the liver is removed, resulting in hypocalcemia and secondary myocardial depression. Treatment with periodic calcium chloride administration should be guided by ionized calcium concentration measurements to avoid hypercalcemia. On the other hand, acid metabolites originating from intestine and lower body cannot be cleared in the absence of liver, resulting in progressive acidosis. Sodium bicarbonate therapy should be guided by arterial blood gas analysis, because excessive administration may result in hypernatremia, hyperosmolality and metabolic alkalosis. Although hypoglycemia can occur, hyperglycemia is more likely, due to the large amounts of transfused blood products. Glucose containing solutions are not used unless hypoglycemia is documented. Due to absence of a liver produced plasminogen activator inhibitor, fibrinolysis may begin during this phase. During this significantly notable phase platelets and coagulation factors continue to decrease. Antifibrinolytic strategies differs among centers. Postreperfusion syndrome may follow within a few minutes with severe hypotension, decreased heart rate, and a major decrease in systemic vascular resistance together with an increase in pulmonary artery pressure (Table 3).

After hepatectomy, vascular anastomoses of the supra- and infra-hepatic inferior vena cava and the portal vein are performed.

3.4.3 The neohepatic phase

In neohepatic phase, when the venous anastomosis to the graft is completed, the liver is flushed with blood and the clamp on IVC is released. This reperfusion of the graft is

associated with elevations of potassium and hydrogen ion concentrations, increase in preload and decrease in systemic vascular resistance with a decrease in blood pressure. Aggarwal described 'postreperfusion syndrome' (PRS) in 1987, and defined it as at least 30% decrease in mean systemic blood pressure for more than 1 min during the first 5 min following reperfusion and by initial reperfusion of the hepatic artery (Aggarwal et al, 1987; Moreno et al, 2006). Despite the restoration of blood volume, electrolyte and acid base balance, hypotension and bradycardia are also common. Avoiding excessive fluid administration and hypervolemia is one of the intraoperative aims, maintaining a low CVP via IV volume restriction or phlebotomy (Massicotte et al, 2006). Besides volume restriction, plebotomy or using both; nitroglycerin may become a pharmacological alternative, in patients whose blood pressures are tolerable, to keep the CVP low (Massicotte et al, 2006; Hannaman & Hevesi, 2011).

Significant hemodynamic changes such as decreased cardiac output, increased splanchnic and lower caval pressures, decreased renal perfusion pressure, and reduced systemic arterial pressure may be seen during this period. Mediators may be released by the ischemic liver including xanthine oxidase, a generator of cytotoxic oxygen radicals which may produce myocardial and cellular damage. Strong vasopressors with an alpha-agonist action such as norepinephrine may be required (Ramsay et al, 1992). These changes related to reperfusion may be decreased by using the piggy back technique contributing to a side-bite of IVC allowing the venous return to continue (Findlay, 2002; Gurusamy et al, 2011). Initial arterial reperfusion may be preferable only in patients with poor cardiac reserve (Moreno et al, 2006). Hypothermia is a marker for the presence of graft outflow into the central circulation. Calcium chloride should be given for treating life-threatening hyperkalemia and possibly bicarbonate administration must be considered. Electrocardiographic changes require prompt treatment. Even in the absence of treatment, increased potassium levels fall spontaneously within minutes due to redistribution. The severe coagulation disorder seen in this period is mainly multifactorial in its etiology including reperfusion hypothermia, ionized hypocalcemia, dilutional coagulopathy, quantitative and qualitative defects in platelets, heparin effect, fibrinolysis and humoral substances released by the grafted liver (Pvalizza et al, 2001; Hannaman & Hevesi, 2011). Infrequently, excessive activation of coagulation may occur during this phase (Gologorsky et al, 2001).

Coagulation disorders should be corrected during this phase of the surgery to obtain sufficient results. However, using blood products may result in hypervolemic state and a paradoxical increase in blood requirement. Fibrinolysis, shown by the thromboelastograph, should be reversed with aminocaproic acid and antifibrinolytics or cryoprecipitate may be required. Thromboelastograph helps assessing both cellular and humoral components of whole blood coagulation and fibrinolysis; and also the effects of antifibrinolytic therapy, cryoprecipitate, fresh frozen plasma, platelet and protamin as treatment strategies. However, there have been some case reports of thromboembolic complications in patients treated with antifibrinolytic agents (Manji et al, 1998; Sopher et al, 1997; O'Connor et al, 2000; Gologorsky et al; 2007; Ramsay et al, 2004; Ellenberger et al, 2006). A systematic review and meta-analysis of the safety of antifibrinolytic drugs has not shown any relationship with thrombosis in liver transplantation (Molenaar et al 2007). Certainly, antifibrinolytics should not be used prophylactically in patients with a history of thrombotic events.

Signs of liver function which may be seen in the operating room are decreased calcium requirements, improvement in acidosis, increased urine output, rising core temperature and

biliary output from the graft. As the graft begins functioning the coagulopathy gradually improves. Fibrinolysis and heparin effect ceases within 2 hours, coagulation factors and platelets begin to increase toward baseline levels; prothrombin time and activated partial thromboplastin time reach their baseline values within a few days (Hannaman&Hevesi, 2011) (Table 3).

Preanhepatic phase	• Baseline Tests
	• Intravenous antibiotics
	• Warmers
	• Incision
	• Induction of anesthesia
	• Invasive monitoring (arterial catheter, pulmonary catheter)
	• Lower CVP (5 cm H_2O), restriction of iv fluid administration, phelobotomy if Hgb>10 g/dl
	• Vasopressin (Norepinephrine) to keep mean blood pressure (BP)>60 mmHg
	• Epinephrine or dopamine to preserve cardiac output (C.O)>5L/min
	• Maintain Hgb >7 g/dl, platelets>40.000, MA (TEG)>45mm, fibrinogen>100 mg/dl
	• Prior to clamping, iv mannitol 0.5 g/kg
	• Just before clamping
	  • IV Heparin 3-5000 U
	  • Increase CVP to 10 cm H_2O
	  • In severe hypoalbuminemia 25%

Anhepatic Phase	• Maintain Hgb >7 g/dl • IV fluids to keep CVP around 5 cm H_20 • Vasopressin/Norepinephrine to preserve BP>60 mmHg and C.O>5 L/min • Correct base deficit • Normocalcemia

Neohepatic Phase	• Reperfusion
	• IV vasopressin 1-5 U bolus to keep BP>60 mmHg
	• Euvolemia (CVP 5-10 cm H_2O)
	• Dopamine/epinephrine to preserve C.O>5L/min
	• Norepinephrine/vasopressin to preserve BP>60 mmHg
	• Transesophageal Echocardiography if necessary
	• Maintain Hgb>7 g/dl, platelets>40.000, fibrinogen>100 mg/dl
	• TEG:
	  • Protamin 30 mg iv if R is more
	  • Maintain MA>45 mm with platelet infusion
	  • If Ly30>8% iv EACA 5gr
	• Consider fast tracking

Table 3. The evidence-based protocol for liver transplantation that is outlined by University of Wisconsin (Hannaman & Hevesi, 2011)

3.5 Sodium, potassium and glucose management in organ transplantation

A lot of recent studies have investigated electrolyte and glucose management in organ transplantation. In the last decade, anesthesiologists paid attention to intraoperative glucose management. Interaction between glycemic control and organ transplantation were recently reviewed (Marwin&Morton, 2009). Hyperglycemia increases the expression of adhesion molecules and the production of cytokines, increases ischemic damage and the inflammatory response to ischemia/reperfusion (Marwin & Morton, 2009). In addition to this; the documented risk of hypoglycemia with intensive insulin therapy has led to the modification of more conservative glycemic targets, but investigation of glycemic control in transplant recipients are limited. Although transplant patients were not specifically mentioned, The American Association of Clinical Endocrinologists (AACE) and the American Diabetes Association (ADA) reported a consensus statement on glycemic control (Moghissi et al, 2009). A perioperative 'middle ground' target glucose of between 140 and 180 mg/dl seems appropropriate (Keegan & Wright, 2010; Lazar et al, 2009). However, studies of glucose management in transplant recipients are limited.

In transplant patients, predictors and potential treatments especially for perioperative hyperkalemia has been documented, because hyperkalemia leading to ventricular fibrillation is still reported, although in most patients a progressive decrease is seen. A retrospective analysis of 1124 liver recipients showed that 10.2% of patients had hyperkalemia with high baseline potassium values (Xia et al, 2007). High recipient potassium concentrations were found to be an independent predictor of death within the first year after liver transplantation (Dawwas et al, 2009). Bank blood transfusion imposes a severe potassium increase, and hyperkalemia may complicate the status of the transplant patient, especially in the presence of renal impairment and acidemia (Nakasuji& Bookallil, 2000). Serum potassium concentrations must be checked periodically and prereperfusion hyperkalemia must be corrected aggressively. Treatments are administration of insulin–glucose and/or salbutamol, furosemide, washing of bank blood using cell-salvage equipment, and hemodiafiltration. The effectiveness of different volumes of 5% albumin solution for the washout of preservation fluid in liver transplant grafts prior to reperfusion was measured; proposing the minimal washout fluid volume as 500 ml to reduce the risk of postreperfusion syndrome and hyperkalemia (Homvises et al, 2008). During neohepatic period baseline hypokalemia, low body weight (pediatric patients), administration of fresh-frozen plasma units and absence of ascites at surgery were independent predictors for hypokalemia (Xia et al, 2006). Potassium should be administered carefully, since it leads to hyperkalemia easily and is more likely to be dangerous than no treatment.

Besides, hyponatremia occurs in approximately 20% of patients with decompensated cirrhosis and has been shown to be a predictor of death for patients with end-stage liver disease listed for liver transplantation (Kim et al, 2008). The brain adapts to hyponatremia by losing intracellular solutes limiting brain edema (Gines P & Guevara, 2008). Treating hyponatremia may prevent hepatic encephalopathy. While correcting hyponatremia rapidly in the presence of lack of adaptation by the brain may lead to osmotic demyelination syndrome. Central pontine myelinolysis (CPM) in liver transplant recipients appear to be associated with a rapid rise in serum sodium concentration in previously hyponatremic patients (Zhang et al, 2009). This syndrome is often associated with neurological morbidity and mortality. A review of 1247 patients undergoing liver transplantation reported 11

patients diagnosed with CPM by neurological imaging findings (Lee et al, 2009). Patients with hyponatremia can be successfully operated but they are at increased risk of cerebral demyelination syndromes. In one report rapid correction of hyponatremia causing a perioperative rise of 21-32 Meq/L in the serum sodium were associated with central pontine myelinolysis, while an increase of 16 mEq/L was not (Wszolek et al, 1989). The first step in management of hyponatremia is determination of the patient's volume status (Gines P & Guevara, 2008).

3.6 Early-extubation (fast-tracking)

In general, fast tracking of a patient refers to improvement in quality of care, short length of stays in ICU and hospital and reduced costs of total treatment. Prolonged mechanical ventilation is no longer desired, for a group of patients devoid of risk factors, following orthotopic liver transplantation (OLT). 'Fast-tracking' defined as tracheal extubation at the conclusion of surgery before leaving the operating room, varies widely among the centers for OLT recipients. In liver transplantation the aim is rapid progress from preoperative preparation throughout the surgery and early discharge from hospital. Because of the nature of this procedure; awaiting recipients for a cadaver liver donor graft, fast-tracking contributes to intra- and post-operative surgical and anesthesiological strategies; meaning generally a reduction in the postoperative ventilation time (Glanemann, 2007).

In recent years there is a gradual increase in the number of early extubated recipients approaching to approximately 70-80% (Forraz-Neto et al, 1999; Park et al, 2000; Biancofiore et al, 2005; Salizzoni et al, 2005). Postoperative positive airway pressure ventilation combined with sedation has been known to decrease surgical stress response, improve haemodynamic stability and facilitate early recovery; however leading to elevated intrathoracic pressures it causes an increase in pulmonary vascular resistance which in turn rises right ventricular afterload. The possible associated tricuspit regurgitation there may occur venous congestion in the graft (Jullien et al, 1995). On the other hand, spontaneous breathing has been shown to reduce intrapleural pressures, improving venous return and hepatic blood flow; leading to a better recovery (Kaisers et al, 1995). Fast-tracking combined with the restrictive fluid management have been shown to result in rapid recovery (Rossaint et al, 1990).

Fast-tracking of the patients with liver transplantation is usually safe and well tolerated; postoperative mechanical ventilation is no longer required for the majority of patients who are devoid of risk factors (Glanemann, 2007). However, as the Model for end-stage liver disease (MELD)-score based organ allocation system has been introduced, the number of patients to be the candidates for fast-tracking is decreased; because it has been shown that early extubation also has its own complications including postoperative ventilatory failure resulting in impaired oxygen delivery to the new graft and reintubation for early surgical complications such as bleeding, bile leak, thrombosis or retransplantation (Mandell et al, 2007).

Risk factors for prolonged mechanical ventilation after liver transplantation has been described in a statistical analysis. Acccording to this analysis, encephalopathy and a body mass index >34 were significantly associated with failure, thus cannot be extubated in the operating room. Primary graft dysfunction, renal failure, cardiovascular failure, neurological

impairment, use of >12 units of red blood cells and pulmonary edema cannot tolerate extubation within 3 hours postoperatively. Acute liver failure, retransplantation, severe preservation injury to the graft, mechanical ventilation prior to surgery and use of >15 units of red blood cells and fresh frozen plasma require mechanical ventilation at least 24 hours postoperatively (Mandell, 2002).

3.7 Pain management

Orthotopic liver transplant patients were reported to experience less pain and use less morphine in the postoperative period compared to liver resection patients (Moretti et al, 2002). Chen et al have found morphine consumption significantly lower in patients with end-stage liver disease undergoing living-donor liver transplantation; compared with healthy living liver donors and patients with liver cirrhosis due to chronic hepatitis B or C virus infection and hepatocellular carcinoma undergoing hepatectomy, only on the first postoperative day (Chen et al, 2010).

Epidural anesthesia and analgesia provide a good quality of pain relief after major surgery. Cosidering the well known coagulation disorders associated with liver surgery, epidural hematoma formation is a major risk. Nowadays, although it seems that epidural analgesia for living liver donor is a safe method for analgesia, it may be hazardous for recipients because of unpredictable coagulopathies after liver transplantation (Lukanovic et al, 2008).

The type of surgical incision, in particular upper midline compared with subcostal incision affects postoperative pain, but the safety and efficacy needs to be further evaluated (Kim et al, 2009).

3.8 Postoperative adverse events

3.8.1 Early complications (Table 4)

Hypothermia: In the intensive care units it takes 3-8 hours to warm a patient; during this period of hypothermia and warming there is always a risk of arrhtyhmias. Also during this period, shivering causes the metabolic rate to increase.

Prolonged mechanical ventilation: Over 72 hours of mechanical ventilation is required for 15% of the patients; mainly due to preoperative malnutrition, postoperative hemmorhage and primary non-functioning donor graft.

Bleeding: The requirements for blood transfusion continues also in the postoperative period. In reoperations a bleeding site is often found, however in a majority of patients this can also be caused by coagulopathy. Prothrombin time may remain high in the postoperative period and also trombocytopenia may develop because of the sequestration during recirculation. Thromboelastogram is a useful monitor also for the coagulopathy in the postoperative period.

Hypertension: The hyperdynamic circulatory state tends to become normal during the posttransplantation period, however in 55-85% of patients hypertension may develop, resulting in intracranial hemorrhage.

Impaired liver function tests: Within the first 48-72 hours, inadequate perfusion of the graft, venous congestion and edema may lead to functional impairment. However, in the following period coagulopathy improves, whereas aminotransferase, alkalene phosphatase and bilirubin levels start to decrease.

Malnutrition: The patients with end-stage liver disease are often malnourished and have depleted protein stores. Following transplantation protein catabolism occurs, leading to a negative nitrogen balance within a month. This protein catabolism results in an increase in urinary 3-methylhistidine levels, revealing this catabolism originates from muscle.

The primary non-functioning of donor graft: The increase in the levels of liver enzymes within 1 week after transplantation refers to acute cellular rejection, which requires biopsy for the definitive diagnosis. Retransplantation is necessary before the other organs are affected.

Sepsis: Most of the patients are transfered back to the ICU because of sepsis, following their discharge from ICU. Selective intestine decontamination may limit bacterial infections, however non-bacterial organisms contribute to a major problem.

Others: Bile leak, thrombosis of hepatic artery and portal vein may be major complications during this period. Moreover, hyperglycemia, renal insufficiency and neurologic impairments may also occur, as side-seffects of immunsuppressive therapy.

3.8.2 Late complications (Table 4)

Obesity (30-40%), hyperlipidemia (30%), diabetes (13-30%), osteoporosis and malignancy may occur due to the long-term immunsuppressive therapy (Lopez et al, 2006).

Early Complications	• Hypothermia • Prolonged mechanical ventilation • Bleeding • Hypertension • Impaired Liver Function Tests • Malnutrition • The primary non-functioning of donor graft • Sepsis • Others
Late Complications	• Obesity • Hyperlipidemia • Diabetes • Osteoporosis • Malignancy

Table 4. Postoperative Adverse Events after liver transplantation

4. Anesthesia for non-transplant surgery in liver-transplanted patients

Liver transplant recipients may return to the operating room for reexploration, which is frequently for biliary reconstruction. In these cases, liver usually functions normally, leading

to anesthetic considerations including regional techniques such as epidural catheters similar to any abdominal procedure (Baker et al, 2005). The liver grafts that are functioning appropriately can metabolize the drugs effectively, however this functioning should be assessed. Coagulation abnormalities should be treated with vitamin K or FFP, ascites with diuretics or paracenthesis and encephalopathy should be avoided with lactulose administration and careful use of sedatives; these may improve outcome in these patients. Renal functions should also be assessed, moreover hypertension may be a common problem in these patients. Stress dose of corticosteroids may be required for patients who receive chronic supplementation. Because of the powerful immunsuppressive therapy, steril conditions should be optimized for the placements of venous and epidural catheters. Drugs that may decrease hepatic blood flow should be avoided (Steadman, 2004).

5. References

Adachi T. (2003) Anesthetic principles in living-donor liver transplantation at Kyoto University Hospital: experiences of 760 cases. *J Anesth.* 2003; 17:116-24. ISSN: 0913-8668 (Print), 1438-8359 (Electronic).

Aggarwal S, Kang Y, Freeman JA, Fortunato FL, Pinsky MR. (1987) Postreperfusion syndrome: cardiovascularcollapse following hepatic reperfusion during liver transplantation.*Transplant Proc* 1987; 19:54–55. ISSN: 0041-1345.

Alessandra C, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. (2007) Noradrenalin vs Terlipressin in patients with hepatorenal syndrome: A prospective, randomized, unblinded pilot study. *J Hepatol* 2007; 47: 499-505

Aniskevich S, Shine TS, Feinglass NG, Stapelfeldt WH. (2007) Dynamic left ventricular outflow tract obstruction during liver transplantation: the role of transesophageal echocardiography *Cardiothorac Vasc Anesth.* 2007; 21:577-80. ISSN: 1053-0770 (Print) 1532-8422 (Electronic).

Arnal D, Garutti I, Perez-Pena J, Olmedilla L, Tzenkov IG. (2005) Radial to femoral arterial blood pressure differences during liver transplantation. *Anaesthesia* 2005; 60:766–771.ISSN:0003-2409

Baker J, Yost CS, Niemann CU (2005). Organ Transplantation. In: *Miller's Anesthesia* Ed. Miller RD. 6th edition. Pp. 2231-2283 Elsevier Churchill Livingstone, USA ISBN: 0-443-06618-3

Balci ST, Pirat A, Torgay A, Cinar O, Sevmis S, Arslan G. (2008) Effect of restrictive fluid management and acute normovolemic intraoperative hemodilution on transfusion requirements during living donor hepatectomy. *Transplant Proc.* 2008 ; 40:224-7. ISSN: 0041-1345.

Biais M, Nouette-Gaulain K, Cottenceau V, Vallet A, Cochard JF, Revel P, Sztark F. (2008) Cardiac outputmeasurement in patients undergoing liver transplantation: pulmonary artery catheter versus uncalibrated arterial pressure waveform analysis. *Anesth Analg* 2008; 106:1480–1486. ISSN: 0003-2999.

Biancofiore G, Bindi Ml, Romanelli AM, Boldrini A, Bisà M, Esposito M, Urbani L, Catalano G, Mosca F, Filipponi F. (2005) Fast-track in liver Transplantation: 5 years' experience. *Eur J Anesthesiol* 2005; 22: 584-90 ISSN: 0265-0215 (Print) ISSN: 1365-2346 (Electronic)

Biancofiore G, Critchley LAH, Lee A, Bindi L, Bisà M, Esposito M, Meacci L, Mozzo R, DeSimone P, Urbani L, Filipponi F. (2009) Evaluation of an uncalibrated arterial pulse contour cardiac output monitoring system in cirrhotic patients undergoing liver surgery. *Br J Anesth* 2009; 102:47–54. ISSN: 0007-0912.

Brown BR, Frink EJ. (1993)Anesthesia considerations in patients with liver disease. *Anesthesiol Rev* 1993; 20 (6):213-220. ISSN: 0093-4437

BurtenshawAJ, Isaac JL. (2006) The role of trans-oesophageal echocardiography for perioperative cardiovascular monitoring during orthotopic liver transplantation.*Liver Transpl* 2006; 12:1577–1583. ISSN: 1527-6473 (Electronic).

Chari RS, Gan TJ, Robertson KM, Bass K, Camargo CA Jr, Greig PD, Clavien PA. (1998) Venovenous bypass in adult orthotopic liver transplantation: routine or relective use? *J Am Coll Surg* 1998; 186:683-690. ISSN: 1072-7515

Chen CL, Chen YS, de Villa VH, Wang CC, Lin CL, Goto S, Wang SH, Cheng YF, Huang TL, Jawan B, Cheung HK. (2000) Minimal blood loss living donor hepatectomy. *Transplantation*. 2000 27; 69:2580-6. ISSN: 0041-1337. Electronic ISSN: 1534-0608.

Chen JP, Jawan B, Chen CL, Wang CH, Cheng KW, Wang CC, Concejero AM, Villagomeza E, Huang CJ. Comparison of postoperative morphine requirements in healthy living liver donors, patients with hepatocellular carcinoma undergoing partial hepatectomy, and liver transplant recipients.*Transplant Proc.* 2010 Apr; 42 (3):701-2. ISSN: 0041-1345

Chibber A, Dziak J, Kolano J, Norton JR, Lustik S. (2007) Anesthesia care for adult live donor hepatectomy: our experiences with 100 cases. *Liver Transpl.* 2007 ; 13:537-42. ISSN: 1527-6473 (Electronic)

Choi SJ, Gwak MS, Ko JS, Kim GS, Ahn HJ, Yang M, Hahm TS, Lee SM, Kim MH, Joh JW. (2007) The changes in coagulation profile and epidural catheter safety for living liver donors: a report on 6 years of our experience. *Liver Transpl*. 2007 ; 13:62-70. ISSN: 1527-6465 (Print) ISSN: 1527-6473 (Electronic)

Costa MG, Della Rocca G, Chiarandini P, Mattelig S, Pompei L, Barriga MS, Reynolds T, Cecconi M, Pietropaoli P. (2008) Continuous and intermittent cardiac output measurement in hyperdynamic conditions: pulmonary artery catheter vs lithium dilution technique. *Int Care Med* 2008; 34:257–263. ISSN: 1525-1489 (Electronic)

Davis CL, Gonwa TA, Wilkinson AH. (2009) Identification of patients best suited for combined liver-kidney transplantation. Part II *Liver Transpl* 2004; 10 (10 Supll 2) S 54-8 ISSN: 1527-6465 (Print) ISSN: 1527-6473 (Electronic)

Dawwas MF, Lewsey JD, Watson CJ, Gimson AE. (2005) The impact of serum potassium concentration on mortality after liver transplantation: a cohort multicenter study. *Transplantation* 2009; 88:402–410. ISSN: 0041-1337 (Print) ISSN: 1534-0608 (Electronic)

De Boer MT, Molenaar IQ, Hendriks HGD, Slooff MJ, Porte RJ. (2005) Minimizing blood loss in liver transplantation: progress through research and techniques.*Dig Surg* 2005; 22:265-75. ISSN: 0253-4886 (Print) ISSN:1421-9883 (Electronic)

Della Rocca G, Costa MG, Chiarandini P, Bertossi G, Lugano M, Pompei L, Coccia C, Sainz-Barriga M, Pietropaoli P. (2008) Arterial pulse cardiac output agreement with thermodilution in patients in hyperdynamic conditions. *J Cardiothor Vasc Anesth* 2008; 22:681–687. ISSN: 1053-0770 (Print) 1532-8422 (Electronic).

Duvoux C, Zanditenas D, Hezode C, Chauvat A, Monin JL, Roudot-Thoraval F, Mallat A, Dhumeaux D (2002). Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome; a pilot study. *Hepatology* 2002; 36 (2):374-80 ISSN: 0270-9139 (Print) ISSN:1527-3350 (Electronic)

Ellenberger C, Mentha G, Giostra E, Licker M. (2006) Cardiovascular collapse due to massive pulmonary thromboembolism during orthotopic liver transplantation.*J Clin Anesth* 2006; 18:367–371. ISSN: 0952-8180 (Print) ISSN:1873-4529 (Electronic)

Erdem N, Kanbak M, Saricaoglu F, Ocal T, Tanyel C, Ertas N, Berkkan A, Aypar U (2006). Extrahepatic metabolism and renal effects of sevoflurane in a case of liver transplantation. *Transplant Proc*. 2006 Jun; 38 (5): 1463-6 ISSN: 0041-1345

Ferraz-Neto BH, Silva ED, Afonso RC, Gregory FH, Goehler F, Meira-Filho SP, Macedo CP, Leitao RM, Parra OM, Saad WA. (1999) Early extubation in liver transolantation. *Transplant Proc*. 1999; 31: 3067-8s ISSN: 0041-1345

Findlay JY. (2002) Anesthesia for Liver Transplantation. In: *Anesthesiology Review*, Faust RJ, Cucchiara RF, Rose SH, Spackman TN, Wedel DJ, Wass CT. pp 525-526, Churchill livingstone, ISBN 0-443-06601-9, USA

Gines P, Guevara M, Perez-Villa F (2004). Management of hepatorenal syndrome: another piece of the puzzle. *Hepatology* 2004; 40 (1); 16-8 ISSN: 0270-9139 (Print) 1527-3350 (Electronic)

Gines P, Guevara M. (2008) Hyponatremia in Cirrhosis: Pathogenesis, clinical significance, and management. *Hepatology* 2008; 48: 1002-1010 ISSN: 0270-9139 (Print) 1527-3350 (Electronic)

Glanemann M, Busch T, Neuhaus P, Kaisers U (2007). Fast-tracking in liver transplantation. Immediate postoperative tracheal extubation: Feasibility and clinical impact. *Swiss Med Wkly* 2007; 137: 187-191 ISSN:1424-7860

Glauser FL. (1990) Systemic hemodynamic and cardiac function changes in patients undergoing orthotopic liver transplantation. *Chest*. 1990; 98:1210-5. ISSN: 0012-3692 (Print) ISSN: 1931-3543 (Electronic).

Gologorsky E, De Wolf AM, Scott V, Aggarwal S, Dishart M, Kang Y. (2001) Intracardiac thrombus formation and pulmonary thromboembolism immediately after graft reperfusion in 7 patients undergoing liver transplantation. *Liver Transpl* 2001; 7:783-9. ISSN: 1527-6473 (Electronic).

Grande L, Rimola A, Cugat E, Alvarez L, Garcia-Valdecasas JC, Taura P, Beltrán J, Fuster J, Lacy AM, González FJ, Tabet J, Cifuentes A, Rull R, Ramos C, Visa J, Rodés J. (1996) Effect of venovenous bypass on perioperative renal function in liver transplantation: results of a randomised, controlled trial. *Hepatology* 1996; 23:1418-142. ISSN: 0270-9139 (Print) 1527-3350 (Electronic)

Gurusamy KS, Pamecha V, Davidson BR (2011). Piggy-back graft for liver transplantation. *Cochrane Database Syst Rev* 2011 Jan 19; (1):CD008258. ISSN: 1469-493X (Electronic).

Gwak MS, Kim JA, Kim GS, Choi SJ, Ahn H, Lee JJ, Lee S, Kim M. (2007) Incidence of severe ventricular arrhythmias during pulmonary artery catheterization in liver allograft recipients. *Liver Transpl* 2007; 13:1451–1454. ISSN: 1527-6473 (Electronic).

Hannaman MJ, Hevesi ZG. (2011) Anesthesia Care for Liver Transplantation *Transplantation Reviews* 2011; 25: 36-43 ISSN: 0955-470X

Henriksen JH, Bendtsen F, Sorensen TIA, Stadeager C, Ring-Larsen H (1989). Reduced central blood volume in cirrhosis. *Gastroenterology* 1989; 97:1506–1513. ISSN: 0016-5085

Homvises B, Sirivatanauksorn Y, Limsrichamrern S, Pongraweewan O, Sujirattanawimol K, Raksakietisak M (2008) The minimal flush volume for washout of preservation fluid in liver transplantation. *Transplant Proc* 2008; 40:2123–2126 ISSN: 0041-1345

Hwang GS, McCluskey SA. (2010) Anesthesia and outcome after partial hepatectomy for adult-to-adult donor transplantation. *Curr Opin Organ Transplant* 2010; 15; 377-382 ISSN: 1087-2418 (Print) ISSN: 1531-7013 (Electronic)

Jullien T, Valtier B, Hongnat Jm Dubourg O, Bourdarias JP, Jardin F (1995). Incidence of Tricuspit regurgitation and venacaval backward flow in mechanical ventilated patients. A color Doppler and contrast echocardiographic study. *Chest* 1995; 9: 301-6 ISSN: 0012-3692 (Print); ISSN: 1931-3543 (Electronic).

Kim SH, Cho SY, Lee KW, Park SJ, Han SS (2009). Upper midline incision for living donor right hepatectomy. *Liver Transpl* 2009; 15: 193-8 ISSN: 1527-6473 (Electronic).

Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, Edwards E, Therneau TM (2008). Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med* 2008; 359:1018–1026. ISSN: 0028-4793

Krowka MJ, Plevak DJ, Findlay JY, Rosen CB, Wiesner RH, Krom RA (2000). Pulmonary hemodynamics and perioperative cardiopulmonary-related mortality in patients with portopulmonary hypertension undergoing liver transplantation. *Liver Transpl*. 2000; 6:443-50. ISSN: 1527-6473 (Electronic).

Lazar HL, McDonnell M, Chipkin SR, Furnary AP, Engelman RM, Sadhu AR, Bridges CR, Haan CK, Svedjeholm R, Taegtmeyer H, Shemin RJ; Society of Thoracic Surgeons Blood Glucose Guideline Task Force. (2009) The Society of Thoracic Surgeons practice guideline series: blood glucose management during adult cardiac surgery. *Ann Thorac Surg* 2009; 87:663–669. ISSN: 0003-4975 (Print) ISSN: 1552-6259 (Electronic)

Lee EM, Kang JK, Yun SC, Kim KH, Kim SJ, Hwang KS, Lee SG (2009). Risk factors for central pontine and extrapontine myelinolysis following orthotopic liver transplantation. *Eur Neurol* 2009; 62:362–368. ISSN: 0014-3022 (Print) ISSN: 1421-9913 (Electronic)

Liu H, Gaskari SA, Lee SS (2006). Cardiac and vascular changes in cirrhosis:pathogenic mechanisms. World J Gastroenterol 2006; 12:837–842 ISSN 1007-9327

Lopez PM, Martin P. Update on liver transplantation: indications, organ allocation, and long-term care. Mt Sinai J Med. 2006 Dec; 73 (8):1056-66. ISSN: 0027-2507 (Print)

Lukanovic NP (2008). Hot topics in liver anesthesia. *Transplant Proc*. 2008; 40 (4):1187-9. ISSN: 0041-1345

Lukin C, Hein HAT, Swygert TH, Gunning TC, Valek TR, Donica SK, Nelson RB 3rd, Ramsay MA. (1995) Duration of vecuronium induced neuromuscular blockade as a predictor of liver allograft dysfunction. *Anesthesia Analgesia* 1995; 80 (3):526-533. ISSN: 0003-2999

Lutz JT, Valentín-Gamazo C, Görlinger K, Malagó M, Peters J (2003). Blood-transfusion requirements and blood salvage in donors undergoing right hepatectomy for living related liver transplantation. *Anesth Analg*. 2003; 96 (2):351-5. ISSN: 0003-2999.

Mandell MS, Lezotte D, Kam I, Zamudio S (2002) Reduced use of intensive care after liver transplantation: Patient attributes that determine early transfer to surgical wards. *Liver Transpl* 2002; 8 682-7 ISSN: 1527-6465 (Print) ISSN: 1527-6473 (Electronic).

Mandell MS, Stoner TJ, Barnett R, Shaked A, Bellamy M, Biancofiore G, Niemann C, Walia A, Vater Y, Tran ZV, Kam I. A multicenter evaluation of safety of early extubation in liver transplant recipients. *Liver Transpl*. 2007 Nov; 13 (11):1557-63 ISSN: 1527-6465 (Print). ISSN: 1527-6473 (Electronic)

Manji M, Isaac JL, Bion J. (1998) Survival from massive intraoperative pulmonary thromboembolism during orthotopic liver transplantation. *Br J Anesth* 1998; 80:685–687. ISSN: 0007-0912 (Print) ISSN: 1471-6771 (Electronic)

Marcel RJ, Ramsay MA, Hein HA, Nguyen AT, Ramsay KJ, Suit CT, Miller RD (1997). Duration of rocuronium-induced neuromuscular block during liver transplantation: a predictor of primary allograft function. *Anesth Analg*. 1997 Apr; 84 (4):870-4. ISSN 0003-2999

Marvin M, Morton V. (2009) Glycemic control and organ transplantation. *J Diabetes Sci Technol* 2009; 3:1365–1372. ISSN: 1932-2968 (Electronic)

Massicotte L, Lenis S, Thibeault L, Sassine MP, Seal RF, Roy A. (2006) Effect of low central venous pressure and phlebotomy on blood product transfusion requirements during liver transplantation. *Liver Transpl* 2006; 12:117-23. ISSN: 1527-6473 (Electronic).

Merritt WT (2004). Living donor surgery: overview of surgical and anesthesia issues. *Anesthesiol Clin North America*. 2004; 22:633-50. ISSN: 0889-8537.

Moghissi E, Korytkowski M, DiNardo M, Einhorn D, Hellman R, Hirsch IB, Inzucchi SE, Ismail-Beigi F, Kirkman MS, Umpierrez GE. (2009) American Association of Clinical Endocrinologists and American Diabetes Association Consensus statement on inpatient glycemic control. *Endocrine Pract* 2009; 15:353–369. ISSN: 1530-891X

Molenaar IQ, Warnaar N, Groen H, Tenvergert EM, Slooff MJ, Porte RJ. (2007) Efficacy and safety of antifibrinolytic drugs in liver transplantation: a systematic review. *Am J Transplant* 2007; 7:185–194. ISSN: 1600-6135 (Print) ISSN: 1600-6143 (Electronic)

Moller S, Henriksen JH. (2008) Cardiovascular complications in cirrhosis. *Gut* 2008; 57:268–278. ISSN: 0017-5749 (Print). ISSN:1468-3288 (Electronic)

Moreno C, Sabate A, Figueras J, Camprubí I, Dalmau A, Fabregat J, Koo M, Ramos E, Lladó L, Rafecas A (2006). Hemodynamic profile and tissular oxygenation in orthotopic liver transplantation: influence of hepatic artery or portal vein revascularization of the graft. *Liver Transpl* 2006; 12:1607–1614. ISSN: 1527-6465 (Print) ISSN: 1527-6473 (Electronic)

Moretti EW, Robertson KM, Tuttle-Newhall JE, Clavien PA, Gan TJ. Orthotopic liver transplant patients require less postoperative morphine than do patients undergoing hepatic resection. *J Clin Anesth.* 2002; 14 (2):416-20 ISSN: 0952-8180

Murray DJ, Pennell BJ, Weinstein SL, Olson JD (1995). Packed red cells in acute blood loss: dilutional coagulopathy as a cause of surgical bleeding.*Anesth Analg* 1995; 80:336-42. ISSN 0003-2999.

Nakasuji M, Bookallil M. (2009) Pathophysiological mechanisms of postrevascularization hyperkalemia in orthotopic liver transplantation. *Anesth Analg* 2000; 91:1351–1355. ISSN 0003-2999.

Nasraway SA, Klein RD, Spanier TB (1995). Hemodynamic correlates of outcome in patients undergoing orthotopic liver transplantation. Evidence for early postoperative myocardial depression. *Chest* 1995; 107:218–224. ISSN: 0012-3692 (Print); ISSN: 1931-3543 (Electronic)

Niemann CU, Eilers H. (2010) Abdominal organ transplantation. *Minerva Anesthesiol* 2010; 76: 266-275 ISSN: 0375-9393 (Print) ISSN:1827-1596 (Electronic)

O'Connor CJ, Roozeboom D, Brown R, Tuman KJ. (2000) Pulmonary thromboembolism during liver transplantation: possible association with antifibrinolytic drugs and novel treatment options. *Anesth Analg* 2000; 91:296–299. ISSN: 0003-2999 (Print); ISSN: 1526-7598 (Electronic)

Ozier Y, Klinck JR. (2008) Anesthetic management of hepatic transplantation. *Curr Opin Anesthesiol* 2008 21: 391-400 ISSN: 0952-7907 (Print). ISSN: 1473-6500 (Electronic)

Park GR, Evans TN, Hutchins J, Borissov B, Gunning KE, Klinck JR (2000). Reducing the demand for admission to intensive care after major abdominal surgery by a change in anaesthetic practice and the use of remifentanil. *Eur J Anaesthesiol* 2000; 17: 111-9 ISSN: 0265-0215 (Print) ISSN: 1365-2346 (Electronic)

Peterfreund RA, Allain RM (2003). *Liver Transplantation*. In: Yao&Artusio's Anesthesiology problem-oriented patient management. 5th ed., Yao FSF. pp 485-509, Lippincott Williams & Wilkins, ISBN 0-7817-3641-2, USA

Pickett JA, Wheeldon D, Oduro A (1994). Multi-organ transplantations: donor management. *Curr Op Anaesthesiol* 1994; 7:80-83. ISSN: 0952-7907

Puhl G, Schaser KD, Pust D, Köhler K, Vollmar B, Menger MD, Neuhaus P, Settmacher U. Initial hepatic microcirculation correlates with early graft function in human liver transplantation, Liver Transpl 2005, 11:555–563. ISSN (electronic): 1527-6473. ISSN: 1527-6465 (Print).

Pvalizza EG, Raber MK, Ozaki CF, Lappin JA, Wood RP. (2001) Heparin effect after hepatic artery anastomosis during liver transplantation. *Anesth Analg* 2001; 93:798-804.ISSN 0003-2999

Rabie M, Negmi H, Hammad Y, Al Oufi H, Khalaf H (2006). Living donor hepatectomy (LDH)--comparative study between two different anesthetic techniques. *Middle East J Anesthesiol*. 2006 ; 18:743-56. ISSN: 0544-0440.

Ramsay MA (2000). Perioperative mortality in patients with portopulmonary hypertension undergoing liver transplantation. *Liver Transpl*. 2000 ; 6:451-2. ISSN: 1527-6465 (Print) ISSN: 1527-6473 (Electronic)

Ramsay MAE, Swygert TG (1992). Anaesthesia for hepatic trauma, hepatic resection and liver transplantation. *Bailliere's Clinical Anaesthesiology* 1992; 6 (4):863-894. ISSN: 0950-3501

Ramsay MAE, Randall HB, Burton E. (2004) Intravascular thrombosis and thromboembolism during liver transplantation: antifibrinolytic therapy implicated. *Liver Transpl* 2004; 10:310–314. ISSN:1527-6465 (Print). ISSN: 1527-6473 (Electronic).

Reddy K, Mallett S, Peachey T. (2005) Venovenous bypass in orthotopic liver transplantation: Time for a Rethink? *Liver Transplantation* 2005; 11 (7):741-749. ISSN (electronic): 1527-6473.

Rossaint R, Slama K, Jaeger M, Konrad M, Pappert D, Bechstein W, Blumhardt G, Neuhaus P, Falke KJ. (1990) Fluid restriction and early extubation for successful liver transplantation.*Transplant Proc*.1990; 22:1533–4. ISSN: 0041-1345.

Salizzoni M, Cerruti E, Romagnoli R, Lupo F, Franchello A, Zamboni F, Gennari F, Strignano P, Ricchiuti A, Brunati A, Schellino MM, Ottobrelli A, Marzano A, Lavezzo B, David E, Rizzetto M. (2005) The first one thousand liver transplants in Turin: a single-center experience in Italy. *Transpl Int* 2005; 18: 1328-35 ISSN: 0934-0874 (Print). ISSN: 1432-2277 (Electronic)

Scott VL, De Wolf AM, Kang Y, Altura BT, Virji MA, Cook DR, Altura BM. (1996) Ionized hypomagnesemia in patients undergoing orthotopic livertransplantation: a complication of citrate intoxication. *Liver Transpl Surg* 1996; 2 (5):343-7. ISSN:1074-3022 (Print) ; ISSN:1074-3022 (Electronic)

Schumann R (2003). Intraoperative resource utilization in anesthesia for liver transplantation in the United States: a survey. *Anesth Analg* 2003; 97:21-8. ISSN: 0003-2999 (Print); ISSN: 1526-7598 (Electronic)

Shaw BW Jr, Martin DJ, Marquez JM, Kang YG, Bugbee AC Jr, Iwatsuki S, Griffith BP, Hardesty RL, Bahnson HT, Starzl TE. (1984) Venous bypass in clinical liver transplantation. *Ann Surg* 1984; 200:524-534. ISSN: 0003-4932 (Print) ISSN: 1528-1140 (Electronic)

Shippy CR, Apple PL, Shoemaker WC. (1984) Reliability of clinical monitoring to assess blood volume in critically ill patients. *Crit Care Med* 1984; 12:107–112. ISSN: 0090-3493 (Print) ISSN: 1530-0293 (Electronic).

Sopher M, Braunfeld M, Shackleton C, Busuttil RW, Sangwan S, Csete M. (1997) Fatal embolism during liver transplantation. Anesthesiology 1997; 87:429–432. ISSN: 0003-3022 (Print) ISSN: 1528-1175 (Electronic)

Stamenkovic DM, Jankovic ZB, Toogood GJ, Lodge JP, Bellamy MC (2011). Epidural analgesia and liver resection: postoperative coagulation disorders and epidural catheter removal. *Minerva Anestesiol*. 2011; 77:671-9. ISSN: 0375-9393 (Print) ISSN: 1827-1596 (Electronic)

Steadman RH. (2004) Anesthesia for liver transplant surgery. *Anesthesiol Clin N Am* 2004, 22: 687-711 ISSN: 0889-8537

Takizawa D, Sato E, Hiraoka H, Tomioka A, Yamamoto K, Horiuchi R, Goto F (2005). Changes in apparent systemic clearance of propofol during transplantation of living related donor liver. *Br J Anaesth*. 2005 ; 95:643-7. ISSN: 0007-0912.

Topal A, Çelik JB. Anesthetic management for liver transplantation. Türkiye Klinikleri J Anest Reanim-Special Topics. 2009 2 (2): 25-38. ISSN: 1308-1195

Vater Y, Levy A, Martay K, Hunter C, Weinbroum AA (2004). Adjuvant drugs for end-phase liver failure and transplantation. *Med Sci Monit* 2004; 10: RA 77-88 ISSN:1234-1010 (Print) ISSN:1643-3750 (Electronic).

Veroli P, el Hage C, Ecoffey C. (1992) Does adult liver transplantation without venovenous bypass result in renal failure? *Anesth Analg* 1992; 75:489-494. ISSN 0003-2999.

Wadei HM, Mai ML, Ahsan N, Gonwa TA (2006). Hepatorenal syndrome Pathophysiology and management. *Clin J Am Soc Nephrol* 2006; 1; 1066-79 ISSN: 1555-9041 (Print) 1555-905X (Electronic)

Wong F, Pantea L, Sniderman K (2004). Midodrine, octreotide, albumin, and TIPS in selected patients with cirrhosis and type I hepatorenal syndrome. *Hepatology* 2004; 40 (1): 55-64 ISSN: 0270-9139 (Print) ISSN: 1527-3350 (Electronic)

Wszolek ZK, McComb RD, Pfeiffer RF, Steg RE, Wood RP, Shaw BW Jr, Markin RS (1989). Pontine and extrapontine myelinolysis following livertransplantation. Relationship

to serum sodium. *Transplantation* 1989; 48 (6):1006-12. ISSN: 0041-1337 (Print) ISSN: 1534-0608 (Electronic)

Xia VW, Du B, Tran A, Liu L, Hu KQ, Hiatt JR, Busuttil RW, Steadman RH. (2006) Intraoperative hypokalemia in pediatric liver transplantation: incidence and risk factors. *Anesth Analg* 2006; 103:587–593. ISSN: 0003-2999 (Print); ISSN: 1526-7598 (Electronic)

Xia VW, Ghobrial RM, Du B, Chen T, Hu KQ, Hiatt JR, Busuttil RW, Steadman RH. (2007) Predictors of hyperkalemia in the prereperfusion, early postreperfusion, and late postreperfusion periods during adult liver transplantation. *Anesth Analg* 2007; 105:780–785. ISSN: 0003-2999 (Print); ISSN: 1526-7598 (Electronic)

Zhang ZW, Kang Y, Deng LJ, Luo CX, Zhou Y, Xue XS, Wang D, Yin WH. (2009) Therapy of central pontine myelinolysis following living donor liver transplantation: report of three cases. *World J Gastroenterol* 2009; 15:3960–3963. ISSN: 1007-9327

17

Cardiovascular Monitoring and Substitution of the Blood Volume During Liver Transplantation

Peter Nissen, Hans J. Frederiksen and Niels H. Secher
Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark

1. Introduction

Orthotopic liver transplantation (OLT) became a treatment modality in Denver (Starzl *et al.*, 1963) and Cambridge, England in 1968 (Calne, 2008) although exposing critically ill patients to extensive surgery was a challenge and in the 1970s, the one year survival rate remained in the vicinity of only 25% (Starzl *et al.*, 1981). One problem was to manage the often impressive blood loss and rapid infusion devices were developed to provide large amounts of blood products at body temperature (Stammers *et al.*, 2005). During OLT the blood loss has, fortunately, declined and in the 1980s, the average number of blood units administered was reduced to 20 and in some centres, ~ 80% of patients are now without a need for administration of blood (Massicotte *et al.*, 2004). Yet, there remains differences among centres and for a number of patients, OLT is associated with a significant blood loss (Massicotte *et al.*, 2004). Furthermore, haemodynamic challenges are inevitable during the operation. Corresponding to the metabolic activity of the liver, the hepatectomy is likely to reduce cardiac output (CO) and in cases for which caval and portal clamping are used, blood accumulates in the splanchnic region and in the lower part of the body which together leads to a decline in CO by 40%-50% (Ozier & Klinck, 2008). Conversely, CO often doubles during reperfusion of the grafted liver as peripheral vasodilatation is provoked by the release of blood from the splanchnic region mixed with the chilled outflow from the liver, potentially with a high potassium concentration (Ozier & Klinck, 2008). Also it may be that the heart is unable to respond with an adequate increase in CO to reperfusion of the grafted liver, or that there is a reduction in CO despite an, apparently, adequate central blood volume (CBV), accepting that reperfusion of the liver and re-establishing splanchnic blood flow may release some cardio-inhibiting factor (Jordan *et al.*, 1999). Given redistribution of the blood volume during OLT, monitoring of the circulation is, ideally, directed to secure CBV rather than the total blood volume.

As for other types of surgery, there is no universally accepted strategy for haemodynamic monitoring during OLT, save a mandatory arterial line and recording of heart rate (HR) by ECG (Ozier & Klinck, 2008). Yet, mean arterial pressure (MAP) and HR are inadequate for monitoring CBV (Bundgaard-Nielsen *et al.*, 2007a). For example, there may be no significant deviations in these variables until a hypovolaemic shock is provoked (Murrell *et al.*, 2009).

Furthermore during surgery, HR and especially MAP are affected by the anaesthetic agents and by the surgical stress (Ejlersen *et al.*, 1995a). Despite these limitations in the use of HR and MAP to detect deviations in CBV, the capability to balance CBV is of importance for tissue perfusion and oxygenation and notably for oxygenation of the brain (S_cO_2) (Nissen *et al.*, 2009a), indicating that advanced cardiovascular monitoring is required to secure the well-being of the patient (Yao *et al.*, 2004;Bundgaard-Nielsen *et al.*, 2007a;Murkin *et al.*, 2007)

In order to maintain CBV during surgery, it is important that normovolaemia is defined. For supine humans the heart operates on the upper flat part of the Frank-Starling curve (Harms *et al.*, 2003) and to establish and to maintain a maximal resting stroke volume for the heart (or CO) secures that the patient remains normovolaemic during the operation and that fluid administration strategy reduces postoperative complications to an extent that affects the hospital stay (Bundgaard-Nielsen *et al.*, 2007a). Such goal directed fluid therapy was introduced by Shoemaker et al. (Shoemaker, 1972;Shoemaker *et al.*, 1988) in regard to CO but without taking the individual and partly genetically determined differences in CO (Snyder *et al.*, 2006) into account. Accordingly, this chapter focuses on how normovolaemia can be established and maintained during OLT despite the difficulties confronting the definition of normovolaemia by the spontaneous changes in stroke volume, CO and (mixed) venous oxygen saturation (S_vO_2) during the different phases of the operation. A second goal of this chapter is to introduce devices that can be applied for intraoperative monitoring of CBV and S_cO_2. Focus is on the importance of maintaining a normal CBV to secure cerebral blood flow (CBF) and S_cO_2 since these variables are taken to express the integrity of the cardiovascular system and their defence, at least potentially, prevents postoperative complications and cognitive dysfunction (Murkin *et al.*, 2007). In addition, the volume administration strategy applied during the operation is addressed.

2. Normovolaemia

By definition it seems difficult to defend that any patient should be provided with a fluid overload or be maintained hypovolaemic and yet, even hypovolaemic shock is sometimes treated with sympatomimetic drugs (De Backer D. *et al.*, 2010). For balancing volume administration it is of interest that normovolaemia can not only be defined but also defended during surgery. For healthy supine humans stroke volume, CO, and S_vO_2 do not respond to expansion of CBV and, therefore for supine humans, a volume administration strategy that secures that the heart operates on the upper ceiling of the Frank-Starling curve maintains the patient normovolaemic (Harms *et al.*, 2003;Jans *et al.*, 2008;Bundgaard-Nielsen *et al.*, 2009c) (Fig. 1).

For individualized goal-directed fluid therapy, volume is administered until a flow related parameter such as stroke volume, CO or S_vO_2, reaches a maximal value (Jenstrup *et al.*, 1995), i.e. volume is administrated until cardiac function does not depend on preload to the heart. This volume administration strategy is of interest not only because it remains elusive what volume rate a fixed volume administration strategy should aim at (Bundgaard-Nielsen *et al.*, 2009b), but also because an individualized fluid administration strategy, in contrast to a fixed volume strategy, consistently improves outcome for surgical patients (Bundgaard-Nielsen *et al.*, 2007a; Lopes *et al.*, 2007; Donati *et al.*, 2007; Abbas & Hill, 2008; Mayer *et al.*, 2010).

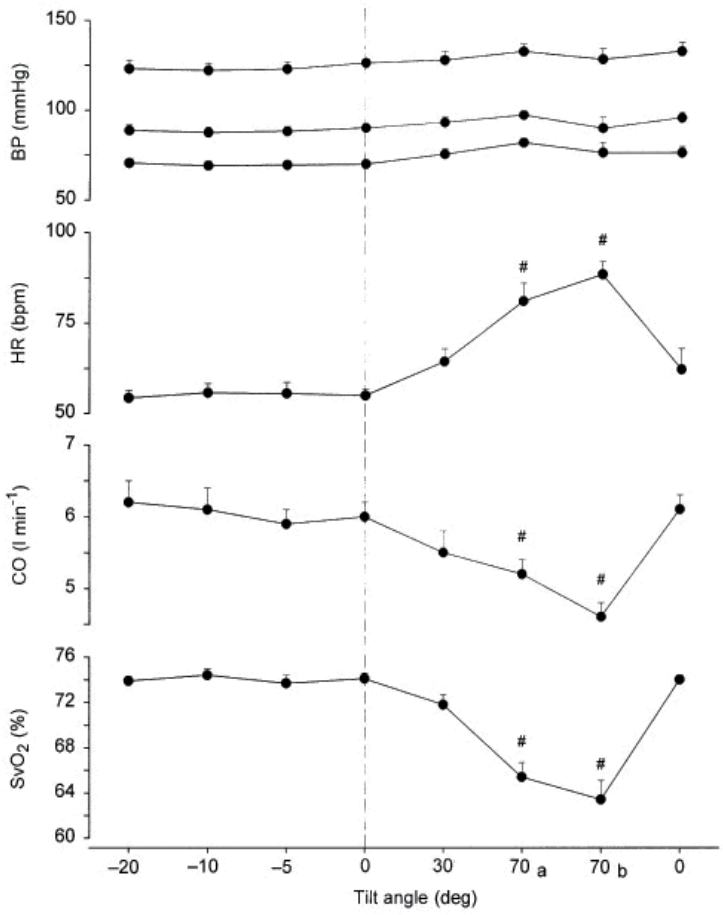


Fig. 1. Relationship between systemic haemodynamic variables, mixed venous oxygen saturation and tilt angle. BP, systolic, mean and diastolic blood pressure; HR, heart rate; CO, cardiac output; S_vO_2 mixed venous oxygen saturation. Variables are mean ± S.E.M. # $P < 0.05$ vs. 0 deg. Dashed line represents the supine position; 70 a, 70 deg head-up tilt for 10 min; 70 b, last minute of 70 deg head-up tilt for nine subjects (From Harms et al., 2003 with permission)

Based on administration of a crystalloid or a colloid, an inherent difficulty for individualized goal directed fluid therapy is, however, that a reduction in haematocrit is associated with an increase in CO, i.e. normovolaemic haemodilution increases CO (Krantz *et al.*, 2005). In other words, it is not CO but S_vO_2 that is the regulated variable since the red cells create their own flow regulation through the release of ATP and NO when oxygen is released from oxyhaemoglobin (Gonzalez-Alonso *et al.*, 2006). To direct fluid administration on the basis of establishing maximal values for stroke volume or CO requires that there is added a rule to limit the fluid administered. A common algorithm implies that a 10%, or larger increase in stroke volume justifies further administration of 200–250 ml of colloid, thereby minimizing the risk of creating a fluid overload (Bundgaard-Nielsen *et al.*, 2007a). In contrast, during isovolaemic haemodilution, S_vO_2 remains stable until the haemoglobin level

is reduced by approximately 50% (Krantz *et al.*, 2005) and volume administration based on the recording of S_cO_2 is therefore widely independent of the type of fluid used.

When CBV is normalized by fluid to establish a maximal S_vO_2, the administration of 100 ml fluid results a ~1% increase in S_vO_2 for the adult patient (~ 70 kg) (Ejlersen *et al.*, 1995a) and that relationship applies also to children when the volume is adjusted according to body weight. For supine humans, S_vO_2 is on an average 75% (Harms *et al.*, 2003) but for patients undergoing OLT, S_vO_2 is typically ~ 85% (Ejlersen *et al.*, 1995b) reflecting that for these patients CO is larger (7-9.5 l/min) (Table 1) than for a reference population (6.5 l/min).

	Dissection phase	**Anhepatic phase**	**Reperfusion**	**End of operation**
HR (bpm)	95	90	87	97
CI (l m^{-1} min^{-1})	4.4	3.3	5.0	4.5
S_vO_2 (%)	85	81	86	82
TA (Ohm)	29	28	29	26
MAP (mmHg)	88	89	85	87
CVP (mmHg)	10	10	14	11
PAMP (mmHg)	18	17	26	21
SVRI (mmHg m^2 min l^{-1})	142	195	105	120
PVRI (mmHg m^2 min l^{-1})	11	14	9	12
Temperature	36	35	35	35

HR, hear rate; CI, cardiac index; S_vO_2, mixed venous saturation; TA, thoracic electric admittance; MAP, mean arterial blood pressure; CVP, central venous pressure; PAMP, pulmonal arterial mean pressure; SVRI, systemic vascular resistance index; PVRI, pulmonal vascular resistance index (Modified from Skak et al., 1997).

Table 1. Cardiovacular variables during OLT

Reperfusion of the grafted liver is associated with peripheral vasodilatation and although CO is likely to increase (Table 1), situations associated with peripheral vasodilatation, as during heating (Wilson *et al.*, 2009), are likely to reduce CBV. The importance of establishing normovolaemia from the induction of anaesthesia is illustrated by an increase in S_cO_2 as determined by near infrared spectroscopy (NIRS) and similarly determined muscle oxygenation (S_mO_2) since both these indices of tissue oxygenation increase in parallel with CO as the heart becomes filled with blood and decrease with the filling of the heart during a bleeding episode (Fig. 2).

Conversely, to maintain the commonly accepted 70% value for S_vO_2 (Rivers, 2006) is likely to represent a 1.5 l volume deficit for the patient undergoing OLT, considering the 1% reduction in S_vO_2 to 100 ml blood volume relationship during hypovolaemia (Ejlersen *et al.*, 1995a). A 1.5 l volume deficit is so large that it compromises MAP and S_cO_2 (Secher *et al.*, 1992) since a ~30 % reduction of the blood volume and, hence CBV elicits a Bezold-Jarisch-like reflex including a critical reduction in CBF (van Lieshout *et al.*, 2003;Secher & van

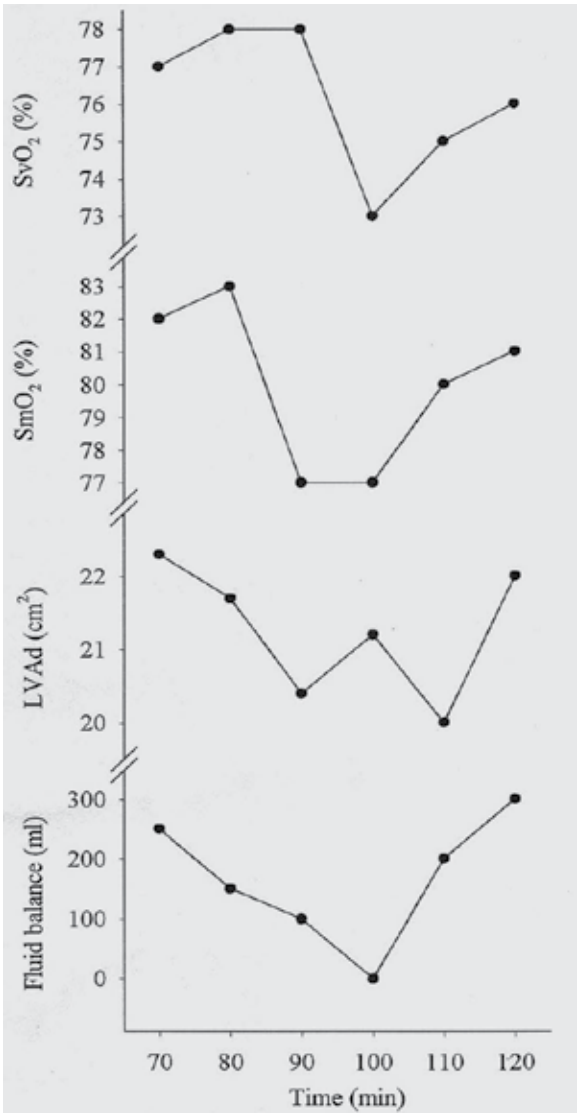


Fig. 2. Venous (S_vO_2) and muscle oxygen saturation (S_mO_2) plotted together with filling of the left ventricle (LVA_d), and volume balance in a patient exposed to an episode of intraoperative bleeding and following volume expansion (C. Tollund unpublished)

Lieshout, 2009;Madsen & Secher, 1999). The Bezold-Jarisch-like reflex also provokes a marked (30-fold) increase in plasma vasopressin (Sander-Jensen *et al.*, 1986) with long-lasting effect on urine production and could explain a potential difficulty in maintaining a reasonable urine production after surgery. Typically, the patients are in need of 0.5 l of volume before OLT (Ejlersen *et al.*, 1995b), a value that corresponds to that found also for other groups of patients before surgery (Jenstrup *et al.*, 1995;Bundgaard-Nielsen *et al.*, 2007b;Bundgaard-Nielsen *et al.*, 2009a). If it is felt desirable to maintain urine production, it is important that such an initial volume deficit is corrected and, eventually, a larger

fluid load may be required than that which establishes that the patient has been provided with a normal blood volume. For additional volume threatment of patients, the administration of lactated Ringer solution is preferable to the administration of saline (Waters et al., 2001).

3. Cardiac output

With a definition of normovolaemia based on the ability of the heart to establish an adequate flow, CO is of interest. As mentioned, patients undergoing OLT present a large CO while MAP may be low (el-Masry *et al.*, 2009; Ejlersen *et al.*, 1997). Yet MAP often normalises when the blood volume, and hence CBV, is expanded to an extent that it does not limit CO, i.e. the patients are provided with the CBV that healthy subjects are provided with when supine (Harms *et al.*, 2003). However, significant ascites may affect venous return to the heart by pressure on the inferior caval vein that can be relieved by tilting the patient to the left - as known from women before birth - but that pressure is eliminated as the abdominal cavity is opened at start of surgery. As indicated, however, OLT is associated with significant changes in the cardiovascular system from the dissection phase to the anhepatic phase and following reperfusion of the donor liver and CO normalises only slowly by the end of the operation (Table 1).

To attenuate a reduction in venous return to the heart from the clamped inferior caval vein during the hepatectomy and insertion of the donated liver and thereby stabilize CO, an extra-corporeal veno-venous bypass shunt (VVBP) can secure portal and femoral venous drainage to one or two veins on the arm or to a central venous access (Ozier & Klinck, 2008). Veins on the arm are preferred to central veins to avoid that blood accumulates in the mediastinum in the case the catheter(s) has perforated the vein(s). Before a central vein receives blood from the shunt, it needs to be secured that blood can be aspirated from the catheter. The VVBP has a flow of 1.5-3 l/min, but for children and patients with a portal hypertension, venous return to the heart may be maintained by spinal and abdominal veins and veins along the oesophagus and there may, accordingly, be no need for a VVBP in these patients accepting substantial accumulation of blood in the splanchnic region. Yet surgical bleeding may be reduced if blood does not accumulate in distended abdominal veins.

At any rate, blood accumulates in the splanchnic region while the portal vein is clamped for establishing the VVBP and when it is seponated the accumulated amount of blood helps to fill the donor liver. Alternatively, the "piggyback" technique for which the inferior caval vein is side-clamped can reduce the restrain on venous return to the heart during the hepatectomy and surgery on the vessels to the donor liver. Notably, stability of the circulation is secured if reperfusion of the liver is graded by declamping the caval vein above the liver followed by declamping the vein below the liver and thereafter establishing its arterial flow.

3.1 Monitoring of cardiac output

For monitoring of CO several methods are applicable and not all will be addressed here. Ideally the chosen method should be reliable, continuous, and easy to set-up and to use and

at the same time possess the capability for a fast response time. Of these priorities, the accuracy of the absolute value is of least relevance due to the marked inter-individual variations in CO among the patients, e.g. 2.5 to 17 l min^{-1} (Nissen *et al.*, 2009b) and the often 2-fold change in CO during the operation, but the method should be able, at all times, to report the changes in CO faithfully and most importantly so during reperfusion of the grafted liver.

A pulmonary artery catheter (PAC) determines CO by thermodilution based on the Henriques-Stewart-Hamilton equation (Pinsky, 2007) and is for clinical use regarded as the golden standard, but it then requires an average of three or probably four determinations (Nilsson et al., 2004) based on bolus injection of (10 ml) cooled saline. Furthermore, it remains a problem that the baseline temperature is often too unstable to make a thermodilution determination of CO possible within the first minutes after reperfusion of the grafted liver. That problem is exaggerated with the version of PAC that uses heating filaments for "continuous" CO determination because the appreciated change in temperature is small and it may take 15 –20 min, or more, after reperfusion of the grafted liver before such a CO can be determined (De Wolf, 2006; Bao & Wu, 2008).

The advantage of transoesophageal echocardiography (TEE) is that it provides a real-time image of the heart and thereby on-line information not only about filling of the heart but also about the structure and contractility of the myocardium (Della *et al.*, 2009) of relevance especially during reperfusion of the grafted liver in case CO does not increase. Thus, TEE allows for distinction between a need for administration of volume versus sympatomimetic drugs. Unfortunately, however, mainly cardiac anaesthesiologists are familiar with the use of TEE and it remains a concern that TEE requires high costs and a need for training (Della *et al.*, 2009). It should also be mentioned that although individualized goal directed fluid administration aims at filling the heart with blood (Figs. 1 and 2), a detailed TEE evaluation of whether that is the case requires off-line evaluation.

Studies comparing CO determined by transoesophageal echo-Doppler (TED) against PAC show conflicting results (Shimamoto et al., 1992; Boucaud et al., 2008; Laupland & Bands, 2002; Colbert et al., 1998) although there seems to be agreement to that changes in CO are reflected by TED. A disadvantage with TED is, however, frequent dislocation of the ultrasound probe (Lefrant et al., 1998) of relevance for the in general long-lasting OLT and TED is counter-indicated in patients with oesophageal disorders of relevance for many of the OLT patients and once a Doppler probe is in place it hinders TEE (de Waal *et al.*, 2009).

For continuous recording of CO, several methods appreciate the arterial waveform. The PiCCO and LiDCO generate a continuous CO by analysis of the arterial pulse pressure (PP). For both methods, an independent technique is used to calibrate the continuous CO analysis since the arterial pulse-pressure analysis does not account for variables such as changing compliance of the vascular bed. Recalibration of CO is recommended after changes in patient position, therapy, or condition (de Waal *et al.*, 2009) of relevance for OLT encompassing marked changes in the vasculature through the different phases of the operation.

In the case of PiCCO, transpulmonary thermodilution is used for calibration. As with the PAC, PiCCO appreciates transpulmonary thermodilution according to the Henriques-Stewart-Hamilton principle but considers that a determination of a change in temperature from a central venous line to an arterial line (e.g. femoral or axillary) is less invasive than a determination based on a PAC catheter. The CO derived from this cold-saline

thermodilution is used to calibrate the arterial pulse-pressure contour that then provides the continuous CO. The PiCCO algorithm appreciates the blood pressure waveform morphology (i.e. mathematical analysis of the pulse-pressure waveform) and calculates a continuous CO as described by Wesseling et al (Wesseling *et al.*, 1993). Transpulmonary thermodilution includes both the right and left side of the heart as well as the pulmonary circulation and that allows for further analysis of the thermodilution curve with measures of the cardiac filling volume, the intrathoracic blood volume, and the extravascular lung water, the latter addressing wheather pulmonary oedema is developed (Oren-Grinberg, 2010; Costa *et al.*, 2007). Accepting that transpulmonary thermodilution may be a less invasive procedure for determination of CO than one based on PAC, it is also less accurate and still requires central venous and arterial lines.

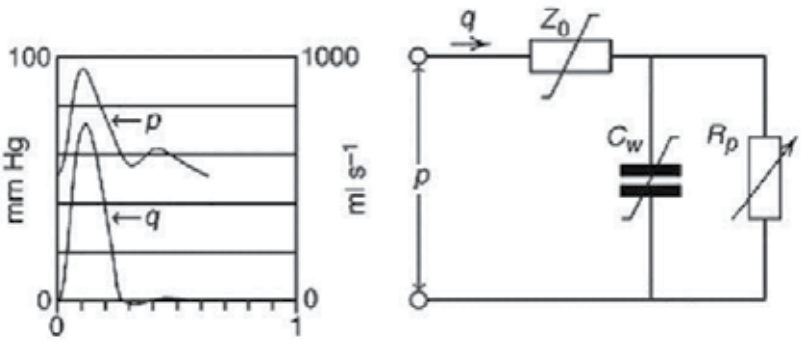
In the case of LiDCO, the independent calibration technique is lithium dilution, again according to the Henriques-Stewart-Hamilton principle. LiDCO uses lithium dilution from a peripheral vein to an arterial line and, thereby, does not express information on cardiac filling volumes or the extravascular lung water. It is also a concern that calibration cannot be performed frequently and calibration may be unreliable in patients with grave hyponatreamia (Morgan et al., 2008) that may manifest in some OLT patients. The PulseCO algorithm used by LiDCO is based on pulse power derivation rather than on waveform morphology.

Continuous tracking of changes in stroke volume by arterial pulse wave analysis in Modelflow provides flow from pressure and has the potential to appreciate the importance of systemic blood flow (Wesseling *et al.*, 1993;Harms *et al.*, 1999). With Modelflow®, beat-to-beat CO is estimated from the arterial pressure wave (Wesseling *et al.*, 1993). Pressure from an arterial line is, after calibrating and zeroing to the mid-axillary level used as input to the model to calculate CO. The method uses a non-linear three-element model of the aortic input impedance and simulates the aortic flow waveform from the pressure signal. Two of the three model elements (aortic characteristic impedance and arterial compliance) depend on the elastic properties of the aorta and are computed using a built-in database of arctangent aortic pressure-area relationships given age and gender of the patient. Integrating the aortic flow waveform per beat provides left ventricular stroke volume and CO is the product of stroke volume and HR while the third model element, peripheral vascular resistance is calculated for each heartbeat as the quotient of arterial pressure to CO (Fig. 3A). The software used is an online real-time version of Beatscope® (FMS, Amsterdam, The Netherlands).

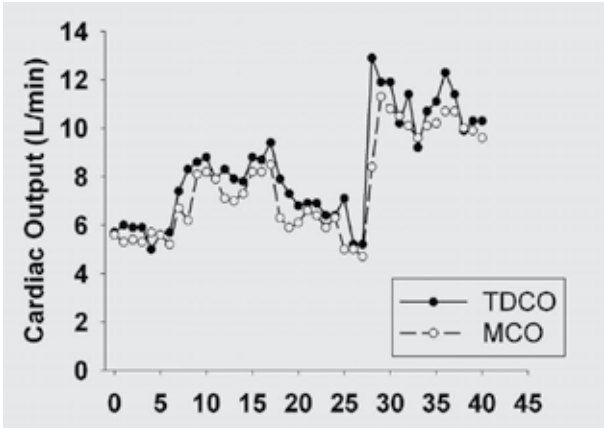
The Modelflow method is fully automatic, self-recording, has a fast response time, a high precision, and has been successfully validated against a thermodilution estimate of CO during cardiac surgery (Jansen *et al.*, 2001), intensive care medicine (Jansen *et al.*, 2001;Jellema *et al.*, 1999), and notably during OLT (Nissen *et al.*, 2009b) and, surprisingly, without a need for taking potential differences in vascular tone during the operation into account (Fig. 3 B). That is the case although Modelflow appears to underestimate deviations in CO during manipulation of body temperature with a significant increase in vascular conductance during heating (Shibasaki *et al.*, 2011).

4. Thoracic electric admittance

With the large spontaneous changes in CO during OLT, it may be an advantage to monitor CBV separately and, as indicated, evaluate the pulmonary water content. According to



(a)



(b)

Fig. 3. a) Diagram of the three-element non-linear, self-adapting model (right) and input pressure and simulated flow pulse (left) used for Modelflow. Arterial pressure p is applied to the model input. Z_o, characteristic impedance of the proximal aorta; C_w, arterial Windkessel compliance; R_p total systemic peripheral resistance. The non-linear properties of Z_0 and C_w are indicated by a stylised "S" symbol. R_p has an arrow indicating that it adapts to changes in systemic resistance. The result of the model simulation is a flow curve q. Integrated per beat (area under the curve) yields stroke volume. (From Wesselring et al. 1993 with permission). b) Cardiac output followed by Modelflow (MCO) and thermodilution (TDCO) during liver transplantation surgery. (From Nissen et al., 2009 b with permission)

Ohm's law changes in CBV can be assessed by thoracic electrical admittance (TA) and the obtained value is expressed in milli-Siemens (mS). Using a low (e.g. 1.5 kHz) and a high frequency current (e.g. 100 kHz), TA distinguishes between the extracellular ($TA_{1.5}$) and total water (TA_{100}) content. Accordingly, changes in the difference between the high and the low frequency current reflects those in the intracellular water content (TA_{ICW}) (Cai *et al.*, 2000), i.e. red cell volume within the thoracic region (Fig. 4) and TA_{ICW} can, thereby, indicate a need for transfusion of blood while $TA_{1.5}$ monitors the (pulmonary) water content.

With the use of goal-directed approach to the administration of fluid and blood information about CBV becomes important in the anhepatic phase of OLT when approximately one third

of the circulation is eliminated causing a similar decrease in CO (Table 1), not necessarily due to hypovolaemia. Similarly when the donor liver is reperfused, an increase in CO requires that CBV is maintained despite the blood needed to fill the liver, while care is directed also to avoid distension of the liver by administrating a too large volume load.

With TA, evaluation of CBV is continuous with the use of, e.g. two ECG electrodes on the right side of the neck and two other electrodes placed high in the midaxillay line on the left side of the thorax (Ejlersen *et al.*, 1997;Matzen *et al.*, 1991) in order to include the heart and central vessels in the evaluation and at the same time exclude the abdominal fluid content. With a four electrode arrangement, skin resistance is eliminated from the evaluation making changes in TA an almost perfect report of those in the thoracic fluid / blood volume (Krantz *et al.*, 2000). Similarly, it is possible to monitor accumulation of blood in the legs during cross-clamping of inferior caval vein by changes in TA over one leg or the glutal region (Ejlersen *et al.*, 1997).

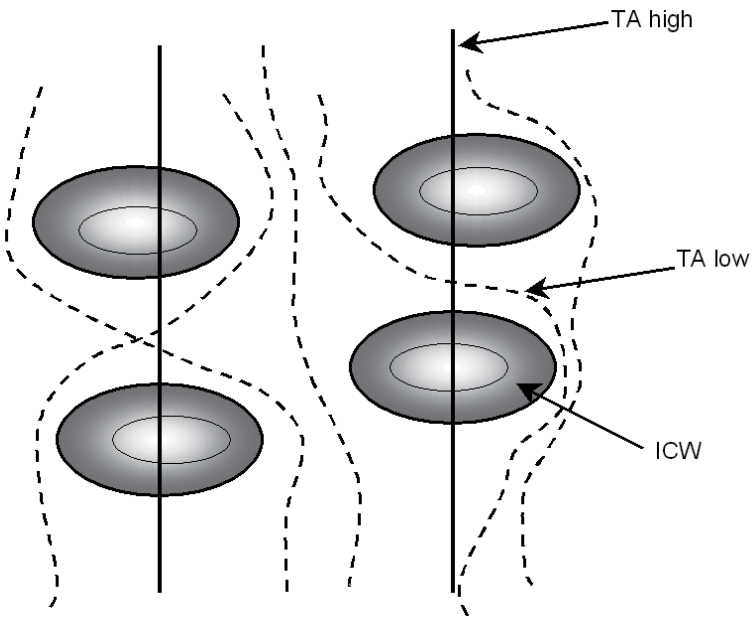


Fig. 4. At low and high frequencies, thoracic electric admittance (TA) distinguishes between the extracellular (ECW) and total body water (TBW). The difference reflects the intracellular water content (ICW) and changes respond to haemorrhage versus administration of blood

When volume is administered according to individualised goal directed fluid therapy, an increase in TA reflects that CBV is increased, as does pulmonary artery mean and wedge pressures and that is the case although there may be no changes in HR, central venous pressure (CVP), or MAP as CO increases during OLT (Ejlersen *et al.*, 1995b).

5. Venous saturation

For continuous reading of S_vO_2, a PAC catheter is ideal but a central S_vO_2 may be similarly obtained from a central venous line using a catheter with the same type of fiberoptic

oxymeter. Central S_vO_2 values are about 5% larger than those obtained from the PAC catheter (Krantz *et al.*, 2005; Rivers, 2006) but with parallel changes in response to deviations in blood volume (el-Masry *et al.*, 2009; Krantz *et al.*, 2005). Alternatively, S_vO_2 may be obtained by blood sampling during the different phases of the operation or, eventually, at times when there is a need to check volume administration during a major blood loss.

Thus, in order to evaluate whether a hypotensive incident is due to a reduced CO or to a reduced afterload, monitoring of S_vO_2 is well suited (Fig. 2). Considering that the patient's (basal) metabolic rate (Vo_2) does not change during the operation (despite the hepatectomy), there is a direct relationship between S_vO_2 and CO as described by Fick's equation: (Vo_2 = (C_a – C_v) Q), where C_a and C_v represent the arterial and venous oxygen content. Therefore, a continuous recording of S_vO_2 may be applied in situations where devices for continuous recording of stroke volume or CO are not available of relevance, as mentioned, especially for the first minutes after reperfusion of the donor liver. Typically, a low arterial pressure reflects a blood loss in the dissection phase of the operation, while reperfusion of the liver is likely to be associated with a low blood pressure due to loss of peripheral resistance as detected by an increase in muscle blood flow as recorded easily by an increase in muscle oxygenation (S_mO_2). Surprisingly, also CBF increases during reperfusion of the grafted liver despite MAP may decrease to a level that is lower than normally considered to represent the lower level of cerebral autoregulation (60 mmHg) and an increase in the arterial carbon dioxide tension (P_aCO_2) seems only partly to explain an elevated CBF during reperfusion of the liver (Pott *et al.*, 1995; Larsen *et al.*, 1999). However, during reperfusion of the liver, function of the heart may be affected by a lowering of HR in response to an abrupt increase in plasma potassium as detected by blood sampling or indicated by the ECG and before the liver is reperfused, a small dose of adrenaline (4-6 µg kg^{-1} h^{-1}) prevents this slowing of the heart since adrenaline promotes clearance of potassium from the circulation (Struthers et al., 1983) besides its chronotropic effect.

Generally, a reduction in S_vO_2 should be considered as an indication for a low CO due to hypovolaemia and treated accordingly by applying the principles of individualised goal directed fluid therapy. That is the case although with the often marked increase in CO during early reperfusion of the grafted liver, S_vO_2 may reach extreme values (>95%) and, as mentioned, TA can indicate whether CBV is maintained or whether further volume treatment would be likely to increase CO even more and thereby stabilize MAP. Thus S_vO_2 values derived from either a PAC or a central venous catheter have potential as variables for goal directed fluid therapy (Fig. 2) although outcome and comparative studies are available only in regard to monitoring stroke volume or CO (Bundgaard-Nielsen *et al.*, 2007a).

6. Vascular pressures

Monitoring arterial pressure is often by way of a catheter in the radial artery, but in case of central hypovolaemia, the radial artery constricts to dilate again as the hypovolaemic shock develops (Iversen et al., 1995) making the pressure in the radial artery a potential underestimate of the systemic pressure (De Wolf, 2006; Krenn & De Wolf, 2008). In contrast, the use of a femoral artery catheter is reliable for recording of MAP (Arnal et al., 2005) but never the less, a radial artery catheter can be used for blood sampling and as back-up for the determination of MAP. For example in case the hepatic artery needs to be grafted to the

aorta, partial or complete clamping of the abdominal aorta may eliminate pressure recording in the femoral artery.

The CVP is often taken to express filling of the right ventricle but CVP is not well correlated with preload to the heart as expressed by its diastolic filling (De Wolf, 2006). A correlation between CO and CVP is established during acute changes in the CBV as induced by head-up-tilt (Ogoh *et al.*, 2003) or lower body negative pressure (Murray *et al.*, 1999), while for most patients CO is not related to CVP although there is a relation between CO and diastolic filling of the heart as indicated by echocardiography (Thys et al., 1987).

In order to limit the blood loss during surgery, a strategy of keeping CVP low has been suggested and studies on liver resection patients show a reduction in the blood loss, in morbidity, as in the hospital stay when the intraoperative CVP is kept < 5 mmHg (Jones et al., 1998;Chen et al., 2000). Implementing the same strategy to OLT, one study found that a low CVP increased morbidity as expressed as postoperative renal failure and mortality (Schroeder et al., 2004), while an other study came to the opposite conclusion (Massicotte et al., 2006). We hold it that volume therapy during OLT is better detected by flow related parameters than by vascular pressure(s) (Bundgaard-Nielsen *et al.*, 2007a;Jenstrup *et al.*, 1995;Ejlersen *et al.*, 1995b).

A PAC allows for monitoring pulmonary artery (mean) pressure (PAMP) as well as wedge pressure (PAWP). These values are relevant to patients undergoing OLT since some patients develop both portal and pulmonary hypertension (Krenn & De Wolf, 2008). It remains, however, elusive whether monitoring of PAWP provides advantages over the continuous recording of PAMP. At least it should be considered that a determination of PAWP carries the risk of rupturing a branch of the pulmonal artery and that is likely to be fatal.

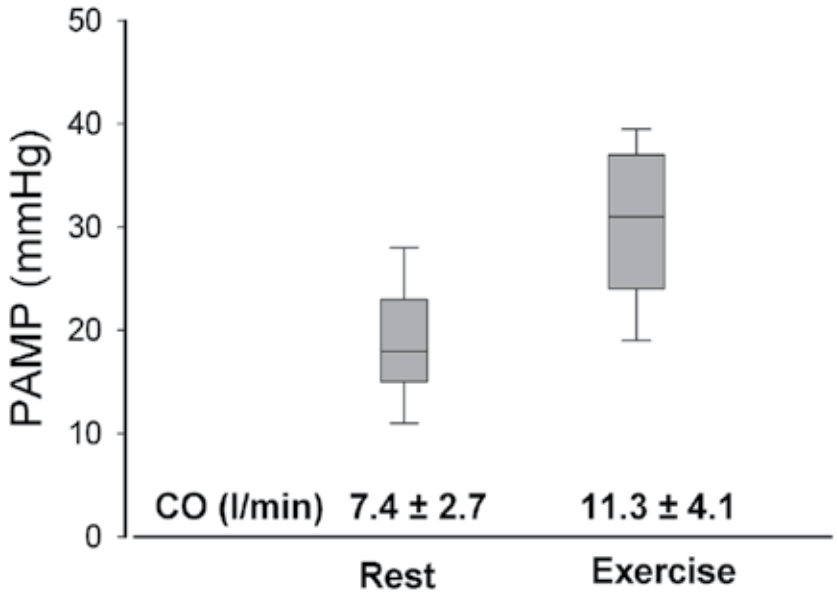


Fig. 5. Pulmonary artery mean pressure (PAMP) (±SD and 5th and 95th percentile) and cardiac output (CO) (±SD) for 33 candidates for OLT at rest and during exercise (P. Nissen, unpublished)

A PAMP limit of 30 mmHg may be considered to indicate pulmonary hypertension, but it should be taken into account that there is a direct relationship between PAMP and CO as illustrated during physical exercise (Tolle *et al.*, 2008) and that is also the case for patients under evaluation for OLT (Fig. 5).

If a candidate OLT patient presents a high PAMP, it should accordingly be considered whether the high pulmonary pressure is because of a large CO and thereby unrelated to pulmonary disease. As for other patient categories, it seems more important that the heart is able to generate a reasonable CO than at what pressures it operates. For example, a successful OLT is reported for a patient with a PAMP of 44 mmHg and a CO of 5.7 l/min and with an ability to increase CO to 10 l/min (at a PAMP of 51 mmHg during reperfusion of the liver) (Liu *et al.*, 1996). Similarly, there is even for patients without pulmonary hypertension an often impressive increase in PAMP concomitant with the increase in CO during reperfusion of the grafted liver (Table 1). Furthermore hypoxia leads to pulmonary hypertension of relevance for OLT patients with associated significant pulmonary shunt and at least in case of hypoxia, pulmonary hypertension seems to be related to the patient's iron status (Smith *et al.*, 2008). Accordingly, the iron status of the OLT candidate with pulmonary hypertension and hypoxia should be checked.

7. Heart rate

It is probably without exception that HR is monitored during OLT and deviations in HR in response to variation in CBV are therefore of interest. In textbook descriptions of hypovolaemic shock it is stated often that tachycardia is the arterial baroreceptor response to a low blood pressure (Secher & Bie, 1985). Yet the common, both experimental and clinical finding is that the HR response to central hypovolaemia encompasses three stages (Secher *et al.*, 1992) (Fig. 6).

With a small reduction in CBV, there is a moderate increase in HR, most often to less than 100 bpm and as indicated, the increase in HR may be so small that it does not become statistically significant (Murrell *et al.*, 2009). However, with a 30% reduction of CBV, both HR and MAP decrease as known from a vasovagal syncope and cerebral perfusion and oxygenation become affected (Madsen & Secher, 1999;van Lieshout *et al.*, 2003). Such an incident is fatal if CBV is not restored immediately (Madsen *et al.*, 1998), but with partial restoration of CBV, intense sympathetic activation in response to (central) hypovolaemia elicits a marked increase in HR (>120 bpm) may be in response to cerebral hypoperfusion. Thus, adequate restoration of CBV in stage II of shock secures the well-being of the patient, while manifest tachycardia (stage III) appears to indicate a transition to an irreversible stage of shock since the patient then is likely to need eventually prolonged intensive care and is then exposed to the associated grave prognosis (De Backer D. *et al.*, 2010). It should also be noted that the administration of atropine to treat bradycardia during haemorrhage is likely to enhance the haemorrhage and, thereby, could be fatal (Bertolini, 1995). At any rate, the administration of atropine hinders the use of HR to monitor an eventual volume deficit. In case of a low HR, volume should be administrated with an expected moderate increase in HR before the "resting" HR is established (Fig. 6). Conversely, it should be checked whether any increase in HR is due to an otherwise undetected (small) volume deficit by supplementing (100-200 ml) of volume and thereby keep HR low and the volume administration can then stop when HR does not decrease further.

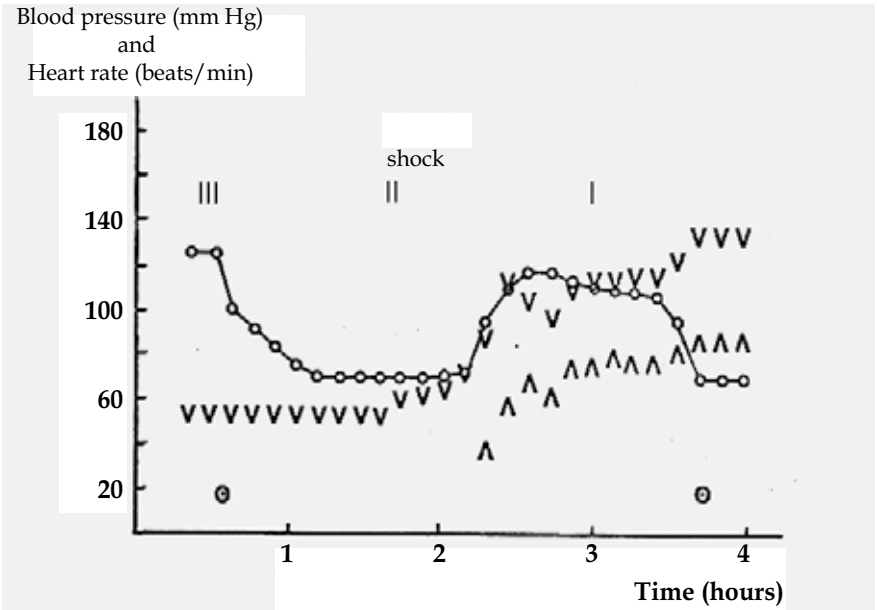


Fig. 6. Heart rate (HR) and blood pressure (systolic **v** and diastolic **^**) responses of a patient treated for a ruptured abdominal aneurism. At admission the patient had thacycardia and low blood pressure (stage III of hypovolaemic shock). During volume loading a decrease in HR (stage II) is seen followed by an increase (stage I, preshock) as blood pressure began to increase before stable values were reached; beginning and end of the operation indicated by circles. (From Jacobsen & Secher, 1992 with permission)

8. Cerebral autoregulation

Central to this chapter is the ability to secure CBF and S_cO_2 during the OLT and thereby, presumably, maintain the patient's well being after the operation (Murkin *et al.*, 2007).

Interest in recording CBF or S_cO_2 for the OLT patient is relevant not only because these variables may be affected by hypotensive events during the operation in case a low blood pressure reflects a reduced CO, but also because some acute liver disease patients demonstrate impaired cerebral autoregulation (Larsen *et al.*, 1999; Nissen P. *et al.*, 2009; Ejlersen *et al.*, 1994) (Fig. 7).

Cerebral perfusion may be followed by transcranial Doppler (TCD) derived middle cerebral artery mean blood velocity (MCA V_{mean}) (Pott *et al.*, 1995) and evaluation of cerebral tissue flow by clearance of ^{133}Xe has been carried out during OLT(Larsen *et al.*, 1999). However, it remains that NIRS is, by far the most feasible method for routine monitoring of cerebral perfusion during surgery (Nissen *et al.*, 2009a;Steiner *et al.*, 2009). NIRS reflects changes in brain capillary saturation and mitochondrial oxygen tension in response to manipulation of the inspired oxygen and CO_2 tensions (Rasmussen *et al.*, 2007) although a potential influence of skin blood flow needs to be considered (Sato et al., 2011).

While a determination of CBF requires extensive apparatus and calculations, the recording of S_cO_2 is as readily available as the recording of arterial oxygen saturation by pulsoximetry

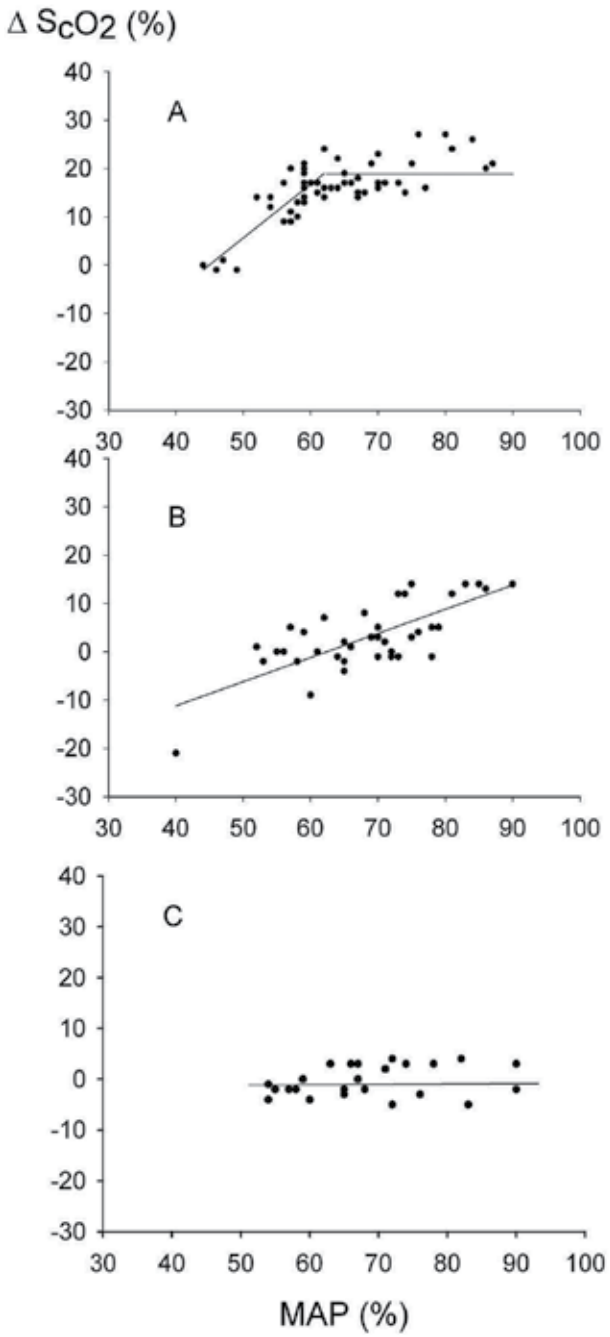


Fig. 7. Changes in frontal lobe cerebral oxygen saturation (ΔS_cO_2) related to mean arterial pressure (MAP) for three patients undergoing liver transplantation (A) A patient for whom a lower limit of cerebral autoregulation can be defined. (B) A patient who demonstrates no cerebral autoregulation. (C) A patient for whom no lower limit of cerebral autoregulation was detected (From Nissen et al., 2009 with permission)

and builds on the same technology of protons absorbance in the near infrared spectrum (NIRS) (Madsen & Secher, 1999). However in contrast to pulsoximetry, S_cO_2 is not coupled to the recording of pulse and, thereby, expresses an average rather than a maximal oxygen concentration of the tissue, i.e. of the brain or skeletal muscles. With spatial resolution NIRS, light is sampled at two distances from the emitter to prioritise absorption of light in the deep tissue, for the head assumed to represent the cerebral cortex (S_cO_2) and over a muscle (S_mO_2) oxygen saturation of haemoglobin and myoglobin. Yet, a sustained subcutaneous fat deposit may hinder detection of S_mO_2 and the thenar muscle is ideal for monitoring S_mO_2 since there is no subcutaneous fat over that muscle group (Thomson et al., 2009).

Thus frontal lobe oxygenation by NIRS is a non-invasive recording of changes in CBF (Madsen & Secher, 1999) with a correlation between S_cO_2 and MCA V_{mean} (Steiner *et al.*, 2009). Also changes in S_cO_2 parallel those in internal jugular venous O_2 saturation (Pott *et al.*, 1995;Skak *et al.*, 1997) and NIRS is able to detect cerebral hypoperfusion (Plachky *et al.*, 2004). The NIRS determined S_cO_2 is based on the absorption of light in the spectra for oxygenated and deoxygenated haemoglobin and reports tissue oxygenation as a percentage of light absorption by oxygenated to total haemoglobin. An emitter generates light at, e.g. 733 and 808 nm and the reflection is registered by two or more optodes placed at a distance of, e.g. 3 and 4 cm from the emitter to allow for the subtraction of reflections derived from superficial tissues of the scalp and the skull for detection of S_cO_2 (Grubhofer *et al.*, 1997) (Fig. 8). Thus with increasing distance between the emitter and the optodes, light penetrates deeper into the tissues and with evaluation of absorption at two distances (spatial resolution), absorption in deep tissue, i.e. brain, is appreciated.

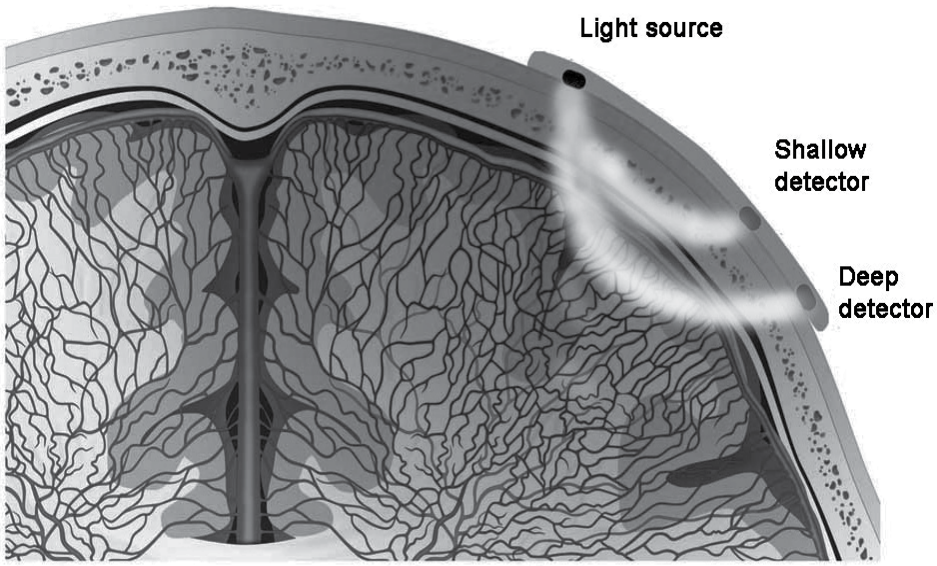


Fig. 8. Near infrared spectroscopy applied to the brain. Distance between the light emitter and the optodes 3 and 4 cm. By subtracting the superficial from the deeper reflections, oxygenation of brain cortex is appreciated (from Covidien, Denmark with permission)

Of relevance for the recording of S_cO_2 during OLT, bilirubin absorbs light in the same wavelength as haemoglobin and depending of the wavelength used to derive S_cO_2, there

may be a negative influence of plasma bilirubin of the detected S_cO_2 (Madsen *et al.*, 2000). However, even when plasma bilirubin is elevated and the reported S_cO_2 is low, the derived value reacts on changes imposed by bleeding and changes in P_aCO_2.

The significant haemodynamic changes associated with OLT may lead to neurological complications and increased mortality in reflection of reduced cerebral vascular resistance in the first hour after reperfusion of the liver exposing the brain to hyperperfusion (Ardizzone et al., 2006). Thus in case of lacking cerebral autoregulation, CFB is affected both by a low and a high blood pressure and during OLT, blood pressure is likely to increase markedly when surgery leads to manipulation of the adrenal gland with, presumably, release of adrenaline into the circulation. Normally CBF is considered to be maintained within a MAP range from approximately 60-150 mmHg (Paulson et al., 1990). However, cerebral perfusion decreases already at a MAP of 80 mmHg when the decrease in blood pressure is caused by a low CBV and thereby a lowered CO (Madsen *et al.*, 1995). On the other hand, cerebral perfusion and S_cO_2 may be preserved even at a MAP below 40 mmHg if CBV is not affected (Nissen P. *et al.*, 2009) (Fig. 9).

A given MAP therefore does not guarantee that cerebral perfusion is secured leading to the conclusion that CBF or, more likely, S_cO_2 should be monitored during the operation (Nissen *et al.*, 2009a).

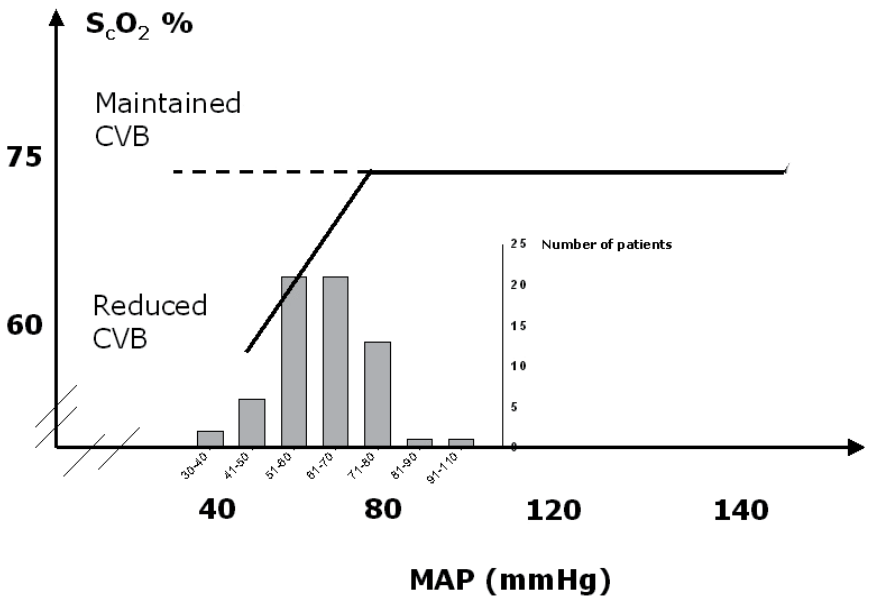


Fig. 9. Cerebral oxygen saturation (S_cO_2) related to mean arterial pressure (MAP) including data obtained with a maintained central blood volume (CBV) during anaesthesia (broken line) and from subjects for whom the central blood volume was reduced deliberately during head-up tilt (full line). Normogram illustrates distribution of the lowest MAP in the anaesthetized patients (Modified from Nissen et al., 2009 with permission)

Also it is to be considered that administration of phenylephrine in case of a low blood pressure, in an attempt to increase blood pressure to above what is might present the lower limit of cerebral autoregulation, is associated with a decrease rather than with the probably

intended increase in S_cO_2 (Nissen et al., 2010). Alternatively, hypotension should be considered in relation to a decrease in plasma calcium in response to administration of blood products and calcium should be supplemented to restore the physiological level (1.2 mM). However, the use of phenylephrine may be indicated during reperfusion of the donor liver to reduce peripheral vasodilatation and thereby to centralise of blood accumulated in the splanchnic region. Alternatively, the administration of phenylephrine to increase the blood pressure may be replaced by the use of ephedrine that does not demonstrate the same negative influence on S_cO_2 (Nissen *et al.*, 2010; Meng *et al.*, 2011).

P_aCO_2 has a significant influence on CBF and P_aCO_2 is regularly monitored by a continuous recording of the end-tidal CO2 tension to maintain a value of, e.g. 4,5 kPa. In that regard OLT is no exception, but with the reduction of the metabolic rate during the anhepatic phase of the OLT, a given setting of ventilation may lower P_aCO_2 and ventilation then needs to be reduced in order to maintain CBF and S_cO_2 (Madsen & Secher, 1999). Conversely, with reperfusion of the donor liver, P_aCO_2 increases again and often to values that exceed the level established in the dissection phase of the operation. Accordingly, ventilation should be increased at, or likely before reperfusion of the liver in order to prevent cerebral hyperperfusion and ventilation is thereafter gradually reduced towards the end of the operation guided by S_cO_2 as the CO_2 load is eliminated by the exhaled air.

With optodes placed over a muscle NIRS monitors muscle oxygen saturation (S_mO_2) and decreases before central hypovolaemia affects blood pressure. However, haemorrhagic hypotension is likely to be caused by a Bezold-Jarisch-like reflex including loss of sympathetic activity and, therefore, an increase in muscle blood flow and in turn S_mO_2 (Madsen *et al.*, 1995). S_mO_2 effectively detects central hypovolaemia (Soller *et al.*, 2008) (Fig. 2) and supplements, or may be used as an alternative non-invasive monitoring modality to S_vO_2 for detection of a blood loss. Ideally S_mO_2 provides for an early warning of ongoing haemorrhage and allows for direction of fluid administration before the blood loss affects CBF and in turn S_cO_2.

9. Support to the central blood volume

An OLT may be associated with a severe blood loss in about 20% of the patients (Massicotte *et al.*, 2004). Bleeding during OLT is most common during the dissection phase for the cirrhotic liver associated with distended abdominal veins. Accordingly, rapid infusion systems have been developed which that can deliver 1.5 l of heated fluid per minute through 7 F venous catheter is recommended for infusion of fluids and blood products. The rapid infusion machine reports the accumulated amount of fluid (blood) administered and is also suited for infusion of small volumes to children as the fluid can be administrated with an accuracy of millilitres. To adults it is common to administer bolus infusions of 100 ml (occasionally 500 ml) over 1 min since, as mentioned, a lowering of S_vO_2 by 1 % corresponds typically to a volume deficit of ~100 ml (Ejlersen et al., 1995a). Furthermore, one or two i.v. lines can be placed for supplementary administration of fluid and medication.

Since patients with end-stage liver decease present with serious haemostatic defects, management of coagulation during OLT is a challenge. Coagulopathy can be aggravated by hypothermia and acidemia, associated with increased risk of uncontrolled bleeding and mortality (Lier et al., 2008). Accordingly fluid, including blood products, is preheated and the patients are provided with a warm airflow blanket (e.g. “Bair Hugger”) covering the

upper part of thorax, the neck, and arms and that arrangement is usually able to maintain the patient's temperature above 36°. Furthermore, frequent adjustment of CBV according to the individualized goal directed fluid administration strategy prevents marked changes in pH and arterial lactate and frequent monitoring of the plasma calcium level, with appropriate substitutions, supports coagulation competence.

Yet, in order to preserve coagulation competence, there remains some disagreement. While one review finds that correction of coagulation defects with fresh frozen plasma (FFP) and platelets does not reduce the blood loss and has a negative influence on outcome (Dalmau et al., 2009), the local experience is that timely administration of FFP and platelets improves outcome (Johansson *et al.*, 2010), a strategy supported by other studies demonstrating a decrease in blood loss and improvement in 24 hour and 30-day survival following early use of plasma and platelets (Holcomb, 2010; Holcomb et al., 2008). Discrepancy between views likely relate to which patients are addressed with the need for FFP and platelets indicated only for patients presenting with a deficit in consequence of their liver disease or provoked by major haemorrhage.

It is important to monitor the OLT patient's coagulation competence and in that regard a determination of the protrombin time (PT) and activated partial protrombin time (APPT) seems inadequate since these variables do not correlate well with clinically coagulopathy or bleeding condition (Murray *et al.*, 1999; Segal & Dzik, 2005). For on-line evaluation of haemostasis, thrombelastographhy (TEG) is used as pioneered during OLT by Kang et al (Kang *et al.*, 1985). TEG records the viscoelastic changes during coagulation by analysing whole blood placed in a rotating cup (Fig. 10) while a pin suspended in the blood from a torsion wire records the resistance to motion.

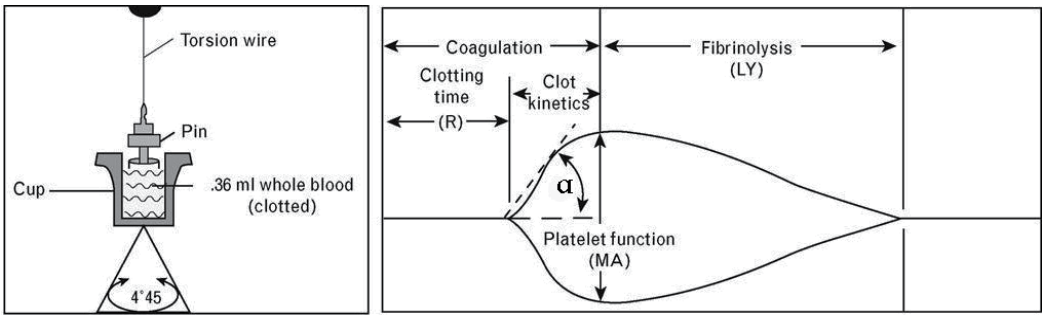


Fig. 10. Thrombelastograph technology and measured variables: The clotting time (R), the angle (α) representing the progressive increase in clot strength, the maximal clot strength (MA) and the fibrinolysis (LY) (From Johannson et al,. 2010 with permission)

Four TEG parameters are regularly reported: the clotting time (R), the angle (α) representing the progressive increase in clot strength, the maximal clot strength (MA), and fibrinolysis (LY) (Fig. 10) (Johansson, 2009). The TEG reported values correlate well with the clinical bleeding conditions and are recommended to direct blood product treatment together with a platelet count and check of the haemoglobin concentration (Plotkin et al., 2008). The TEG analysis can be performed in the laboratory and is locally displayed in real-time in the operating theatre to enable for early intervention, e.g. by administration of platelets and plasma in case of significantly attenuated coagulation competence. In this endeavour, it is considered that transfusion of platelets to 100 X 10^9 L^{-1} (rather than to the commonly used

guideline of 50 X 10^9 L^{-1}) secures coagulation competence and additional administration of platelets is routinely performed before the reperfusion of the donor liver associated with significant use of platelets (Johansson *et al.*, 2010). Thus, the coagulation competence of the patient's blood is accentuated by the administration of FFP and saline-adenine-glucose-mannitol (SAGM) erythrocyte suspension to a haemoglobin concentration of 6 mM (haematocrit 30%), making sure that plasma calcium does not decrease and calcuimcloride is administered frequently to maintain a reference value of 1.2 mM.

Yet it has to be accepted that some patients have intraoperative increased consumption of fibrinogen and if a diffuse bleeding in combination with a reduced α and MA manifest, we suggest monitoring functional fibrinogen to decide whether the reduction in MA relates to platelet or to fibrinogen function. Attention has also been directed to the endothelial barrier function in response to haemorrhagic shock and the role of glycocalyx appears important to endothelial permeability, intracellular dysfunction, and oedema (Holcomb, 2011).

Until recently, infusion of aprotinin was an option for OLT and aprotinin reduces haemorrhage by hindering fibrinolyses and thereby stabilizes the formed blood clots. Aprotinin has, however, been withdrawn from the marked (Dietrich, 2009) and tranexamic acid is the (cheaper) alternative to be administrated before surgery and again before reperfusion of the donor liver (Takagi et al., 2009).

Further refinement of treatment includes control of plasma concentration of magnesium (Skak et al., 1996) and corrected if low (reference value 0.8 mM) and maintenance of the blood glucose or, conversely, administration of insulin in case the blood glucose level increases beyond 10 mM. Surprisingly, the blood glucose level does not decrease during the anhepatic phase of OLT, presumably because the kidneys supplement glucose production (Lauritsen *et al.*, 2002). Also it should be considered that during massive administration of blood products, HR might be affected by the potassium concentration of Sag-M blood of approximately 50 mM. It is advised that massive administration of blood is paralleled by infusion of adrenaline (4-6 mg kg^{-1} min^{-1}) to eliminate potassium from blood.

10. Conclusion

During OLT it is possible to maintain coagulation competence by timely administration of fresh frozen plasma and platelets together with SAGM-blood while body temperature and plasma pH and calcium are controlled. Notably platelets are supplemented to a reference value of 100 X 10^9 L^{-1} rather than 50 X 10^9 L^{-1} seems to increase survival. Even massive bleeding can be coped with if CBV and thereby S_cO_2, at all times is kept within narrow limits. To maintain a stable central blood volume requires that treatment of patients with liver disease is focused on eventually high maximal values for CO and S_vO_2.

11. References

Abbas SM & Hill AG (2008). Systematic review of the literature for the use of oesophageal Doppler monitor for fluid replacement in major abdominal surgery. *Anaesthesia* 63, 44-51.

Ardizzone G, Arrigo A, Schellino MM, Stratta C, Valzan S, Skurzak S, Andruetto P, Panio A, Ballaris MA, Lavezzo B, Salizzoni M, & Cerutti E (2006). Neurological complications of liver cirrhosis and orthotopic liver transplant. *Transplant Proc* 38, 789-792.

Arnal D, Garutti I, Perez-Pena J, Olmedilla L, & Tzenkov IG (2005). Radial to femoral arterial blood pressure differences during liver transplantation. *Anaesthesia* 60, 766-771.

Bao FP & Wu J (2008). Continuous versus bolus cardiac output monitoring during orthotopic liver transplantation. *Hepatobiliary Pancreat Dis Int* 7, 138-144.

Bertolini A (1995). The opioid/anti-opioid balance in shock: a new target for therapy in resuscitation. *Resuscitation* 30, 29-42.

Boucaud C, Bouffard Y, Dumortier J, Gaillac N, Sagnard P, Graber MC, Adham M, & Boillot O (2008). Transoesophageal echo-Doppler vs. thermodilution cardiac output measurement during hepatic vascular exclusion in liver transplantation. *Eur J Anaesthesiol* 25, 485-489.

Bundgaard-Nielsen M, Holte K, Secher NH, & Kehlet H (2007a). Monitoring of peri-operative fluid administration by individualized goal-directed therapy. *Acta Anaesthesiol Scand* 51, 331-340.

Bundgaard-Nielsen M, Jorgensen CC, Secher NH, & Kehlet H (2009a). Functional intravascular volume deficit in patients before surgery. *Acta Anaesthesiol Scand*.

Bundgaard-Nielsen M, Ruhnau B, Secher NH, & Kehlet H (2007b). Flow-related techniques for preoperative goal-directed fluid optimization 3450. *Br J Anaesth* 98, 38-44.

Bundgaard-Nielsen M, Secher NH, & Kehlet H (2009b). 'Liberal' vs. 'restrictive' perioperative fluid therapy--a critical assessment of the evidence. *Acta Anaesthesiol Scand* 53, 843-851.

Bundgaard-Nielsen M, Sorensen H, Dalsgaard M, Rasmussen P, & Secher NH (2009c). Relationship between stroke volume, cardiac output and filling of the heart during tilt. *Acta Anaesthesiol Scand* 53, 1324-1328.

Cai Y, Holm S, Jenstrup M, Stromstad M, Eigtved A, Warberg J, Hojgaard L, Friberg L, & Secher NH (2000). Electrical admittance for filling of the heart during lower body negative pressure in humans. *J Appl Physiol* 89, 1569-1576.

Calne RY (2008). Early days of liver transplantation. *Am J Transplant* 8, 1775-1778.

Chen H, Merchant NB, & Didolkar MS (2000). Hepatic resection using intermittent vascular inflow occlusion and low central venous pressure anesthesia improves morbidity and mortality. *J Gastrointest Surg* 4, 162-167.

Colbert S, O'Hanlon DM, Duranteau J, & Ecoffey C (1998). Cardiac output during liver transplantation. *Can J Anaesth* 45, 133-138.

Costa MG, Chiarandini P, & Della RG (2007). Hemodynamics during liver transplantation. *Transplant Proc* 39, 1871-1873.

Dalmau A, Sabate A, & Aparicio I (2009). Hemostasis and coagulation monitoring and management during liver transplantation. *Curr Opin Organ Transplant* 14, 286-290.

De Backer D., Biston P, Devriendt J, Madl C, Chochrad D, Aldecoa C, Brasseur A, Defrance P, Gottignies P, & Vincent JL (2010). Comparison of dopamine and norepinephrine in the treatment of shock. *N Engl J Med* 362, 779-789.

de Waal EE, Wappler F, & Buhre WF (2009). Cardiac output monitoring. *Curr Opin Anaesthesiol* 22, 71-77.

De Wolf AM (2006). Perioperative assessment of the cardiovascular system in ESLD and transplantation. *Int Anesthesiol Clin* 44, 59-78.

Della RG, Brondani A, & Costa MG (2009). Intraoperative hemodynamic monitoring during organ transplantation: what is new? *Curr Opin Organ Transplant* 14, 291-296.

Dietrich W (2009). Aprotinin: 1 year on. *Curr Opin Anaesthesiol* 22, 121-127.

Donati A, Loggi S, Preiser JC, Orsetti G, Munch C, Gabbanelli V, Pelaia P, & Pietropaoli P (2007). Goal-directed intraoperative therapy reduces morbidity and length of hospital stay in high-risk surgical patients. *Chest* 132, 1817-1824.

Ejlersen E, Larsen FS, Pott F, Gyrtrup HJ, Kirkegaard P, & Secher NH (1994). Hepatectomy corrects cerebral hyperperfusion in fulminant hepatic failure. *Transplant Proc* 26, 1794-1795.

Ejlersen E, Skak C, Moller K, Pott F, & Secher N (1995a). Central cardiovascular responses to surgical incision at a maximal mixed venous oxygen saturation. *Transplant Proc* 27, 3500.

Ejlersen E, Skak C, Mooller K, Pott F, & Secher N (1995b). Central cardiovascular variables at a maximal mixed venous oxygen saturation in severe hepatic failure. *Transplant Proc* 27, 3506-3507.

Ejlersen E, Sode P, Skak C, Rasmussen A, Espersen K, Kirkegaard P, & Secher NH (1997). Lower body impedance for the evaluation of venovenous bypass flow. *Liver Transpl Surg* 3, 153-159.

el-Masry A, Mukhtar AM, el-Sherbeny AM, Fathy M, & el-Meteini M (2009). Comparison of central venous oxygen saturation and mixed venous oxygen saturation during liver transplantation. *Anaesthesia* 64, 378-382.

Gonzalez-Alonso J, Mortensen SP, Dawson EA, Secher NH, & Damsgaard R (2006). Erythrocytes and the regulation of human skeletal muscle blood flow and oxygen delivery: role of erythrocyte count and oxygenation state of haemoglobin. *J Physiol* 572, 295-305.

Grubhofer G, Lassnigg A, Manlik F, Marx E, Trubel W, & Hiesmayr M (1997). The contribution of extracranial blood oxygenation on near-infrared spectroscopy during carotid thrombendarterectomy. *Anaesthesia* 52, 116-120.

Harms MP, van Lieshout JJ, Jenstrup M, Pott F, & Secher NH (2003). Postural effects on cardiac output and mixed venous oxygen saturation in humans. *Exp Physiol* 88, 611-616.

Harms MP, Wesseling KH, Pott F, Jenstrup M, Van Goudoever J, Secher NH, & van Lieshout JJ (1999). Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)* 97, 291-301.

Holcomb JB (2010). Optimal use of blood products in severely injured trauma patients. *Hematology Am Soc Hematol Educ Program* 2010, 465-469.

Holcomb JB (2011). A Novel and Potentially Unifying Mechanism for Shock Induced Early Coagulopathy. *Ann Surg*.

Holcomb JB, Wade CE, Michalek JE, Chisholm GB, Zarzabal LA, Schreiber MA, Gonzalez EA, Pomper GJ, Perkins JG, Spinella PC, Williams KL, & Park MS (2008). Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg* 248, 447-458.

Iversen HK, Madsen P, Matzen S, & Secher NH (1995). Arterial diameter during central volume depletion in humans. *Eur J Appl Physiol Occup Physiol* 72, 165-169.

Jans O, Tollund C, Bundgaard-Nielsen M, Selmer C, Warberg J, & Secher NH (2008). Goal-directed fluid therapy: stroke volume optimisation and cardiac dimensions in supine healthy humans. *Acta Anaesthesiol Scand* 52, 536-540.

Jansen JR, Schreuder JJ, Mulier JP, Smith NT, Settels JJ, & Wesseling KH (2001). A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *Br J Anaesth* 87, 212-222.

Jellema WT, Wesseling KH, Groeneveld AB, Stoutenbeek CP, Thijs LG, & van Lieshout JJ (1999). Continuous cardiac output in septic shock by simulating a model of the aortic input impedance: a comparison with bolus injection thermodilution. *Anesthesiology* 90, 1317-1328.

Jenstrup M, Ejlersen E, Mogensen T, & Secher NH (1995). A maximal central venous oxygen saturation (SvO2max) for the surgical patient. *Acta Anaesthesiol Scand Suppl* 107, 29-32.

Johansson PI (2009). Hemostatic strategies for minimizing mortality in surgery with major blood loss. *Curr Opin Hematol* 16, 509-514.

Johansson PI, Ostrowski SR, & Secher NH (2010). Management of major blood loss: an update. *Acta Anaesthesiol Scand* 54, 1039-1049.

Johansson PI, Stensballe J, Rosenberg I, Hilslov TL, Jorgensen L, & Secher NH (2007). Proactive administration of platelets and plasma for patients with a ruptured abdominal aortic aneurysm: evaluating a change in transfusion practice. *Transfusion* 47, 593-598.

Jones RM, Moulton CE, & Hardy KJ (1998). Central venous pressure and its effect on blood loss during liver resection. *Br J Surg* 85, 1058-1060.

Jordan JE, Zhao ZQ, & Vinten-Johansen J (1999). The role of neutrophils in myocardial ischemia-reperfusion injury. *Cardiovasc Res* 43, 860-878.

Kang YG, Martin DJ, Marquez J, Lewis JH, Bontempo FA, Shaw BW, Jr., Starzl TE, & Winter PM (1985). Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. *Anesth Analg* 64, 888-896.

Krantz T, Cai Y, Lauritsen T, Warberg J, & Secher NH (2000). Accurate monitoring of blood loss: thoracic electrical impedance during hemorrhage in the pig. *Acta Anaesthesiol Scand* 44, 598-604.

Krantz T, Warberg J, & Secher NH (2005). Venous oxygen saturation during normovolaemic haemodilution in the pig. *Acta Anaesthesiol Scand* 49, 1149-1156.

Krenn CG & De Wolf AM (2008). Current approach to intraoperative monitoring in liver transplantation. *Curr Opin Organ Transplant* 13, 285-290.

Larsen FS, Ejlersen E, Strauss G, Rasmussen A, Kirkegaard P, Hansen BA, & Secher N (1999). Cerebrovascular metabolic autoregulation is impaired during liver transplantation. *Transplantation* 68, 1472-1476.

Laupland KB & Bands CJ (2002). Utility of esophageal Doppler as a minimally invasive hemodynamic monitor: a review. *Can J Anaesth* 49, 393-401.

Lauritsen TL, Grunnet N, Rasmussen A, Secher NH, & Quistorff B (2002). The effect of hepatectomy on glucose homeostasis in pig and in man. *J Hepatol* 36, 99-104.

Lefrant JY, Bruelle P, Aya AG, Saissi G, Dauzat M, de La Coussaye JE, & Eledjam JJ (1998). Training is required to improve the reliability of esophageal Doppler to measure cardiac output in critically ill patients. *Intensive Care Med* 24, 347-352.

Lier H, Krep H, Schroeder S, & Stuber F (2008). Preconditions of hemostasis in trauma: a review. The influence of acidosis, hypocalcemia, anemia, and hypothermia on functional hemostasis in trauma. *J Trauma* 65, 951-960.

Liu G, Knudsen KE, & Secher NH (1996). Orthotopic liver transplantation in a patient with primary pulmonary hypertension. *Anaesth Intensive Care* 24, 714-716.

Lopes MR, Oliveira MA, Pereira VO, Lemos IP, Auler JO, Jr., & Michard F (2007). Goal-directed fluid management based on pulse pressure variation monitoring during high-risk surgery: a pilot randomized controlled trial. *Crit Care* 11, R100.

Madsen P, Lyck F, Pedersen M, Olesen HL, Nielsen H, & Secher NH (1995). Brain and muscle oxygen saturation during head-up-tilt-induced central hypovolaemia in humans. *Clin Physiol* 15, 523-533.

Madsen P, Svendsen LB, Jorgensen LG, Matzen S, Jansen E, & Secher NH (1998). Tolerance to head-up tilt and suspension with elevated legs. *Aviat Space Environ Med* 69, 781-784.

Madsen PL & Secher NH (1999). Near-infrared oximetry of the brain. *Prog Neurobiol* 58, 541-560.

Madsen PL, Skak C, Rasmussen A, & Secher NH (2000). Interference of cerebral near-infrared oximetry in patients with icterus. *Anesth Analg* 90, 489-493.

Massicotte L, Lenis S, Thibeault L, Sassine MP, Seal RF, & Roy A (2006). Effect of low central venous pressure and phlebotomy on blood product transfusion requirements during liver transplantations. *Liver Transpl* 12, 117-123.

Massicotte L, Sassine MP, Lenis S, & Roy A (2004). Transfusion predictors in liver transplant. *Anesth Analg* 98, 1245-51.

Matzen S, Perko G, Groth S, Friedman DB, & Secher NH (1991). Blood volume distribution during head-up tilt induced central hypovolaemia in man. *Clin Physiol* 11, 411-422.

Mayer J, Boldt J, Mengistu A, Rohm KD, & Suttner S (2010). Goal-directed intraoperative therapy based on autocalibrated arterial pressure waveform analysis reduces hospital stay in high-risk surgical patients: a randomized, controlled trial. *Crit Care* 14, R18.

Meng L, Cannesson M, Alexander BS, Yu Z, Kain ZN, Cerussi AE, Tromberg BJ, & Mantulin WW (2011). Effect of phenylephrine and ephedrine bolus treatment on cerebral oxygenation in anaesthetized patients. *Br J Anaesth* 107, 209-217.

Morgan P, Al-Subaie N, & Rhodes A (2008). Minimally invasive cardiac output monitoring. *Curr Opin Crit Care* 14, 322-326.

Murkin JM, Adams SJ, Novick RJ, Quantz M, Bainbridge D, Iglesias I, Cleland A, Schaefer B, Irwin B, & Fox S (2007). Monitoring brain oxygen saturation during coronary bypass surgery: a randomized, prospective study. *Anesth Analg* 104, 51-58.

Murray D, Pennell B, & Olson J (1999). Variability of prothrombin time and activated partial thromboplastin time in the diagnosis of increased surgical bleeding. *Transfusion* 39, 56-62.

Murrell C, Cotter JD, George K, Shave R, Wilson L, Thomas K, Williams MJ, Lowe T, & Ainslie PN (2009). Influence of age on syncope following prolonged exercise: differential responses but similar orthostatic intolerance. *J Physiol* 587, 5959-5969.

Nilsson LB, Nilsson JC, Skovgaard LT, & Berthelsen PG (2004). Thermodilution cardiac output--are three injections enough? *Acta Anaesthesiol Scand* 48, 1322-1327.

Nissen P., Nielsen HB, van Lieshout JJ, & Secher NH (2009). Frontal lobe oxygenation is maintained during hypotension following propofol-phentanyl anesthesia. *AANA Jour (in press)* 77, 271-276.

Nissen P, Brassard P, Jorgensen TB, & Secher NH (2010). Phenylephrine but not Ephedrine Reduces Frontal Lobe Oxygenation Following Anesthesia-Induced Hypotension. *Neurocrit Care* 12, 17-23.

Nissen P, Pacino H, Frederiksen HJ, Novovic S, & Secher NH (2009a). Near-Infrared Spectroscopy for evaluation of cerebral autoregulation during orthotopic liver transplantation. *Neurocrit Care* 11, 235-241.

Nissen P, van Lieshout JJ, Novovic S, Bundgaard-Nielsen M, & Secher NH (2009b). Techniques of cardiac output measurement during liver transplantation: arterial pulse wave versus thermodilution. *Liver Transpl* 15, 287-291.

Ogoh S, Volianitis S, Nissen P, Wray DW, Secher NH, & Raven PB (2003). Carotid baroreflex responsiveness to head-up tilt-induced central hypovolaemia: effect of aerobic fitness. *J Physiol* 551, 601-608.

Oren-Grinberg A (2010). The PiCCO Monitor. *Int Anesthesiol Clin* 48, 57-85.

Ozier Y & Klinck JR (2008). Anesthetic management of hepatic transplantation. *Curr Opin Anaesthesiol* 21, 391-400.

Paulson OB, Strandgaard S, & Edvinsson L (1990). Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* 2, 161-192.

Pinsky MR (2007). Hemodynamic evaluation and monitoring in the ICU. *Chest* 132, 2020-2029.

Plachky J, Hofer S, Volkmann M, Martin E, Bardenheuer HJ, & Weigand MA (2004). Regional cerebral oxygen saturation is a sensitive marker of cerebral hypoperfusion during orthotopic liver transplantation. *Anesth Analg* 99, 344-9.

Plotkin AJ, Wade CE, Jenkins DH, Smith KA, Noe JC, Park MS, Perkins JG, & Holcomb JB (2008). A reduction in clot formation rate and strength assessed by thrombelastography is indicative of transfusion requirements in patients with penetrating injuries. *J Trauma* 64, S64-S68.

Pott F, Larsen FS, Ejlersen E, Linkis P, Jorgensen LG, & Secher NH (1995). Cerebral perfusion during human liver transplantation. *Clin Physiol* 15, 119-130.

Rasmussen P, Dawson EA, Nybo L, van Lieshout JJ, Secher NH, & Gjedde A (2007). Capillary-oxygenation-level-dependent near-infrared spectrometry in frontal lobe of humans. *J Cereb Blood Flow Metab* 27, 1082-1093.

Rivers E (2006). Mixed vs central venous oxygen saturation may be not numerically equal, but both are still clinically useful. *Chest* 129, 507-508.

Sander-Jensen K, Secher NH, Astrup A, Christensen NJ, Giese J, Schwartz TW, Warberg J, & Bie P (1986). Hypotension induced by passive head-up tilt: endocrine and circulatory mechanisms. *Am J Physiol* 251, R742-R748.

Sato K, Ogoh S, Hirasawa A, Oue A, & Sadamoto T (2011). The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J Physiol* 589, 2847-2856.

Schroeder RA, Collins BH, Tuttle-Newhall E, Robertson K, Plotkin J, Johnson LB, & Kuo PC (2004). Intraoperative fluid management during orthotopic liver transplantation. *J Cardiothorac Vasc Anesth* 18, 438-441.

Secher NH & Bie P (1985). Bradycardia during reversible haemorrhagic shock--a forgotten observation? *Clin Physiol* 5, 315-323.

Secher NH, Jacobsen J, Friedman DB, & Matzen S (1992). Bradycardia during reversible hypovolaemic shock: associated neural reflex mechanisms and clinical implications. *Clin Exp Pharmacol Physiol* 19, 733-743.

Secher NH & van Lieshout JJ (2009). Hypovolaemic shock and pain. *Exp Physiol* 94, 626.

Segal JB & Dzik WH (2005). Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. *Transfusion* 45, 1413-1425.

Shibasaki M, Wilson TE, Bundgaard-Nielsen M, Seifert T, Secher NH, & Crandall CG (2011). Modelflow underestimates cardiac output in heat-stressed individuals. *Am J Physiol Regul Integr Comp Physiol* 300, R486-R491.

Shimamoto H, Kito H, Kawazoe K, Fujita T, & Shimamoto Y (1992). Transoesophageal Doppler echocardiographic measurement of cardiac output by the mitral annulus method. *Br Heart J* 68, 510-515.

Shoemaker WC (1972). Cardiorespiratory patterns of surviving and nonsurviving postoperative patients. *Surg Gynecol Obstet* 134, 810-814.

Shoemaker WC, Appel PL, Kram HB, Waxman K, & Lee TS (1988). Prospective trial of supranormal values of survivors as therapeutic goals in high-risk surgical patients. *Chest* 94, 1176-1186.

Skak C, Ejlersen E, Mogensen T, Winkel P, & Secher NH (1996). Plasma magnesium during human liver transplantation. *Clin Transplant* 10, 157-159.

Skak C, Rasmussen A, Kirkegaard P, & Secher NH (1997). Cerebral oxygen saturation and blood flow during liver transplantation. *Anesth Analg* 84, 730-733.

Smith TG, Balanos GM, Croft QP, Talbot NP, Dorrington KL, Ratcliffe PJ, & Robbins PA (2008). The increase in pulmonary arterial pressure caused by hypoxia depends on iron status. *J Physiol* 586, 5999-6005.

Snyder EM, Beck KC, Dietz NM, Eisenach JH, Joyner MJ, Turner ST, & Johnson BD (2006). Arg16Gly polymorphism of the beta2-adrenergic receptor is associated with differences in cardiovascular function at rest and during exercise in humans. *J Physiol* 571, 121-130.

Soller BR, Yang Y, Soyemi OO, Ryan KL, Rickards CA, Walz JM, Heard SO, & Convertino VA (2008). Noninvasively determined muscle oxygen saturation is an early indicator of central hypovolemia in humans. *J Appl Physiol* 104, 475-481.

Stammers AH, Murdock JD, Klayman MH, Trowbridge C, Yen BR, Franklin D, & Elmore J (2005). Utilization of rapid-infuser devices for massive blood loss. *Perfusion* 20, 65-69.

Starzl TE, Klintmalm GB, Porter KA, Iwatsuki S, & Schroter GP (1981). Liver transplantation with use of cyclosporin a and prednisone. *N Engl J Med* 305, 266-269.

Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, & Brittain.R.S (1963). Homotransplantation of the liver in humans. *Surg Gynecol Obstet* 117, 659-676.

Steiner LA, Pfister D, Strebel SP, Radolovich D, Smielewski P, & Czosnyka M (2009). Near-infrared spectroscopy can monitor dynamic cerebral autoregulation in adults. *Neurocrit Care* 10, 122-128.

Struthers AD, Reid JL, Whitesmith R, & Rodger JC (1983). Effect of intravenous adrenaline on electrocardiogram, blood pressure, and serum potassium. *Br Heart J* 49, 90-93.

Takagi H, Manabe H, Kawai N, Goto SN, & Umemoto T (2009). Aprotinin increases mortality as compared with tranexamic acid in cardiac surgery: a meta-analysis of randomized head-to-head trials. *Interact Cardiovasc Thorac Surg* 9, 98-101.

Thomson SJ, Cowan ML, Forton DM, Clark SJ, Musa S, Grounds M, & Rahman TM (2009). A study of muscle tissue oxygenation and peripheral microcirculatory dysfunction in cirrhosis using near infrared spectroscopy. *Liver Int*.

Thys DM, Hillel Z, Goldman ME, Mindich BP, & Kaplan JA (1987). A comparison of hemodynamic indices derived by invasive monitoring and two-dimensional echocardiography. *Anesthesiology* 67, 630-634.

Tolle JJ, Waxman AB, Van Horn TL, Pappagianopoulos PP, & Systrom DM (2008). Exercise-induced pulmonary arterial hypertension. *Circulation* 118, 2183-2189.

van Lieshout JJ, Wieling W, Karemaker JM, & Secher NH (2003). Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol* 94, 833-848.

Waters JH, Gottlieb A, Schoenwald P, Popovich MJ, Sprung J, & Nelson DR (2001). Normal saline versus lactated Ringer's solution for intraoperative fluid management in patients undergoing abdominal aortic aneurysm repair: an outcome study. *Anesth Analg* 93, 817-822.

Wesseling KH, Jansen JR, Settels JJ, & Schreuder JJ (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* 74, 2566-2573.

Wilson TE, Brothers RM, Tollund C, Dawson EA, Nissen P, Yoshiga CC, Jons C, Secher NH, & Crandall CG (2009). Effect of thermal stress on Frank-Starling relations in humans. *J Physiol* 587, 3383-3392.

Yao FS, Tseng CC, Ho CY, Levin SK, & Illner P (2004). Cerebral oxygen desaturation is associated with early postoperative neuropsychological dysfunction in patients undergoing cardiac surgery. *J Cardiothorac Vasc Anesth* 18, 552-558.

18

The Post-Reperfusion Syndrome (PRS): Diagnosis, Incidence and Management

Kyota Fukazawa and Ernesto A. Pretto, Jr.
University of Miami, Miller School of Medicine, Miami, Florida USA

1. Introduction

Despite the remarkable advances in the peri-operative management of the liver transplant recipient, the post-reperfusion syndrome (PRS) continues to be an important intra-operative risk factor for impaired graft function, and morbidity and mortality of the recipient. In order to institute preventive measures several studies have attempted to elucidate risk factors for PRS. Those identified risk factors and proposed mechanisms underlying the development of PRS, as well as current issues with PRS will be discussed in the following three sections.

2. Diagnosis

The post-reperfusion syndrome (PRS) which occurs during liver transplantation was first diagnosed by Aggarwal et al in 1987 and described as cardiovascular collapse following revascularization of the liver graft. They defined PRS as severe hemodynamic instability, persistent hypotension (a greater than 30% drop below the anhepatic mean arterial blood pressure (MAP) within 5 minutes of reperfusion and sustained for at least 1 minute), accompanied by asystole, or significant arrhythmias as well as development of significant, fibrinolysis requiring treatment. Up until now this definition remains the same with some modifications. Hilmi et al defined *mild* PRS as a drop in MAP to less than 30% of mean baseline MAP observed during the anhepatic stage associated with bradycardia and sustained for less than 5 minutes, and requiring calcium or epinephrine boluses, but without the need for continuous vasopressor infusion. *Severe* PRS was defined as persistent severe hypotension with a greater than 30% reduction in MAP from mean baseline MAP during the anhepatic stage, associated with asystole, significant arrhythmias, and requiring prolonged vasopressor infusion (until end of surgery) and fibrinolysis. Other reports have used only persistent hypotension as the defining endpoint for PRS. Therefore, inconsistency of definitions makes it difficult to draw conclusions concerning the precise incidence of PRS, its clinical presentation and causes.

These definitions rely on the value of the percent change in MAP from the mean baseline MAP observed during the anhepatic stage. It so happens that the anhepatic stage is fraught with hemodynamic fluctuations related to manipulation of the inferior vena cava (IVC), blood loss, veno-venous bypass, hypothermia, metabolic acidosis, etc., which are common during the an-hepatic phase. These multi-factorial hemodynamic perturbations call into

Severe hemodynamic instability
Persistent hypotension
Geater than 30% of the anhepatic MAP within 5 minutes sustained for at least 1 minute
Asystole
Significant arrhythmias
Development of significant fibrinolysis

Table 1. Definition of Post-Reperfusion Syndrome (PRS) *Abbreviations: MAP: Mean Arterial Blood Pressure*

question the reliability and accuracy of PRS incidence and severity based on one parameter namely, % change in MAP. Moreover, pre-treatment with bolus doses of vasopressors, including calcium chloride, vasopressin or methylene blue immediately prior to reperfusion of the portal vein, intended to preempt severe hypotension post-reperfusion also introduce errors into the calculation of % changes in MAP, before and after reperfusion. More importantly, in some instances there may be a complete absence of hemodynamic instability even in the presence of severe graft dysfunction following reperfusion when portal vein flow is inadequate. This is called the no-reflow phenomenon. No-reflow is associated with high vascular resistance in the microcirculation of the graft secondary to multiple factors, such as: tissue edema, leukocyte plugging and the accumulation of pro-inflammatory factors and cellular debris; vasoconstriction of the tissues due to cold preservation, portal vein thrombosis, or presence of large collateral veins (porto-systemic shunts). In those cases, there is a gradual resolution of no-reflow and hemodynamic fluctuations may be delayed beyond the immediate portal vein or even hepatic artery reperfusion periods when the organ is better perfused. For these reasons, PRS incidence may not be accurately ascertained when a narrow window of MAP readings is used in its determination. Likewise, major changes in MAP in the immediate post-reperfusion period may or may not be associated with graft quality. Although cardiovascular collapse following reperfusion is common in liver transplant practice, it is essential to elucidate the underlying mechanisms of PRS, and to determine more accurately the relationship between PRS and graft quality. Is PRS a cause of poor graft quality or is it a consequence? The definitive answer to this question can only be found in the conduct of a blinded controlled prospective study with well-defined end points in a large cohort of patients. The results of such a study would help to broaden the scope of what constitutes the diagnosis of PRS.

3. Incidence of PRS and confounding factors

To date, there is wide variation in the reported incidence of PRS (5.9-61.3%). There are several factors attributable to these wide variations, among these are: differences in surgical technique, intraoperative hemodynamic management, as well as chronological and geographical factors. Piggyback technique is used for implantation of the liver graft without interrupting IVC flow. It was first introduced into human liver transplantation in the late 1980's. Veno-venous bypass (VVB) was also introduced in the 1980's. Those techniques were developed to enable more stable hemodynamics upon manipulation of the IVC during orthotopic liver transplantation. Because volume status of the recipient before reperfusion can be an important risk factor for PRS, more stable hemodynamics before reperfusion may decrease its incidence, although the impact of surgical technique between conventional or

Author	Year	N	Country	Type of Study	Incidence of PRS	Identified Risk Factors for PRS	Outcome
Ryu HG et. al.	2011	62	Korea	Blind Controlled Prospective Study	-	Vasodilator release form graft liver (Kallikrein-Kinin)	-
Garcia-Gil FA et al.	2011	153	Spain	Blind Controlled Prospective Study	-	Type of Preservation Solution	-
Bukowicka B et al.	2011	340	Poland	Retrospective Chart Review	12.1%	Cold Ischemia Time, Transplant Technique*, Operating Time, Transfusion Requirements during	Lower Graft Survival, Higher Incidence of Retransplantation
Fukazawa K et al.	2011	715	USA	Retrospective Chart Review	55.7%	Donor-Recipient Size Mismatch, Donor Age	-
Siniscalchi A et al.	2010	58	Italy	Retrospective Chart Review	41.0%	MELD, Preoperative Creatinine	-
Paugam-Burtz et al.	2009	75	France	Prospective Study	25.0%	PortoCaval Shunt, Cold Ischemia Time	-
Hilmi I et al.	2008	338	USA	Retrospective Chart Review	55.0%	Recipient Age	Higher Transfusion Requirements, Longer Vent Support, ICU stay, and Hospital Stay, Higher Incidence of Retransplantation
Ko JS et. al.	2008	87	Korea	Retrospective Chart Review	28.6% (UW) 61.3% (HTK)	Type of Preservation Solution	-
Homvises B et. al.	2008	20	Thailand	Retrospective Chart Review	-	Volume of Flush Before Reperfusion	-
Pertejo MA et. al.	2007	551	Spain	Retrospective Chart Review	16-27%	-	Initial Poor Graft Function

Table 2. (continues on next page) Reported Incidence of PRS *Transplant technique: conventional inferior vena cava anastomosis versus piggyback anastomosis**Sequence of reperfusion: initial hepatic artery revascularization versus initial portal vein revascularization. *Abbreviations: CVP: central venous pressure, HTK: histidine-triptophan-ketoglutarate solution, ICU: intensive care unit, IVC: inferior vena cava, MELD: models for end stage liver disease, UW: University of Wisconsin solution, VVB: veno-venous bypass*

Moreno et. al.	2006	30	Spain	Randomized Prospective Study	36% (HAR) 42.5% (PVR)	Sequence of Reperfusion**	-
Ayanoglu et al.	2003	145	Turkey	Retrospective Chart Review	48.9%	Long Anhepatic Period, Higher Calcium Requirement, Lower CVP	-
Nanashima et al.	2001	93	Australia	Retrospective Chart Review	29.0%	-	-
Chui A K et al.	2000	321	Australia	Retrospective Chart Review	12.8%	Cold Ischemia Time	Hyperkalemia
Acosta F et al.	1999	32	Spain	Retrospective Chart Review	-	Preemptive Phenylephrine Treatment	-
Acosta F et al.	1999	41	Spain	Retrospective Chart Review	-	Preemptive Atropine Treatment	-
Acosta F et al.	1999	71	Spain	Retrospective Chart Review	-	Transplant technique	-
Garutti M et al.	1997	94	Spain	Retrospective Chart Review	28.7%	Response to IVC clamp	-
Jugan E et al.	1992	58	France	Prospective Study	20.0%	(No difference between with and without VVB)	-

Table 2. (continued) Reported Incidence of PRS *Transplant technique: conventional inferior vena cava anastomosis versus piggyback anastomosis**Sequence of reperfusion: initial hepatic artery revascularization versus initial portal vein revascularization. *Abbreviations: CVP: central venous pressure, HTK: histidine-triptophan-ketoglutarate solution, ICU: intensive care unit, IVC: inferior vena cava, MELD: models for end stage liver disease, UW: university of Wisconsin solution, VVB: veno-venous bypass*

piggy-back techniques on PRS is still in debate. In addition, VVB reduces small bowel edema, which has been suggested as a primary site for the production and release of potent vasoactive inflammatory mediators. With the improvement of transplant outcomes and recognition of risk factors for graft survival, expanded criteria donors (ECD) have been more frequently used due to severe shortages of organ donors. More frequent usage of ECD as well as the introduction of new surgical techniques may affect the incidence of PRS, depending on the era, a chronological factor. Also, geographical areas with low organ donor conversion rates and acute shortage of organs for transplantation will invariably result in higher usage rates of ECD, as well as sicker recipients due to longer waiting times. Therefore, the incidence of PRS will vary with geographical area. The interpretation of those results also needs to take chronological and geographical factors into account as confounding factors.

4. Risk factors for PRS

Well-established risk factors and recognized mechanisms associated with PRS include: i) volume status of the recipient before reperfusion, ii) myocardial depression due to embolization of cold preservation solution into the systemic circulation, and iii) release of vasoactive pro-inflammatory factors originating in activated Kupffer cells of the post-ischemic liver graft.

4.1 Pre-reperfusion volume status

The liver is an important reservoir of blood, containing a total of about 250-500ml or 18-30ml/100g of blood. In liver transplantation, the donor liver graft will be rapidly filled with recipient blood following revascularization of the portal vein, resulting in immediate volume shifts and occasionally, hypotension. De La Morena et al. determined that an insufficient increase in preload is a main causative factor of PRS (or reperfusion hypotension) in an observational study using transesophageal echocardiography (TEE). Maintaining a high cardiac output is essential to ensure adequate perfusion of organs in liver transplantation. However, high cardiac output can be accomplished by maintaining preload, which may be difficult given that the operation itself is associated with major changes in volume and afterload, in addition to blood loss, third space losses, and ongoing ascites production. This result will support the hypothesis that volume shift is one of the main components of reperfusion hypotension and maintaining adequate preload before reperfusion is crucial. Also, size mismatch between donor and recipient can cause additional volume shifts, as evidenced by the fact that a large-for-size donor relative to recipient body size causes more severe reperfusion hypotension. The BSA index (BSAi) may help to more accurately match donor and recipient organs in whole organ liver transplantation.

4.2 Myocardial performance

In addition to volume shifts, a change in myocardial performance is another component of reperfusion hypotension/PRS. Myocardial performance is reduced by a decrease in temperature, acid-base and electrolyte disturbances, which are all caused by the flushing of residual preservation solution into the systemic circulation after revascularization of the graft. Acidosis following reperfusion due to release of acidic fluid from ischemic bowel and liver graft is a common finding in liver transplantation. Acute acidosis can cause

tachycardia, dysrrythmias, and severe myocardial depression by producing changes in resting membrane potential and threshold potential and an increase in the rate of phase IV depolarization. Therefore the use of VVB has been proposed to decrease the incidence of PRS by minimizing small bowel edema/ischemia with varying results.

4.3 Donor factors

Lastly, the release of inflammatory factors from the post-ischemic donor liver graft into the recipient systemic circulation also can trigger a systemic inflammatory chain reaction that can lead to systemic hypotension and multi-organ dysfunction. Therefore, the quality of the donor can be a risk factor for PRS, and postoperative graft function (primary non-function, and graft survival). A donor organ with certain characteristics such as extreme age, adverse past medical history, preexisting liver damage or disease, obesity, hemodynamic instabilities, deceased cardiac donor (DCD), risk of sepsis and malignancies, hypernatremia, and prolonged ICU stay, may be more susceptible to ischemia, and more likely to have higher incidence of primary non-function (PNF), delayed function or subsequent risk for reduction in long-term graft survival. Similarly, several studies have attempted to identify donor risk factors for PRS. Hilmi et al reported warm ischemia time as a risk factor and more recently Paugam-Burtz, et al reported cold ischemia time is also a risk for PRS. In addition to those prior studies, donor age is an important risk factor for PRS. The older donor graft has a lower tolerance for hypoxia, and a greater susceptibility to reperfusion injury, probably due to metabolic changes associated with senescence, age related atherosclerotic changes in vascular structures , or steatotic changes of the parenchyma. The type of preservation solution and the graft flushing techniques have also been shown to affect the severity of PRS.

5. Strategy for the intraoperative management of PRS

Warm recipient blood flows into the cold organ after revascularization of the portal vein, causing immediate volume shifts, re-oxygenation of the ischemic organ, and outflow of cold preservation solution saturated with vasoactive pro-inflammatory factors. Optimization of volume status in the recipient prior to reperfusion may minimize hemodynamic changes related to volume shifts by maximizing hemodynamic capacity to maintain good perfusion pressure throughout the liver graft, especially the large-for-size graft. TEE coupled with close monitoring of hemodynamic parameters with continuous cardiac output (CO)/SvO2, and standard cardiac monitors are particularly helpful to ensure hemodynamic integrity of the recipient. To obtain sufficient perfusion of the liver graft, especially through the hepatic microcirculation, vasodilating agents such as prostaglandin or calcium channel blockers can be used but with caution since those agents can aggravate hemodynamic instability. To minimize the sudden outflow of preservation solution from the liver graft, flushing the organ prior to reperfusion has been used and currently proposed as the most effective way of preventing hemodynamic instability associated with the inadvertent release of preservation solution. Ingredients of the flush solution as well as rate of infusion, temperature of flush solution, and amount of solution are still under investigation. Gradual and homogeneous perfusion of the organ by machine perfusion immediately prior to implantation may improve graft function by effectively removing the pro-inflammatory factors from the graft microcirculation, may reduce PRS and improve short and long term graft survival, especially in the ECD. Preemptive use of vasopressors may help maintain the

systemic blood pressure but may not improve the % change in blood pressure or graft outcome. Preemptive use of oxidative free radical scavenging agents or attempts at counteracting vasoactive inflammatory factors is another area of research. For example, methylene blue has been used to scavenge nitric oxide related vasodilation in various shock states. Also, several oxidative free radical scavengers, vasodilators (inhaled nitric oxide, prostaglandin E), and Ibuprofen (cyclooxygenase inhibitor) have been studied as a way to suppress the pro-inflammatory cascade post-reperfusion. The benefits of these treatment strategies on PRS and postoperative graft function are inconclusive, and larger clinical trials are needed. This will require a consortium of leading academic liver transplant centers to conduct multicenter clinical trials. To minimize the impairment of cardiac performance following reperfusion, electrolyte abnormalities, especially hypocalcemia and hyperkalemia need to be corrected prior to reperfusion. Magnesium replacement may need to be considered, especially when there is hypocalcemia due to intraoperative transfusion and citrate intoxication. Citrate binds magnesium as well as calcium, causing acute hypomagnesemia.

6. Conclusions

After having contributed to the establishment of liver transplantation as a safe and definitive treatment option for patients with end stage liver disease, including those with ESLD complicated by hepatocarcinomas, in the latter half of the 20th century, the next challenge to Transplant Anesthesiologists and Critical Care Specialists in the 21st is to continue to improve the perioperative management of the donor and organ transplant recipient during the most critical period of the liver transplant procedure namely, revascularization, reperfusion and re-oxygenation of the graft. This effort will undoubtedly require multicenter research collaborations, the determination of better and more reliable end-points for PRS and graft function, and the institution and conduct of multicenter clinical trials. Inevitably research will most likely reveal the need for a multi-factorial treatment strategy to preempt or mitigate PRS and the deterioration of graft function, especially of the ECD graft. This effort comprises preconditioning of the donor, preservation and post condition of the recipient transplanted, a process that can be succinctly described as 'organ resuscitation'. Better post-transplant outcomes will decrease the costs involved in re-transplantation, and prolonged ICU and hospital stays.

7. References

Acosta, F., Rodriguez, M. A., Sansano, T., Contreras, R. F., Reche, M., Roques, V., Beltran, R., Robles, R., Bueno, F. S., Ramirez, P. & Parrilla, P. (1999). Influence of surgical technique on postreperfusion syndrome during liver transplantation. *Transplant Proc*, 31, 6, (Sep,1999), pp. 2380-2381,0041-1345 (Print) 0041-1345 (Linking)

Acosta, F., Sansano, T., Contreras, R. F., Reche, M., Beltran, R., Roques, V., Rodriguez, M. A., Robles, R., Bueno, F. S., Ramirez, P. & Parrilla, P. (1999). Atropine prophylaxis of the postreperfusion syndrome in liver transplantation. *Transplant Proc*, 31, 6, (Sep,1999), pp. 2377,0041-1345 (Print) 0041-1345 (Linking)

Acosta, F., Sansano, T., Contreras, R. F., Reche, M., Roques, V., Beltran, R., Rodriguez, M. A., Robles, R., Bueno, F. S., Ramirez, P. & Parrilla, P. (1999). Phenylephrine treatment of the postreperfusion syndrome in liver transplantation. *Transplant Proc*, 31, 6, (Sep,1999), pp. 2373-2374,0041-1345 (Print) 0041-1345 (Linking)

Aggarwal, S., Kang, Y., Freeman, J. A., Fortunato, F. L. & Pinsky, M. R. (1987). Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. *Transplant Proc*, 19, 4 Suppl 3, (Aug,1987), pp. 54-55,0041-1345 (Print) 0041-1345 (Linking)

Ayanoglu, H. O., Ulukaya, S. & Tokat, Y. (2003). Causes of postreperfusion syndrome in living or cadaveric donor liver transplantations. *Transplant Proc*, 35, 4, (Jun,2003), pp. 1442-1444,0041-1345 (Print) 0041-1345 (Linking)

Blankensteijn, J. D., Schlejen, P. M., Groenland, T. H. & Terpstra, O. T. (1992). The effects of long-term graft preservation and prostaglandin E1 on intraoperative hemodynamic changes in liver transplantation. A comparison between orthotopic and heterotopic transplantation in the pig. *Transplantation*, 54, 3, (Sep,1992), pp. 423-428,0041-1337 (Print) 0041-1337 (Linking)

Bukowicka, B., Abi Akar, R., Olszewska, A., Smoter, P. & Krawczyk, M. (2011). The occurrence of postreperfusion syndrome in orthotopic liver transplantation and its significance in terms of complications and short-term survival. *Ann Transplant*, 16, 2, (Jun 30,2011), pp. 26-30,1425-9524 (Print) 1425-9524 (Linking)

Chui, A. K., Shi, L., Tanaka, K., Rao, A. R., Wang, L. S., Bookallil, M., Mayr, M., Chiu, E., Verran, D. J., Mears, D. & Sheil, A. G. (2000). Postreperfusion syndrome in orthotopic liver transplantation. *Transplant Proc*, 32, 7, (Nov,2000), pp. 2116-2117,0041-1345 (Print) 0041-1345 (Linking)

Clements, S. D., Jr. & Hurst, J. W. (1972). Diagnostic value of electrocardiographic abnormalities observed in subjects accidentally exposed to cold. *Am J Cardiol*, 29, 5, (May,1972), pp. 729-734,0002-9149 (Print) 0002-9149 (Linking)

Cordovi de Armas, L., Jimenez Paneque, R. E., Gala Lopez, B., Rapalo Romero, E. I., Anuez Castillo, Y. & Vallongo Menendez, M. B. (2010). Rapid and homogeneous reperfusion as a risk factor for postreperfusion syndrome during orthotopic liver transplantation. *Rev Bras Anestesiol*, 60, 2, (Apr,2010), pp. 154-161, 188-192,1806-907X (Electronic) 0034-7094 (Linking)

de la Morena, G., Acosta, F., Villegas, M., Bento, M., Sansano, T., Bueno, F. S., Ramirez, P., Ruiperez, J. A. & Parrilla, P. (1994). Ventricular function during liver reperfusion in hepatic transplantation. A transesophageal echocardiographic study. *Transplantation*, 58, 3, (Aug 15,1994), pp. 306-310,0041-1337 (Print) 0041-1337 (Linking)

Diaz, J., Acosta, F., Parrilla, P., Sansano, T., Tornel, P. L., Robles, R., Ramirez, P., Bueno, F. S. & Martinez, P. (1996). Serum ionized magnesium monitoring during orthotopic liver transplantation. *Transplantation*, 61, 5, (Mar 15,1996), pp. 835-837,0041-1337 (Print) 0041-1337 (Linking)

Evgenov, O. V. & Bjertnaes, L. J. (2003). Administration of methylene blue in human septic shock: renaissance of an old drug? *Crit Care Med*, 31, 5, (May,2003), pp. 1601-1602; author reply 1602,0090-3493 (Print) 0090-3493 (Linking)

Fukazawa, K., Nishida, S., Volsky, A., Tzakis, A. G. & Pretto, E. A., Jr. (2011). Body surface area index predicts outcome in orthotopic liver transplantation. *J Hepatobiliary Pancreat Sci*, 18, 2, (Mar,2011), pp. 216-225,1868-6982 (Electronic)

Fukazawa, K. & Pretto, E. A. (2011). The effect of methylene blue during orthotopic liver transplantation on post reperfusion syndrome and postoperative graft function. *J Hepatobiliary Pancreat Sci*, 18, 3, (May,2011), pp. 406-413,1868-6982 (Electronic)

Garcia-Gil, F. A., Serrano, M. T., Fuentes-Broto, L., Arenas, J., Garcia, J. J., Guemes, A., Bernal, V., Campillo, A., Sostres, C., Araiz, J. J., Royo, P. & Simon, M. A. (2011). Celsior versus university of wisconsin preserving solutions for liver transplantation: postreperfusion syndrome and outcome of a 5-year prospective randomized controlled study. *World J Surg*, 35, 7, (Jul,2011), pp. 1598-1607,1432-2323 (Electronic) 0364-2313 (Linking)

Grazi, G. L., Cescon, M., Ravaioli, M., Ercolani, G., Pierangeli, F., D'Errico, A., Ridolfi, L., Cavallari, A. & Mazziotti, A. (2001). A revised consideration on the use of very aged donors for liver transplantation. *Am J Transplant*, 1, 1, (May,2001), pp. 61-68,1600-6135 (Print)

Griffith, B. P., Shaw, B. W., Jr., Hardesty, R. L., Iwatsuki, S., Bahnson, H. T. & Starzl, T. E. (1985). Veno-venous bypass without systemic anticoagulation for transplantation of the human liver. *Surg Gynecol Obstet*, 160, 3, (Mar,1985), pp. 270-272,0039-6087 (Print) 0039-6087 (Linking)

Hilmi, I., Horton, C. N., Planinsic, R. M., Sakai, T., Nicolau-Raducu, R., Damian, D., Gligor, S. & Marcos, A. (2008). The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. *Liver Transpl*, 14, 4, (Apr,2008), pp. 504-508,1527-6473 (Electronic) 1527-6465 (Linking)

Himmelreich, G., Hundt, K., Neuhaus, P., Bechstein, W. O., Roissant, R. & Riess, H. (1993). Evidence that intraoperative prostaglandin E1 infusion reduces impaired platelet aggregation after reperfusion in orthotopic liver transplantation. *Transplantation*, 55, 4, (Apr,1993), pp. 819-826,0041-1337 (Print) 0041-1337 (Linking)

Homvises, B., Sirivatanauksorn, Y., Limsrichamrern, S., Pongraweewan, O., Sujirattanawimol, K. & Raksakietisak, M. (2008). The minimal flush volume for washout of preservation fluid in liver transplantation. *Transplant Proc*, 40, 7, (Sep,2008), pp. 2123-2126,0041-1345 (Print) 0041-1345 (Linking)

Ickx, B., Walker, S. & Farman, J. V. (1987). Ionized calcium levels during liver transplantation. *Eur J Anaesthesiol*, 4, 6, (Nov,1987), pp. 421-427,0265-0215 (Print) 0265-0215 (Linking)

Jawan, B., de Villa, V., Luk, H. N., Chen, Y. S., Chiang, Y. C., Wang, C. C., Wang, S. H., Cheng, Y. F., Huang, T. L., Eng, H. L., Liu, P. P. & Chen, C. L. (2003). Ionized calcium changes during living-donor liver transplantation in patients with and without administration of blood-bank products. *Transpl Int*, 16, 7, (Jul,2003), pp. 510-514,0934-0874 (Print) 0934-0874 (Linking)

Jugan, E., Albaladejo, P., Jayais, P. & Ecoffey, C. (1992). The failure of venovenous bypass to prevent graft liver postreperfusion syndrome. *Transplantation*, 54, 1, (Jul,1992), pp. 81-84,0041-1337 (Print) 0041-1337 (Linking)

Kampmann, J. P., Sinding, J. & Moller-Jorgensen, I. (1975). Effect of age on liver function. *Geriatrics*, 30, 8, (Aug,1975), pp. 91-95,0016-867X (Print)

Kawamoto, S., Tashiro, S., Miyauchi, Y. & Inoue, M. (1991). Mechanism for enterohepatic injury caused by circulatory disturbance of hepatic vessels in the rat. *Proc Soc Exp Biol Med*, 198, 1, (Oct,1991), pp. 629-635,0037-9727 (Print) 0037-9727 (Linking)

Kirov, M. Y., Evgenov, O. V., Evgenov, N. V., Egorina, E. M., Sovershaev, M. A., Sveinbjornsson, B., Nedashkovsky, E. V. & Bjertnaes, L. J. (2001). Infusion of

methylene blue in human septic shock: a pilot, randomized, controlled study. *Crit Care Med*, 29, 10, (Oct,2001), pp. 1860-1867,0090-3493 (Print) 0090-3493 (Linking)

Ko, J. S., Kim, G. S., Gwak, M. S., Yang, M., Kim, H. K., Shin, B. S., Kim, J. K. & Lee, S. K. (2008). Greater hemodynamic instability with histidine-tryptophan-ketoglutarate solution than University of Wisconsin solution during the reperfusion period in living donor liver transplantation. *Transplant Proc*, 40, 10, (Dec,2008), pp. 3308-3310,0041-1345 (Print) 0041-1345 (Linking)

Kulpmann, W. R., Rademacher, E. & Bornscheuer, A. (1996). Ionized magnesium concentration during liver transplantation, resection of the liver and cardiac surgery. *Scand J Clin Lab Invest Suppl*, 224, 1996), pp. 235-243,0085-591X (Print) 0085-591X (Linking)

Leggett, R. W. & Williams, L. R. (1991). Suggested reference values for regional blood volumes in humans. *Health Phys*, 60, 2, (Feb,1991), pp. 139-154,0017-9078 (Print) 0017-9078 (Linking)

Leight, L., Roush, G., Rafi, E. & McGaff, C. J. (1963). The Effect of Intravenous Potassium on Myocardial Contractility and Cardiac Dynamics. *Am J Cardiol*, 12, (Nov,1963), pp. 686-691,0002-9149 (Print) 0002-9149 (Linking)

Marczin, N., Tekeres, M., Salzman, A. L. & Szabo, C. (1995). Methylene blue infusion in septic shock. *Crit Care Med*, 23, 11, (Nov,1995), pp. 1936-1938,0090-3493 (Print) 0090-3493 (Linking)

Marino, I. R. & De Luca, G. (1985). Orthotopic liver transplantation in pigs. An evaluation of different methods of avoiding the revascularization syndrome. *Transplantation*, 40, 5, (Nov,1985), pp. 494-498,0041-1337 (Print) 0041-1337 (Linking)

Marquez, J., Martin, D., Virji, M. A., Kang, Y. G., Warty, V. S., Shaw, B., Jr., Sassano, J. J., Waterman, P., Winter, P. M. & Pinsky, M. R. (1986). Cardiovascular depression secondary to ionic hypocalcemia during hepatic transplantation in humans. *Anesthesiology*, 65, 5, (Nov,1986), pp. 457-461,0003-3022 (Print) 0003-3022 (Linking)

Marzi, I., Knee, J., Menger, M. D., Harbauer, G. & Buhren, V. (1991). Hepatic microcirculatory disturbances due to portal vein clamping in the orthotopic rat liver transplantation model. *Transplantation*, 52, 3, (Sep,1991), pp. 432-436,0041-1337 (Print) 0041-1337 (Linking)

Moreno, C., Sabate, A., Figueras, J., Camprubi, I., Dalmau, A., Fabregat, J., Koo, M., Ramos, E., Llado, L. & Rafecas, A. (2006). Hemodynamic profile and tissular oxygenation in orthotopic liver transplantation: Influence of hepatic artery or portal vein revascularization of the graft. *Liver Transpl*, 12, 11, (Nov,2006), pp. 1607-1614,1527-6465 (Print) 1527-6465 (Linking)

Nanashima, A., Pillay, P., Crawford, M., Nakasuji, M., Verran, D. J. & Painter, D. (2001). Analysis of postrevascularization syndrome after orthotopic liver transplantation: the experience of an Australian liver transplantation center. *J Hepatobiliary Pancreat Surg*, 8, 6, 2001), pp. 557-563,0944-1166 (Print) 0944-1166 (Linking)

Oishi, M., Tanaka, N. & Orita, K. (1996). Beneficial effects of prostaglandin E1 on hemodynamic changes during liver transplantation in pigs. *Transpl Int*, 9 Suppl 1, 1996), pp. S100-104,0934-0874 (Print) 0934-0874 (Linking)

Olschewski, P., Gass, P., Ariyakhagorn, V., Jasse, K., Hunold, G., Menzel, M., Schoning, W., Schmitz, V., Neuhaus, P. & Puhl, G. (2010). The influence of storage temperature

during machine perfusion on preservation quality of marginal donor livers. *Cryobiology*, 60, 3, (Jun,2010), pp. 337-343,1090-2392 (Electronic) 0011-2240 (Linking)

Paugam-Burtz, C., Kavafyan, J., Merckx, P., Dahmani, S., Sommacale, D., Ramsay, M., Belghiti, J. & Mantz, J. (2009). Postreperfusion syndrome during liver transplantation for cirrhosis: outcome and predictors. *Liver Transpl*, 15, 5, (May,2009), pp. 522-529,1527-6473 (Electronic) 1527-6465 (Linking)

Pertejo, M. A., Torres, J. G., Gillem, P. G., Jimenez, J. C., Rodriguez, F. S., Argente, G. R. & Aleixandre, I. S. (2007). Initial poor function in the age of old donors: prognostic factors. *Transplant Proc*, 39, 7, (Sep,2007), pp. 2109-2111,0041-1345 (Print) 0041-1345 (Linking)

Pittet, J. F., Morel, D. R., Mentha, G., Le Coultre, C., Suter, P. M. & Rohner, A. (1991). Protective effect of indomethacin in the development of the postreperfusion syndrome during liver transplantation in pigs. *Transplant Proc*, 23, 4, (Aug,1991), pp. 2290-2296,0041-1345 (Print) 0041-1345 (Linking)

Preiser, J. C., Lejeune, P., Roman, A., Carlier, E., De Backer, D., Leeman, M., Kahn, R. J. & Vincent, J. L. (1995). Methylene blue administration in septic shock: a clinical trial. *Crit Care Med*, 23, 2, (Feb,1995), pp. 259-264,0090-3493 (Print) 0090-3493 (Linking)

Pretto, E. A., Jr. (2006). Perioperative management of the recipient of the extended criteria cadaveric donor liver (ECDL): a metabolic approach. *Int Anesthesiol Clin*, 44, 4, (Fall,2006), pp. 79-96,0020-5907 (Print) 0020-5907 (Linking)

Ronholm, E., Tomasdottir, H., Runeborg, J., Bengtsson, A., Bengtson, J. P., Stenqvist, O. & Friman, S. (1994). Complement system activation during orthotopic liver transplantation in man. Indications of peroperative complement system activation in the gut. *Transplantation*, 57, 11, (Jun 15,1994), pp. 1594-1597,0041-1337 (Print) 0041-1337 (Linking)

Rull, R., Vidal, O., Momblan, D., Gonzalez, F. X., Lopez-Boado, M. A., Fuster, J., Grande, L., Bruguera, M., Cabrer, K. & Garcia-Valdecasas, J. C. (2003). Evaluation of potential liver donors: limits imposed by donor variables in liver transplantation. *Liver Transpl*, 9, 4, (Apr,2003), pp. 389-393,1527-6465 (Print) 1527-6465 (Linking)

Ryu, H. G., Jung, C. W., Lee, C. S. & Lee, J. (2011). Nafamostat mesilate attenuates Postreperfusion Syndrome during liver transplantation. *Am J Transplant*, 11, 5, (May,2011), pp. 977-983,1600-6143 (Electronic) 1600-6135 (Linking)

Sankary, H. N., Foster, P., Brown, E. & Williams, J. (1996). Do splanchnic viscera contribute to liver preservation reperfusion injury? *Transplantation*, 61, 8, (Apr 27,1996), pp. 1142-1147,0041-1337 (Print) 0041-1337 (Linking)

Schneider, F. (1995). Methylene blue infusion in septic shock. *Crit Care Med*, 23, 11, (Nov,1995), pp. 1935-1936; author reply 1938,0090-3493 (Print) 0090-3493 (Linking)

Scott, V. L., De Wolf, A. M., Kang, Y., Altura, B. T., Virji, M. A., Cook, D. R. & Altura, B. M. (1996). Ionized hypomagnesemia in patients undergoing orthotopic liver transplantation: a complication of citrate intoxication. *Liver Transpl Surg*, 2, 5, (Sep,1996), pp. 343-347,1074-3022 (Print) 1074-3022 (Linking)

Siniscalchi, A., Dante, A., Spedicato, S., Riganello, L., Zanoni, A., Cimatti, M., Pierucci, E., Bernardi, E., Miklosova, Z., Moretti, C. & Faenza, S. (2010). Hyperdynamic circulation in acute liver failure: reperfusion syndrome and outcome following liver transplantation. *Transplant Proc*, 42, 4, (May,2010), pp. 1197-1199,1873-2623 (Electronic) 0041-1345 (Linking)

Steininger, R., Roth, E., Fugger, R., Winkler, S., Langle, F., Grunberger, T., Gotzinger, P., Sautner, T. & Muhlbacher, F. (1994). Transhepatic metabolism of TNF-alpha, IL-6, and endotoxin in the early hepatic reperfusion period after human liver transplantation. *Transplantation*, 58, 2, (Jul 27,1994), pp. 179-183,0041-1337 (Print) 0041-1337 (Linking)

Therapondos, G., Flapan, A. D., Plevris, J. N. & Hayes, P. C. (2004). Cardiac morbidity and mortality related to orthotopic liver transplantation. *Liver Transpl*, 10, 12, (Dec,2004), pp. 1441-1453,1527-6465 (Print) 1527-6465 (Linking)

Tzakis, A., Todo, S. & Starzl, T. E. (1989). Orthotopic liver transplantation with preservation of the inferior vena cava. *Ann Surg*, 210, 5, (Nov,1989), pp. 649-652,0003-4932 (Print) 0003-4932 (Linking)

Yersiz, H., Shaked, A., Olthoff, K., Imagawa, D., Shackleton, C., Martin, P. & Busuttil, R. W. (1995). Correlation between donor age and the pattern of liver graft recovery after transplantation. *Transplantation*, 60, 8, (Oct 27,1995), pp. 790-794,0041-1337 (Print) 0041-1337 (Linking)

19

Malignant Hyperthermia in Liver Transplantation

Cláudia Regina Fernandes[1],
David Silveira Marinho[2] and Fernanda Paula Cavalcante[2]
[1]*Anesthesiology Residency Training Program at Walter Cantídio University Hospital – Federal University of Ceará*
[2]*Liver Transplantation Center of Ceará. Walter Cantídio University Hospital – Federal University of Ceará*
Brazil

1. Introduction

Malignant hyperthermia (MH) is an inherited, pharmacogenetic disorder of the skeletal muscle, characterized by dangerous hypermetabolic state after anesthesia with succinylcholine and/or volatile halogenated anesthetic agents. MH may also be triggered in susceptible individuals by severe exercise in hot conditions, infections, neuroleptic drugs and overheating in infants[1-4].

MH produces rapid increase in body temperature (by as much as 1ºC in five minutes) and extreme acidosis. These are a result of acute loss of control of intracellular calcium levels and compensatory uncontrolled increases in skeletal muscle metabolism, which may progress to severe rhabdomyolysis. Critical worldwide attention to MH began in 1960 with the reports of Denborough and Lovell. They described MH in a young man who had a history of several deaths of relatives during anesthesia. He developed tachycardia, hot and sweaty skin, peripheral mottling and cyanosis during general anesthesia using halothane. After prompt symptomatic treatment, the episode was aborted[5-6]. The term malignant hyperthermia was first quoted by Wilson and colleagues in 1967[7]. In this same year, Dantrolene Sodium, a hydantoin derivative (1-[5-(4-nitrophenyl)-2-furanyl]methylene]imino]-2,4-imidazolidinedione), was first used because of its possible muscle-relaxing properties[8]. Shortly thereafter, dantrolene was shown to alleviate muscle spasticity effectively in animals[9] and humans[10]. Later, it was shown that dantrolene uncoupled the excitation-contraction process during skeletal muscle stimulation[11]. A few years later, an association between MH and porcine stress syndrome was proposed, providing an animal model for MH[12-13]. Because malignant hyperthermia was thought to result from continuous muscle contraction, perhaps through an abnormality in the excitation-contraction coupling mechanism, the compound was tested as a treatment for this condition[14].

In 1975, Harrison[15] described the efficacy of dantrolene in preventing and treating porcine halothane induced-MH. By 1979 the U.S. Food and Drug Administration (FDA) approved dantrolene for use in humans with MH[16]. The effectiveness of dantrolene in human MH was then confirmed in a multihospital evaluation of dantrolene used to treat anesthetic-induced episodes[17]. More than four decades after its discovery, dantrolene remains the primary basis for successful MH therapy[18].

In the late 1980s, Caffeine Halothane Contracture Test became the gold standard diagnostic test for MH and a variety of neuromuscular disorders associated with MH susceptibility. These disorders include central core disease, Duchenne muscular dystrophy, myotonia congenita, myotonic dystrophy, nonspecific myopathies, and King-Denborough syndrome[19].

During liver transplantation, some commonly employed anesthetic agents may trigger MH in susceptible patients. This occurrence, in such a complex scenario and with such delicate patients, can make anesthesia management even more challenging. Besides this, dantrolene is hepatotoxic, which can pose another injury risk to the graft[20].

2. General Information

2.1 Incidence and prevalence

In North America and Europe, the incidence of MH is currently estimated to be 1:15,000 anesthetics for children and adolescents and 1:50,000–1:150,000 anesthetics for adults[21-23]. The prevalence for this syndrome in the general population is unknown because of lack of universal reporting, but although it may be as common as one in 2000[24]. Malignant hyperthermia is more common in male patients[25]. The incidence and prevalence varies from country to country, based on differences in gene pools[26-30].

Although the incidence of reported episodes of MH has increased, the mortality rate from MH has declined. This may reflect a greater awareness of the syndrome, earlier diagnosis, and better therapy[31].

2.2 Risk factors

Apparently, MH can exist regardless of race or gender although predominance in males and adolescents has been suggested[25]. Family history of fatal general anesthesia complications associated with the use of volatile agents or depolarizing muscle relaxants should make the anesthesiologist aware about the increased risk for MH.

Patients with Duchenne muscular dystrophy, myotonia congenita, myotonic dystrophy, nonspecific myopathies, central core disease, King-Denborough, osteogenesis *imperfecta* and Schwartz-Jampel syndrome have an increased risk for MH syndrome[19]. Patients who develop masseter muscle rigidity (MMR) after administration of succinylcholine have an increased risk to develop MH in the next minutes, and 25% of these will show positive contracture tests to MH[32-33].

2.3 Pathophysiology

Malignant hyperthermia is an inherited pharmacogenetic disorder of skeletal muscle, characterized by an increased calcium release from the skeletal muscle sarcoplasmic reticulum. A mutation in the ryanodine receptor (RyR) may be the main causative factor in many patients and families with MH[34-35]. The ryanodine receptor type 1 (RYR1) gene encodes the human skeletal muscle calcium release channel. RYR1 gene is responsible for the release of sarcoplasmic reticulum stores of calcium. In about 50% of MH susceptible families, there is a mutation in RYR1[36]. The large variability among individuals may be explained by different genes causing MH in different families or by other predisposing factors being expressed differently in susceptible patients[36].

The vast majority of patients susceptible to MH are asymptomatic in the absence of anesthesia. In humans, the defect only appears to be expressed significantly in skeletal muscle, although receptors are present in cardiac muscle[37] and even in the liver[38]. Recently some authors suggested that RyR's play an active role in the Ca^{2+} signaling of hepatocytes, creating local Ca^{2+} microdomains that enhance the responsiveness of neighboring Inositol Trisphosphate Receptors through Ca^{2+}-positive feedback[38].

The exact mechanism by which different substances initiate a MH crisis has not been determined. It can be assumed, though, that a defect of intracellular Ca^{2+} homeostasis plays an important role. Susceptibility to MH is clearly based on an abnormal Ca^{2+} metabolism within the skeletal muscle, most probably caused by a defective Ca^{2+} release channel in the sarcoplasmic reticulum (SR), e.g. the ryanodine receptor which is the footplate protein seated between the dihydropyridine receptor and the sarcoplasmic reticulum[39-41]. The abnormal function of the ryanodine receptor of skeletal muscle in MH causes barely controlled concentration of calcium within the cell when it is not exposed to triggering agents[42,43]. The added loss of control of intracellular calcium on exposure to triggering agents or heat stress leads to marked metabolic stimulation within the cell to provide extra adenosine triphosphate to drive the calcium pumps that restore calcium to its reservoirs (e.g., sarcoplasmic reticulum, mitochondria, extracellular fluid)[44].

On a cellular level, magnesium acts as a physiological calcium inhibitor resulting in less-intense calcium liberation from the sarcoplasmic reticulum. In normal resting muscle, cytosolic Mg^{2+} exerts a potent inhibitory influence on the SR Ca^{2+} release channel (ryanodine receptor, RyR1). Impaired Mg^{2+}-regulation of RyR1 has been proposed as a causal factor in MH. The marked potentiation of SR Ca^{2+} release after a moderate reduction in cytosolic Mg^{2+} suggests that conditions which cause hypomagnesemia will increase the probability and possibly severity of an MH event. Conversely, maintenance of a normal or slightly increased cytosolic Mg^{2+} may reduce the probability of MH[45]. There is increasing evidence to suggest that defective Mg^{2+} regulation of RyR1 confers susceptibility to malignant hyperthermia. At the molecular level, interactions between critical RyR1 subdomains may explain the clustering of RyR1 mutations and associated effects on Mg^{2+} regulation[46].

MH is a syndrome caused by dysregulation of excitation-contraction (EC) coupling in skeletal muscle. The increased activity of pumps and exchangers trying to correct the increase in Ca^{2+} causes a need for ATP, which in turn produces heat. Thus, the end result is hyperthermia. The rigidity that is frequently seen during a fulminant MH episode is the result of the inability of the Ca^{2+} pumps and transporters to reduce the unbound myoplasmic Ca^{2+} below the contractile threshold[44,47]. Human malignant hyperthermia is a heterogeneous disorder, and the down-regulation of sodium channel subunit may be involved in the final common pathway through which mutations in any one of several proteins, including the ryanodine receptor, could render a person susceptible[48]. These changes would prolong the sodium current making the cell membrane depolarized for a longer time, increasing calcium release time period from the terminal cisternae. Patients expressing sodium channel abnormalities are at increased risk for muscle rigidity.

2.4 Triggering agents

All volatile anesthetics are triggers of malignant hyperthermia and must therefore be strictly avoided in malignant hyperthermia-susceptible patients. Furthermore, the depolarizing

muscle relaxant succinylcholine triggers the syndrome[49]. Isoflurane, desflurane and sevoflurane appear to be less potent triggers than halothane, but these agents can produce a more gradual or fast onset of MH[50-56]. The onset may be explosive if succinylcholine is used[57]. Local anesthetics, nondepolarizing muscle relaxants, barbiturates, benzodiazepines, droperidol, ketamine, nitrous oxide, opioids, and propofol are all safe drugs to administer in MH susceptible patients[49].

2.5 Clinical presentation

The commonest clinical presentation of MH is a hypermetabolic state in a genetically susceptible individual in response to certain anesthetic agents, notably succinylcholine or halogenated volatile anesthetics. One of the earliest clinical signs is MMR after succinylcholine. *In vitro* muscle testing in patients who have developed this sign alone reveals that 28–50% are susceptible to MH. In the full-blown syndrome there is a rapid and sustained rise in body temperature, without shivering, either in the operating theatre or in the recovery room, in the absence of any obvious cause such as infection or a hot and humid environment. Tachycardia, cyanosis, generalized muscle rigidity, and cardiac arrhythmias are common clinical signs. There may be heating and rapid exhaustion of the soda-lime canisters. Acidosis is an early finding and there may also be hyperkalemia, hyperphosphatemia, and hypocalcemia from muscle-cell breakdown. Rhabdomyolysis is an important feature of the syndrome and is best demonstrated by measuring serum CK, which usually peaks on the second or third day after the reaction. Tenderness and swelling of muscles may develop, especially in the thighs. Myoglobinemia and myoglobinuria are common and renal failure may result from the rhabdomyolysis. Another complication is disseminated intravascular coagulation[1].

In less obvious cases, MH may present with one or any combination of the above clinical signs. The first indication of MH may be an unexplained cardiac arrest or cardiac arrhythmia. A rise in end-tidal CO_2 is often the earliest indication of MH, and now that this is widely measured in clinical anesthesia MH may be picked up before the more florid signs develop. Previously apparently uncomplicated anesthesia with halothane and/or succinylcholine does not exclude the diagnosis of MH on a subsequent occasion. Factors such as the concentration of the anesthetic drugs used, the duration of the anesthesia, and the degree of MH susceptibility of the patient may explain why one anesthetic procedure is uneventful while another in the same patient is not[1].

When MH was first recognized as a complication of anesthesia the case-fatality rate was 70%. Today, with the use of a specific drug for MH and the introduction of an *in vitro* muscle-contracture test[59] to identify susceptibility to MH in individuals and their relatives, the case-fatality rate is only 5%[1].

3. Sodium dantrolene

3.1 Pharmacokinetics

Flewellen et al showed that after an intravenous dose of dantrolene, therapeutic levels were rapidly achieved and remain stable for around 5.5h. Subsequently, the dantrolene blood level slowly declined following first order kinetics with a half-life elimination of 12h. The mean residual blood dantrolene concentration present 20h after the last dose was 1.7 $mcg.ml^{-1}$ and, after 50h, that level was 0.3 $mcg.ml^{-1}$ [60]. This same study evaluated neuromuscular effects of intravenous dantrolene in conscious patients and showed that

maximal depression of muscle twitch response (75% depression) and grip strength (42% depression) was accomplished after a cumulative dose of 2.4 $mg.kg^{-1}$ body weight. Twenty four hours after such regimen, dantrolene levels were still high enough to cause strength reduction and a subjective weakness complaint only disappeared 48h after the last dose. On the other hand, spontaneous respiratory parameters (peak expiratory flow rate, vital capacity, end-tidal carbon dioxide and respiratory rate) did not change significantly during dantrolene administration. In children, the pharmacokinetic profile is similar, with a half-life of approximately 10 h[61].

Metabolism of dantrolene is achieved microsomally in the liver via oxidative and reductive pathways. Oxidation results in hydroxylation of the hydantoin ring to 5-hydroxydantrolene (5HD), while reduction of the nitro group of dantrolene leads to the formation of aminodantrolene, which is then acetylated to the reduced acetylated derivative (RAD) of dantrolene[62].

5HD is a metabolite with muscle relaxant effects. Compared with dantrolene sodium, it has a longer half-life (15,5h vs. 6h), but its activity is lower and its plasma levels are only 30-50% of its parent drug[63]. As a result, in healthy patients, 5HD can only be considered to play a minor role on the skeletal muscle relaxant properties of dantrolene therapy. The other metabolites have no relaxant effect.

After an oral dose, 70% of dantrolene is absorbed. Twenty-five percent of the dose is excreted in urine, most of it as 5HD (79%) or RAD (17%); only 4% is excreted as unchanged drug[62]. Biliary excretion accounts for 45-50% of the oral dose administered[64]. Specific and detailed excretion studies after intravenous dantrolene are lacking.

3.2 Pharmacodynamics

Dantrolene is a unique muscle relaxant. Unlike neuromuscular blocking agents (site of action of which is at the nicotinic receptor of the neuromuscular junction) or the nonspecific relaxants (which modulate spinal cord synaptic reflexes), several studies have shown that dantrolene interferes with excitation-contraction coupling by reducing the concentration of myoplasmic calcium[65-69]. Consequently, muscle contraction is decreased without an effect on the action potential patterns of the neuromuscular junction[70].

However, the pathway by which dantrolene lowers myoplasmic Ca^{2+} is complex and still not fully understood. The ryanodine receptor of skeletal muscle (RyR1) has traditionally been thought to be the site of action of dantrolene[66], and recent studies have located the molecular target of dantrolene to the area comprising amino acid residues 590 through 609 of RyR1[71], strengthening that hypothesis. Some controversy was shed on that assumption when purified RYR1 was incorporated into an artificial planar lipid bilayer and no effect of dantrolene was detected in channel activity or pharmacology[72]. As a consequence, to date, we lack evidence of a direct action of dantrolene on purified RyR1 channels studied in lipid bilayers, even in the presence of calstabin 1, ATP, and activating concentrations of Ca^{2+}, suggesting that dantrolene's main action is to alter key protein-protein interactions[73].

3.3 Side effects

The two most frequently observed side-effects were muscle weakness in 22% and phlebitis in 10% of the patients.

Besides the intrinsic effect of dantrolene therapy, muscle weakness during MH may have a contribution of the muscle injury that is an integral part of the syndrome. Additionally, prolonged mechanical ventilation may, *per se*, exert deleterious effects on respiratory function. Although some authors objectively demonstrated strength reduction with clinically used doses of dantrolene[60], no studies of pulmonary function have been performed in patients after MH crises, dantrolene therapy and intensive care management. As a result, careful attention with respiratory function is essential in these patients, especially during weaning of mechanical ventilation or in patients with borderline respiratory function, like those neuromuscular disorders. The clinicians treating an MH episode should request repeated measurement of creatine kinase until it returns to normal levels[74].

Because of its high alkalinity (pH = 9.6) after reconstitution, dantrolene should be preferentially administered through a large bore peripheral or central venous access to avoid local inflammatory phlebitis at the infusion site. Moreover, the sites of infusion should be frequently inspected for signs of extravasation and tissue necrosis.

Besides these, the most commonly reported adverse effects can be grouped as of central (drowsiness, weakness, dizziness, malaise, fatigue, diplopia, dysarthria, seizures) and gastrointestinal (nausea, epigastric discomfort, diarrhea, constipation, abdominal pain) origin[70]. Gastrointesinal symptoms are more common with oral therapy[75]. Central nervous system symptoms may be worsened by sedatives and general anesthetics and it is not yet clear whether they are mediated by altered neuronal calcium homeostasis[76].

The side-effects were more commonly reported at the initiation of oral therapy and frequently disappeared with continued therapy and dose titration, although in 2.5% of patients they may be severe and persistent enough to warrant discontinuation of therapy[64].

Once the sarcoplasmic reticulum of heart muscle plays an essential role in the variable calcium release and uptake in excitation-contraction coupling, negative inotropic effects of dantrolene could be expected. The first studies to specifically address the effects of dantrolene on cardiovascular function evaluated healthy anesthetized dogs and showed no relevant effects on arterial pressure, central venous pressure, heart rate, coronary blood flow and cardiac output[77-78]. Later, other authors argumented that those results did not imply absence of effects on cardiovascular functions, since mechanisms of compensation may have had a role in maintaining the stability of the parameters investigated[79]. So, several authors began to study the effects of dantrolene in isolated animal cardiac muscle[79-81], but these investigations resulted in divergent results. The human studies that addressed this issue did not show any relevant effects of therapeutic doses of dantrolene on cardiovascular function [60], even in patients with poor cardiac function[82]. Whether this stability was due to complete absence of action of dantrolene on human myocardium or due to the action of compensating cardiovascular mechanisms is still a matter of debate.

Another relevant cardiovascular issue has recently emerged in a two-decade registry analysis of the complications associated with dantrolene administration[74]. The authors found that the risk of any complication with dantrolene therapy increases with larger doses of dantrolene and fluid administration; on the other hand, this same study showed that the associated use of furosemide decreased that risk. Besides this, considering only the subset of patients with serious underlying disease or complex surgery (like liver transplant), there was a greater incidence of complications and these patients commonly presented more than one type of complication.

The interpretation of these findings has to be undertaken in light of the administration peculiarities of dantrolene. Each vial with 20 mg of dantrolene contains 3 g of mannitol (to improve liposolubility) and has to be reconstituted with 60 mL of sterile water. Thus, the results of the registry analysis would suggest that the mannitol content of dantrolene formulations, when combined with fluid administration, would further aggravate the fluid shifts related to the pathophysiology of MH and major surgeries, justifying the occurrence of complications like pulmonary edema. On the other hand, the careful use of furosemide to maintain urinary output and regulate intravascular volume status decreased these complications and has long been suggested by many authors. In this registry analysis, two of the 386 enrolled patients (0.5%) presented a decrease in cardiac output, but the authors did not sufficiently describe these cases to determine if it may have been the result of direct negative inotropism of dantrolene or due to other possible causes, like fluid overload.

Liver transplantation is a very complex surgery and hepatorenal syndrome and cirrhotic cardiomyopathy are relatively common among liver transplant patients. Besides this, fluid management during liver transplantation, which is especially challenging because of massive bleeding and altered hemodynamics of cirrhotic patients, can become even more challenging with the occurrence of MH. As a result, dantrolene therapy may be especially prone to cardiovascular complications in this population. Because of these concerns, some authors suggest that documentation of cardiac filling pressures and cardiac output with continuous monitors such as echocardiography may improve management of critically ill subjects during MH treatment, although they were unable to demonstrate a reduction in dantrolene-associated complications with these measures. Furthermore, careful titration of the lowest effective dose regimen should always be sought.

Although rarely encountered, chronic oral dantrolene therapy has been linked to different grades of hepatic damage, including fatal hepatitis in 0.1-0.3% of patients[20,83]. As a result, it received a black box warning for hepatotoxicity in 1976, early after its release in 1974[84]. Despite these facts, a few authors suggest that other concomitant therapies may have had a role in that toxicity[75-76]. In addition, *in vivo* experiments in mice have not revealed any toxicity to hepatocytes[85-86]. In fact, recently, it was argued that dantrolene, due to its properties of restoring calcium homeostasis in scenarios of its disruption (like models of ischemia, hypoxia, seizure, trauma, anesthesia, and neurodegenerative diseases), may have cytoprotective effects in different tissue culture or animal models of diseases involving cytotoxicity induced by disruption of intracellular calcium homeostasis in pathogenesis[87].

Although the great majority of the studies agree that dantrolene may induce liver toxicity, the reports regarding intravenous short term dantrolene therapy are scarce, and most of the information is related to the oral long term dantrolene therapy in patients with spasticity disorders[20]. The only study that addressed the hepatic effects of intravenous dantrolene, found no significant differences in liver enzymes after its use, although it employed volunteers without any signs of MH[60]. In publications of oral therapy, some risk factors for dantrolene associated hepatitis have been identified like female sex, patients over the age of 35 years and greater accumulated doses[20,88].

Although larger doses were identified as a risk factor, there is no agreement about the reactions involved in dantrolene hepatotoxicity and, until now, it is not known if the mechanism is dose-related or attributable to hypersensitivity (idiosyncratic reaction after a few doses)[85,89-90]. As described in the pathophysiology section, ryanodine receptors were

recently discovered in hepatocytes[38]. Whether dantrolene causes liver toxicity through these receptors or during its metabolism is unclear.

Most of the patients with dantrolene hepatitis develop only mild and nonspecific symptoms (malaise, weakness, vomiting, fever, vomiting, jaundice)[90], although fatal acute hepatic failure has been described[93]. Laboratory exams show different degrees of alterations in liver enzymes (alkaline phosphatase, AST, ALT) and bilirubin levels[91]. Histological findings of liver biopsies did not show a homogenous pattern, and multiple different descriptions were published (Table 1)[20,88-90,92-95]. If signs of hepatic injury develop during MH therapy, the treatment is mainly supportive and dantrolene should be stopped soon after control of the crisis, as dantrolene hepatitis is usually reversible after its withdrawal.

In the two available reports of the use of dantrolene sodium during liver transplantation, there were alterations in postoperative laboratory exams, but the liver graft recovered uneventfully[56,96]. Actually, although dantrolene may pose an additive threat in the large set of perioperative injuries to the graft, abnormal symptoms and laboratory exams may be masked in the routine postoperative course of hepatic transplantation. Besides this, biopsies may not be of great help because histological patterns of dantrolene hepatitis do not greatly differ from those usually observed postoperatively in liver grafts. Consequently, prevention of dantrolene-induced hepatic injury is crucial. So, if malignant hyperthermia happens during liver transplantation, it seems prudent to, besides supportive treatment, use the lowest effective dose of dantrolene for the shortest time possible.

Less commonly reported effects are acne-like rash, pruritus, urticaria, fever, hypersensitivity pleural effusion with pericarditis.

4. Malignant hyperthermia in liver transplantation

4.1 Preoperative evaluation and investigation of susceptibility

Preoperative evaluation is crucial for all liver transplant candidates and, although involvement of multiple specialties like surgery, gastroenterology, cardiology, nephrology and endocrinology may be beneficial, it does not dispense with a judicious assessment by an anesthesiologist. Before planning anesthesia in a patient with known or suspected susceptibility to malignant hyperthermia, complete information about previous anesthetic procedures including complications or adverse events and other medical reports is needed[97]. Such evaluation is best accomplished and documented with the use of systematic formularies, where all collected data are registered and the anesthetic technique is individualized according to the risk factors for MH. The survey should include questions regarding muscular disorders, complications, deaths, unexplained high fever or dark-colored urine after surgery. Symptoms like fever, cramps, muscular fatigue and weakness may suggest muscular disorders and susceptibility, but are overly common among candidates for liver transplantation and are of limited value.

All common premedications like opioids, benzodiazepines, barbiturates, anticholinergics, and antihistamines are safe, but phenothiazines should not be administered. There is no need for preoperative use of dantrolene, but it must be immediately available in the operating room.

It must be emphasized that uneventful previous anesthetics (even more than once) with MH-triggering agents do not preclude the occurrence of MH in future exposures[56,98].

Some factors may have a role in attenuating MH crisis: pre-exposure hypothermia[99], differential trigger potency for MH[100] and variable genetic penetrance[101]. One of the described cases of MH during liver transplantation occurred in a patient who had previous uneventful general anesthetic[31].

4.2 Factors influencing the choice of anesthetic agents for liver transplantation and alternatives

The choice of anesthetic agents for liver transplant surgery takes into account three key factors: maintenance of hemodynamic stability, lack of hepatic toxicity and pharmacokinetic profile[102].

Circulation of cirrhotic patients is hyperdynamic, showing low systemic vascular resistance and high cardiac output[103-104]. The use of betablockers for the prevention of variceal bleeding may render these patients bradycardic and hypotensive on arrival at the operating room. Besides this, large volume paracentesis, manipulation of major vessels, presence of surgical retractors, high propensity to massive bleeding and the reperfusion syndrome may all, *per se*, result in profound intraoperative hemodynamic changes. To further aggravate the scenario, it is widely known that most drugs used in anesthesia have negative effects on the cardiovascular system. As a result, judicious choice of anesthetic agents may help alleviate the tendency toward hemodynamic instability – the following choices are considered reasonable[105].

- Hypnotic agents for anesthetic induction: propofol in low doses (1.0 – 1.5 $mg.kg^{-1}$)[106]; etomidate (0.2 – 0.3 $mg.kg^{-1}$). Hypnotics are not triggers of MH.
- Analgesic agents: fentanyl, sufentanil and remifentanil in continuous infusion are recommended; doses should be lower than usual, as the hepatic clearance is reduced. If remifentanil is used, postoperative hyperalgesia may be a concern[107] and pain control regimens should receive special attention. Opioids are not triggers of MH.
- Neuromuscular blocking agents: rapid sequence induction is recommended, since these patients commonly present with ascites[108] and gastroparesis[109]. Succinylcholine is a common choice in these cases, although it should be avoided in patients susceptible to MH. Rocuronium is a safe alternative, although its effects may be prolonged because of its hepatic metabolism; furthermore, postoperatively, in case of graft dysfunction, extubation may be delayed[110]. For the maintenance of neuromuscular block, intermittent bolus or continuous infusion of intermediate-acting drugs independent of hepatic metabolism (atracurium or cisatracurium) are a good choice and should be guided by neuromuscular monitoring.
- For maintenance of anesthesia during liver transplantation, halogenated inhalational agents (except halothane) or propofol in continuous infusion can be used. It has been shown that anesthetic requirements during liver transplantation are inversely proportional to the degree of hepatic dysfunction[111], so careful titration of anesthetic doses with monitors like BIS© can minimize the negative cardiovascular effects of these drugs[112]. Isoflurane seems to be the most adequate halogenated agent, because it has few hemodynamic effects, lacks hepatotoxicity and protects hepatocytes from graft reperfusion injury[113-114]. On the other hand, isoflurane may be stronger trigger of MH than sevoflurane[100].
- In such a way, in MH susceptible patients, succinylcholine and halogenated inhalational anesthetics must be avoided and dantrolene must be available in the operating room.

Local anesthetics, nondepolarizing muscle relaxants, barbiturates, benzodiazepines, droperidol, ketamine, nitrous oxide, opioids, propofol and vasoactive drugs are all safe drugs to administer to these patients[115].

4.3 Suspected cases and confounding factors during liver transplantation

MH syndrome exhibits a wide range of symptoms including tachycardia, progressive elevation of the exhaled CO_2, arrhythmias, hyperthermia, profuse sweating, fever up to 40ºC, cyanosis, poor skin perfusion and blood pressure instability[31]. The only physical sign typical of MH is muscular rigidity, although it may be hard to detect due to the limited access for physical evaluation. MMR may be observed upon anesthetic induction and is predictive of the syndrome[32-33].

However, tachycardia, arrhythmias, poor skin perfusion, blood pressure instability and other subtle manifestations of the initial phase of HM are commonly observed during liver transplantation, as it involves large volume paracentesis, manipulation of major vessels, massive bleeding and reperfusion syndrome[116]. Inadequate anesthetic depth and pyrogenic reaction can mimic some of those symptoms.

Most of liver transplant patients are maintained normothermic with the aid of forced warm air mattresses. After the reperfusion, the graft begins to produce heat by its exothermic metabolic reactions and the addition of this new source of heat may lead to hyperthermia. However this temperature rise only begins lately in the course of the surgery, usually is minimized by turning off the mattresses and rarely exceeds 39ºC. Another potential source of confusion in the diagnosis of MH is the use of defective equipment for patient heating (leading to overheating and sweating) or poorly calibrated temperature monitors.

Some situations can induce severe intraoperative Systemic Inflammatory Response Syndrome (SIRS) during liver transplantation, like bacteremia/sepsis, acute rejection and graft non-function. Although SIRS may show up with hyperthermia[117], other MH symptoms like severe hypercapnia are not usually present in these cases.

Hypercapnia may have several causes during liver transplantation, like intrinsic pulmonary diseases, accumulation of lung secretions in the airway, lung compression by retractors, inappropriate mechanical ventilation, exhaustion of soda lime and faulty carbon dioxide monitoring. However, after checking and solving all these issues, the maintenance of a progressive rise on end-tidal carbon dioxide becomes a strong indicator of malignant hyperthermia.

To make diagnosis even problematic, most early laboratory manifestations of malignant hyperthermia, such as respiratory and lactic acidosis and hyperkalemia, are also commonly observed in anesthesia for liver transplantation. The reasons for respiratory acidosis were described above. Lactic acidosis frequently results from the combination of tissue hypoperfusion and decreased hepatic clearance of lactate during the anhepatic phase. Hyperkalemia during liver transplantation may have several reasons, like poor baseline renal function, large and rapid transfusion of red cells and high-potassium content of preservation solutions. Nonetheless, mixed venous oxygen saturation (SvO_2) may have a lower value when MH is suspected. Due to severe increase in cellular oxygen consumption,

the SvO_2 of patients with MH is usually low. Such low values are not usually seen in liver transplantation, since cirrhotic patients generally have systemic shunts and a hyperdynamic circulation, yielding high values of SvO_2[118].

4.4 Intraoperative differential diagnosis

Several disorders share similarities with MH and may be confused with the syndrome. Neuroleptic malignant syndrome is characterized by hyperthermia, acidosis, hyperkalemia and myoglobinuria following use of a wide variety of neuroleptics, especially haloperidol. Patients taking mono-amino-oxidase inhibitors who receive meperidine may present with hyperthermia, acidosis and an increase in creatine kinase concentration, what may become fatal. Other conditions that may resemble the MH situation include – but are not limited to – : iatrogenic overheating, thyroid storm in thyrotoxicosis, hypothalamic lesions, heat illness, pheochromocytoma, and intrathecal injection of high osmolar contrast agents, cocaine or ecstasy overdose, hypoxic encephalopathy and sudden cardiac arrest in a patient with occult myopathy[119]. None of these disorders, however, is frequent in liver transplant patients.

4.5 Investigation of suspected cases

Investigation of susceptibility should begin upon clinical suspicion during preoperative evaluation.

Creatine kinase (CPK) dosage during rest has been suggested as a component of a clinical grading scale to predict malignant hyperthermia susceptibility for patients with positive family history; increased resting values could suggest myopathy and MH susceptibility[120]. The use of this test is not recommended for the general population as it would yield an unacceptably high rate of false positive results. Cirrhotic patients habitually have reduced muscle mass and decreased exercise-tolerance, thus high CPK results in patients with positive family history are suggestive.

Caffeine-Halothane Contracture Test (CHCT) is considered the gold standard for the diagnosis of MH. This test, which uses a small piece of live muscle from biopsy, assesses the muscular contractility in response to increasing concentrations of halothane and caffeine exposure. It has a 97% sensitivity and 78% specificity[121]. Even in typical cases, CHCT is beneficial to guide the necessity of investigation of relatives. MH is inherited in an autosomal dominant pattern, meaning that if one of the parents has the disease, the risk of his or her passing it down to sons or daughters is 50 per cent[122]. Muscle biopsy for CHCT should be avoided in patients whose weight is less than 20 kg, patients under chronic dantrolene or calcium channel blocker therapy and in the first three months after a MH crisis, because muscle lesion may still be present[123]. A muscle sample is removed from the *vastus lateralis* or *medialis* or *rectus abdominis*. As the tests have to be finished up to five hours after its collection, patients have to be transported to specialized MH centers. The procedure is performed under general or regional anesthesia, obviously avoiding potentially trigerring agents and keeping dantrolene immediately available[123]. CHCT is indicated in patients preoperatively deemed at risk for MH, postoperatively in patients with a typical MH crisis during liver transplantation and the relatives of the patients with a positive CHCT.

4.6 Management and treatment of MH during anesthesia for liver transplantation

4.6.1 Intraoperatively

Protocols for MH treatment prioritize four mainstays: immediate discontinuation of trigger agents, administration of antidote (sodium dantrolene), life support measures and prevention of complications[124-125].

In the acute phase of MH the following steps are recommended:

1. **Immediate discontinuation of triggering agents:** some MH crisis may be attenuated or aborted with discontinuation of triggering agents. When MH or MMR is identified soon after induction, postponement of the surgery is commonly recommended[119]. In liver transplant surgery, however, the decision to postpone the procedure is very tough. The anesthesiologist is faced with a patient who has a delicate clinical status that may be worsened either by a MH crisis or by returning to the waiting list. All the medical team should be involved in this sentence.
2. **Call for help:** initiation of measures to treat MH, including the laborious process of dantrolene dilution, may be troublesome for one only anesthesiologist. Consequently the presence of another health professional (preferably an anesthesiologist) may be of valuable help.
3. **Adjust ventilation:** increase minute ventilation to lower $EtCO_2$ and use 100% oxygen. There is no need to change the breathing circuit or the soda lime canister[126].
4. **Administer the antidote:** Dantrolene is the drug of choice in treatment of malignant hyperthermia[127]. The contents of each bottle should be diluted in 60 mL of sterile water rather than solutions such as 5 percent dextrose in water or bicarbonate because the extra molecules in solution lead to a salting-out effect with greater difficulty in dissolving dantrolene. If it does not dissolve immediately, producing a clear yellow to yellow-orange color, it should be heated under tap water or autoclaved for a few minutes[128]. In a dire emergency, it should be administered through a blood filter without concern for crystals. Dantrolene should be preferentially administered through a large bore peripheral or central venous access to avoid local inflammatory phlebitis at the infusion site.

   General dosing regimens recommend an initial bolus of 2.5 $mg.kg^{-1}$, which can be repeated every 5 minutes until normalization of the hypermetabolic state and the disappearance of all MH symptoms. After this initial control, a continuous intravenous dantrolene infusion at 10 $mg.kg^{-1}.day^{-1}$ should be given for at least 24 h after initial successful therapy[76].

   Although this is the classical regimen, it may be excessive and deleterious in liver transplantation patients. In this scenario, although the diseased liver is removed and a new liver graft is transplanted, the transplanted liver unavoidably sustains warm, cold, and reperfusion injuries during graft procurement and transplantation[129]. Dantrolene sodium is considered hepatotoxic and the hepatic effects of dantrolene on such liver allografts are unknown. As a result, it seems prudent to use the lowest effective dose for the shortest time possible.

   There are two published case reports of MH in liver transplantation, with identical clinical presentation and successful treatment with lower than usual dantrolene doses[56,96]. One of the reports used a 1 $mg.kg^{-1}$ dose intraoperatively, followed by 1 $mg.kg^{-1}$ every eight hours for 36 hours; the authors observed signs of hepatic

dysfunction 9 days after the transplant, which was attributed to dantrolene and had spontaneous resolution[56]. In the other report, the same intraoperative dose was used (1 mg.kg^{-1}) and no maintenance dose was used; in this case, no signs of liver graft dysfunction were observed[96].

Therefore, to minimize the risks of graft toxicity by dantrolene, it seems prudent to adopt intraoperative doses of 1 mg.kg^{-1}, which may be repeated every 30 minutes until control of symptoms. Next, the patient should be closely observed for MH recrudescences; if these occur, a regimen of 1 mg.kg^{-1} every eight hours for 36 hours should be instituted.

5. **Begin active cooling measures:** hypothermic blanket under and over the patient (if possible); cold isotonic saline for intravenous infusion and for gastric, vesical, peritoneal or rectal irrigation, as appropriate; ice packs to groin, axilla, and neck. To avoid hypothermia, cooling measures should be stopped when temperature decreases to 38ºC[130].
6. **Treatment of metabolic acidosis:** drugs like sodium bicarbonate or THAM are usually required to keep pH within the acceptable range[132].
7. **Treatment of hyperkalemia:** pH should be raised with the aid of hyperventilation and/or sodium bicarbonate or THAM. Glucose-insulin infusions may be helpful. In the past, authors contraindicated the use of Calcium Chloride during MH because they feared worsening of the crisis[131], on the other hand, today, it seems reasonable to use it to antagonize hyperkalemia-induced electrocardiographic changes[132].
8. **Treatment of cardiac arrhythmias:** the control of hyperkalemia and acidosis usually alleviates this problem. When they persist, the treatment should be guided according to internationally accepted protocols, like Advanced Cardiac Life Support©. Calcium blockers should not be used along with dantrolene, since hyperkalemia (sometimes culminating in cardiovascular collapse) has been described in animals with such a drug combination[132].
9. **Optimize urine output:** an output greater than 2 mL.kg^{-1} should be instituted with the use of fluids and diuretics, like furosemide and mannitol. Such measure prevents the development of renal injury secondary to rhabdomyolysis and helps the control of hyperkalemia[124].
10. **Follow blood gases, electrolytes, creatine kinase, coagulation profile and urine myoglobin.**
11. **Call for specialized center:** several countries have toll-free phone numbers for MH centers. As MH is a rare disorder, few anesthesiologists are fully used to its treatment when it happens. With such call, one may receive valuable instructions.

4.6.2 Postoperatively

1. **Observation and monitoring:** as much as 25% of patients with MH will present a recrudescence of the syndrome several hours after its initial control[133]. As a result, authors recommend observation in Intensive Care Units for 24-72 hours postoperatively[32]. Samples for blood gases, electrolytes, creatine kinase, coagulation profile and blood and urine myoglobin should be collected every 6-12 hours[76,131]. Habitually, immediate postoperative tests show evidence of hepatic injury (increased liver enzymes) and coagulopathy. Such profile mainly results of the ischemic injury to the graft and is self-limited when the graft begins to work. When MH occurs during liver transplantation, it is not clear if dantrolene can make these abnormalities more

severe nor if it can contribute to graft dysfunction. Irrespective of these uncertainties, the postoperative management is similar: graft function should be closely followed, dantrolene doses should be minimized to control symptoms and supportive treatment should be instituted. Liver biopsy has a limited value in this scenario, since the histopathological patterns of dantrolene hepatotoxicity are comparable to the ones usually observed in liver transplantation and the treatment choice does not change. If severe graft dysfunction ensues despite minimal use of dantrolene, retransplantation should be considered.

2. **Postoperative dantrolene therapy:** in liver transplant patients, after initial control of MH, dantrolene should be reserved for recrudescences (to minimize liver toxicity). The dosage regimen is recommended 1 $mg.kg^{-1}$ every eight hours for 36 hours.
3. **Mechanical ventilation weaning:** one of the most reported side-effects of dantrolene is muscle weakness, which may persist up to 24-48h after the last dose. Although some authors objectively demonstrated strength reduction with clinically used doses of dantrolene[60], no studies of pulmonary function have been performed in patients after MH crises, dantrolene therapy and intensive care management. As a result, careful attention with respiratory function is essential in these patients, especially during weaning of mechanical ventilation or in patients with borderline respiratory function, like those neuromuscular disorders.
4. **Refer patient and family to MH Testing Center for contracture or DNA testing[134-135].**

4.7 Perioperative management of liver transplantation candidates susceptible to malignant hyperthermia

Patients at risk for MH may be identified based on data collected on preoperative evaluation, like clinical symptoms, presence of muscular disease and personal and family history of previous anesthetics (see preoperative evaluation section). CHCT is indicated in patients preoperatively deemed at risk for MH, postoperatively in patients with a typical MH crisis during liver transplantation and the relatives of the patients with a positive CHCT. Anesthesia for liver transplantation in patients with some risk factors for MH must be free of succinylcholine and halogenated inhalational agents. Additionally, dantrolene must be immediately available in the operating room. Besides this, some precautions have to be taken with the anesthesia machine. Avoidance of succinylcholine is an easy to deal issue. However, avoidance of vapor anesthetics is more challenging because anesthesia machines retain anesthetic vapors long after discontinuation. Instructions for clearing residual anesthetic gases include removal or disabling of vaporizers, flushing the machine with a fresh gas flow rate more than 10 $L.min^{-1}$ using the ventilator for at least 20 min, and replacement of the fresh gas outlet hose, carbon dioxide absorbent, and anesthesia circuit. The goal is to decrease the residual anesthetic vapor concentration within the breathing circuit. These precautions represent the standard of care for the management of MH-susceptible patients. These instructions for purging anesthetic gases were derived from studies designed to optimize gas clearance in older generation machines. Modern anesthesia workstations are more complex and contain more gas absorbing materials. The current guidelines are inadequate to prepare newer generation workstations, which require more time for purging anesthetic gases, autoclaving or replacement of parts, and modifications to the gas delivery system. As a result, institutions must develop protocols that individualize their own new generation anesthesia machines[136].

5. Conclusion

Malignant hyperthermia is an inherited pharmacogenetic disorder of the skeletal muscle. It can be triggered by any halogenated inhalational agent or by succinylcholine. A mutation on the Ryanodine Receptor (RyR) may be the main causative factor, resulting in a defect of intracellular Ca^{2+} homeostasis. Besides muscle tissue, RyR's are present in hepatocytes. Dantrolene is the only available treatment for MH and is a unique muscle relaxant that interferes with excitation-contraction coupling by reducing the concentration of myoplasmic calcium. This drug seems to be hepatotoxic, although the exact mechanism is unclear. Preoperative evaluation of liver transplant candidates by anesthesiologist is critical to identify patients at risk or susceptible to MH. There is a significant overlap between the MH clinical manifestations and the usual physiological behavior during liver transplantation, and early clinical diagnosis is a challenge. Sustained and progressive increases in $EtCO_2$, despite checking and solving other possible causes, should raise suspicion of MH. Treatment should be aimed towards discontinuing triggering agents, administering the antidote and instituting supportive measures. Due to its possible hepatotoxicity, dantrolene should be used in doses lower than usual and for shorter periods of time.

6. Acknowledgements

The authors would like to thank the bibliographical support kindly and patiently provided by Dr. Amir Mohamed El Shahawy, M.D.

7. References

[1] Denborough M. Malignant hyperthermia. *Lancet*. 1998; 352(9134): 1131-6.
[2] Stratman RC, Flynn JD, Hatton KW. Malignant hyperthermia: a pharmacogenetic disorder. *Orthopedics*. 2009; 32(11): 835.
[3] Muldoon S, Deuster P, Brandom B, Bunger R. Is there a link between malignant hyperthermia and exertional heat illness? *Exerc Sport Sci Rev*. 2004; 32(4): 174-9.
[4] Uchoa RB, Fernandes CR. Exercise-induced rhabdomyolysis and risk for malignant hyperthermia: case report. *Rev Bras Anestesiol*. 2003; 53(1): 63-8.
[5] Denborough MA, Lovell RH. Anaesthetic deaths in a family (Letter). *Lancet* 1960; 276: 45.
[6] Denborough MA, Forster JF, Lovell RRH, Maplestone PA, Villiers JD. Anaesthetic deaths in a family. *Br J Anaesth.* 1962; 34: 395–6.
[7] Wilson RD, Dent TE, Traber DL, McCoy NR, Allen CR. Malignant hyperpyrexia with anesthesia. *JAMA*. 1967; 202(3): 183-6.
[8] Snyder HR Jr, Davis CS, Bickerton RK, Halliday RP. 1-[(5-arylfurfurylidene) amino]hydantoins. A new class of muscle relaxants. *J Med Chem*. 1967; 10(5): 807-10.
[9] Honkomp LJ, Halliday RP, Wessels FL. Dantrolene, 1-[5-(p-nitrophenyl) furfurylidene] amino hydantoin, a unique skeletal muscle relaxant. *Pharmacologist.* 1970; 12: 301.
[10] Chyatte SB, Birdsong JH, Bergman BA. The effect of dantrolene sodium on spasticity and motor performance in hemiplegia. *Southern Med J.* 1971; 64: 180-5.
[11] Ellis KO, Bryant SH. Excitation-contraction uncoupling in skeletal muscle by dantrolene sodium. *Naunyn Schmiedebergs Arch Pharmacol*. 1972; 274: 107–109.
[12] Lister D, Hall GM, Lucke JN. Letter: Malignant hyperthermia: a human and porcine stress syndrome? *Lancet*. 1975; 1(7905): 519.

[13] Williams CH, Houchins C, Shanklin MD. Pigs susceptible to energy metabolism in the fulminant hyperthermia stress syndrome. *Br Med J*. 1975; 3(5980): 411-3.

[14] Benson BE. Dantrolene, in *Critical Care Toxicology: Diagnosis and Management of the Critically Poisoned Patient*, Benson BE, Brent J et al (Eds). Mosby, Philadelphia, PA, 2005: 1589-1594.

[15] Harrison GG: Control of the malignant hyperpyrexic syndrome in MHS swine by dantrolene sodium. *Br J Anaesth*. 1975; 47: 62-65.

[16] Dantrolene sodium approved for malignant hyperthermia. *FDA Drug Bull.* 1979; 9(5): 27.

[17] Kolb ME, Horne ML, Martz R. Dantrolene in human malignant hyperthermia. *Anesthesiology*. 1982; 56: 254-262.

[18] Hirshey Dirksen SJ, Larach MG, Rosenberg H, et al. Future Directions in Malignant Hyperthermia Research and Patient Care. *Anesth Analg*. 2011 Jun 27. [Epub ahead of print]

[19] Heiman-Patterson TD, Rosenberg H, Fletcher JE, Tahmoush AJ. Halothane-caffeine contracture testing in neuromuscular diseases. *Muscle Nerve*. 1988; 11(5): 453-7.

[20] Utili R, Boitnott JK, Zimmerman HJ. Dantrolene-associated hepatic injury: incidence and character. *Gastroenterology*. 1977; 72:610.

[21] Loke J, MacLennan DH. Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. *Am J Med*. 1998; 104: 470–486.

[22] Abraham RB, Adnet P, Glauber V, et al. Malignant hyperthermia. *Postgrad Med J*. 1998; 74: 11–17.

[23] Hogan K. The anesthetic myopathies and malignant hyperthermia. *Curr Opin Neurol*. 1998; 11: 469–476.

[24] Monnier N, Krivosic-Horber R, Payen JF, et al. Presence of two different genetic traits in malignant hyperthermia families: implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. *Anesthesiology*. 2002; 97(5): 1067–1074.

[25] Britt BA, Endrenyi L, Peters PL, et al. Screening of malignant hyperthermia susceptible families by creatine phosphokinase measurement and other clinical investigations. *Can Anaest Soc J*. 1976; 23(3): 263–284.

[26] Sumitani M, Uchida K, Yasunaga H, Horiguchi H, Kusakabe Y, Matsuda S, Yamada Y. Prevalence of malignant hyperthermia and relationship with anesthetics in Japan: data from the diagnosis procedure combination database. *Anesthesiology*. 2011; 114(1): 84-90.

[27] Brady JE, Sun LS, Rosenberg H, Li G. Prevalence of malignant hyperthermia due to anesthesia in New York State, 2001-2005. *Anesth Analg*. 2009; 109(4): 1162-6.

[28] Ording H.Incidence of malignant hyperthermia in Denmark. *Anesth Analg*. 1985; 64(7): 700-4.

[29] Pollock AN, Langton EE, Couchman K, Stowell KM, Waddington M. Suspected malignant hyperthermia reactions in New Zealand. *Anaesth Intensive Care*. 2002; 30(4): 453-61.

[30] Simões CM, Koishi GN, Rozatti M, Amaral JL. Are we prepared to diagnose and manage malignant hyperthermia? *Rev Bras Anestesiol*. 2003; 53(2): 248-57.

[31] Larach MG, Brandom BW, Allen GC, Gronert GA, Lehman EB. Cardiac arrests and deaths associated with malignant hyperthermia in north america from 1987 to 2006:

a report from the north american malignant hyperthermia registry of the malignant hyperthermia association of the United States. *Anesthesiology*. 2008;108(4): 603-11.

[32] Allen GC, Rosenberg H. Malignant hyperthermia susceptibility in adult patients with masseter muscle rigidity. *Can J Anaesth*. 1990; 37(1): 31-5.

[33] Rosenberg H, Fletcher JE. Masseter muscle rigidity and malignant hyperthermia susceptibility. *Anesth Analg*. 1986; 65(2): 161-4.

[34] Muniz VP, Silva HC, Tsanaclis AM, Vainzof M. Screening for mutations in the RYR1 gene in families with malignant hyperthermia. *J Mol Neurosci*. 2003; 21(1): 35-42.

[35] Brandom BW. Genetics of malignant hyperthermia. *Scientific World Journal*. 2006; 6: 1722-30.

[36] Carpenter D, Ringrose C, Leo V, Morris A, Robinson RL, Halsall PJ, Hopkins PM, Shaw MA. The role of CACNA1S in predisposition to malignant hyperthermia. *BMC Med Genet*. 2009; 10: 104.

[37] Sun J, Yamaguchi N, Xu L, Eu JP, Stamler JS, Meissner G. Regulation of the cardiac muscle ryanodine receptor by O(2) tension and S-nitrosoglutathione. *Biochemistry*. 2008; 47(52): 13985-90.

[38] Pierobon N, Renard-Rooney DC, Gaspers LD, Thomas AP. Ryanodine receptors in liver. *J Biol Chem*. 2006; 281(45): 34086-95.

[39] Kim DH, Sreter FA, Ikemoto N. Involvement of the 60 kDa phosphoprotein in the regulation of Ca^{2+} release from sarcoplasmic reticulum of normal and malignant hyperthermia susceptible pig muscles. *Biochim Biophys Acta*. 1988; 945: 246-252.

[40] Mickelson JR, Gallant EM, Rempel WE et al. Effects of the halothane-sensitivity gene on sarcoplasmic reticulum function. *Am J Physiol*. 1989; 257: C787-C793.

[41] Fill M, Stefani E, Nelson TE. Abnormal human sarcoplasmic reticulum Ca^{2+} release channels in malignant hyperthermic skeletal muscle. *Biophys J*. 1991; 59: 1085-1090.

[42] Endo M. Calcium-induced calcium release in skeletal muscle. *Physiol Rev*. 2009; 89(4): 1153-76.

[43] Ogawa Y. Dysregulation of the gain of CICR through ryanodine receptor1 (RyR1): the putative mechanism underlying malignant hyperthermia. *Adv Exp Med Biol*. 2007; 592: 287-94.

[44] Diaz-Sylvester PL, Porta M, Copello JA. Halothane modulation of skeletal muscle ryanodine receptors: dependence on Ca^{2+}, Mg^{2+} and ATP. *Am J Physiol Cell Physiol.* 2008; 294(4): C1103-12.

[45] Duke AM, Hopkins PM, Halsall PJ, Steele DS. Mg^{2+} dependence of Ca^{2+} release from the sarcoplasmic reticulum induced by sevoflurane or halothane in skeletal muscle from humans susceptible to malignant hyperthermia. *Br J Anaesth*. 2006; 97(3): 320-8.

[46] Steele DS, Duke AM. Defective Mg^{2+} regulation of RyR1 as a causal factor in malignant hyperthermia. *Arch Biochem Biophys*. 2007; 458(1): 57-64.

[47] Louis CF, Balog EM, Fruen BR. Malignant hyperthermia: an inherited disorder of skeletal muscle Ca^{2+} regulation. *Biosci Rep*. 2001; 21(2): 155-68.

[48] Fletcher JE, Wieland SJ, Karan SM, Beech J, Rosenberg H. Sodium channel in human malignant hyperthermia. *Anesthesiology*. 1997; 86(5): 1023-32.

[49] Hopkins PM. Malignant hyperthermia: pharmacology of triggering. *Br J Anaesth*. 2011; 107(1): 48-56.

[50] Lee YS, Kim WY, Lee SH, Baek SM, Ok SJ, Kim JH, Park YC. A case of malignant hyperthermia during anesthesia induction with sevoflurane -A case report. *Korean J Anesthesiol*. 2010; 59 Suppl: S6-8.

[51] Chen PL, Day YJ, Su BC, Lee PC, Chen CY. Delayed onset of sevoflurane-induced juvenile malignant hyperthermia after second exposure. *Acta Anaesthesiol Taiwan*. 2007; 45(3): 189-93.

[52] Bonciu M, de la Chapelle A, Delpech H, Depret T, Krivosic-Horber R, Aimé MR. Minor increase of endtidal CO_2 during sevoflurane-induced malignant hyperthermia. *Paediatr Anaesth*. 2007; 17(2): 180-2.

[53] Wedel DJ, Gammel SA, Milde JH, Iaizzo PA. Delayed onset of malignant hyperthermia induced by isoflurane and desflurane compared with halothane in susceptible swine. *Anesthesiology*. 1993; 78(6): 1138-44.

[54] Wappler F, Fiege M. Is desflurane a "weak" trigger of malignant hyperthermia? *Anesth Analg*. 2003; 97(1): 295; author reply 295.

[55] Hoenemann CW, Halene-Holtgraeve TB, Booke M, Hinder F, Daudel F, Reich A, Van Aken H. Delayed onset of malignant hyperthermia in desflurane anesthesia. *Anesth Analg*. 2003; 96(1): 165-7.

[56] Fernandes CR, Azevedo DM, Gomes JM, et al. Malignant hyperthermia in a liver transplant patient: a case report. *Transplant Proc*. 2007; 39(10): 3530-2.

[57] Metterlein T, Schuster F, Kranke P, Hager M, Roewer N, Anetseder M. Magnesium does not influence the clinical course of succinylcholine-induced malignant hyperthermia. *Anesth Analg*. 2011; 112(5): 1174-8.

[58] MacKenzie AE, Allen G, Lahey D, Crossan ML, Nolan K, Mettler G, Worton RG, MacLennan DH, Korneluk R. A comparison of the caffeine halothane muscle contracture test with the molecular genetic diagnosis of malignant hyperthermia. *Anesthesiology*. 1991; 75(1): 4-8.

[59] Metterlein T, Hartung E, Schuster F, Roewer N, Anetseder M. Sevoflurane as a potential replacement for halothane in diagnostic testing for malignant hyperthermia susceptibility: results of a preliminary study. *Minerva Anestesiol*. 2011; 77(8): 768-73.

[60] Flewellen EH, Nelson TE, Jones WP et al. Dantrolene dose response in awake man: Implications for management of malignant hyperthermia. *Anesthesiology* 1983; 39: 275–80.

[61] Lerman J, McLeod ME, Strong HA. Pharmacokinetics of intravenous dantrolene in children. *Anesthesiology*. 1989; 70: 625–9.

[62] Lietman PS, Haslam RH, Walcher JR. Pharmacology of dantrolene sodium in children. *Arch Phys Med Rehabil*. 1974; 55(8): 388-92.

[63] Ellis KO, Wessels FL. Muscle relaxant properties of the identified metabolites of dantrolene. *Naunyn Schmiedebergs Arch Pharmacol*. 1978; 301: 237–240.

[64] Dykes MH. Evaluation of a muscle relaxant: dantrolene sodium (Dantrium). *JAMA*. 1975; 231: 862-4.

[65] Morgan KG, Bryant SH. The mechanism of action of dantrolene sodium. *J Pharmacol Exp Ther*. 1977; 201(1): 138-47.

[66] Yamaguchi N, Igami K, Kasai M. Kinetics of depolarization-induced calcium release from skeletal muscle triads *in vitro*. *J Biochem (Tokyo)*. 1997; 121: 432-439.

[67] Coronado R, Morrissette J, Sukhareva M, Vaughan DM. Structure and function of ryanodine receptors. *Am J Phys*. 1994; 266: C1485–504.

[68] Palnitkar SS, Bin B, Jimenez LS, et al. [3H] Azidodantrolene: synthesis and use in identification of a putative skeletal muscle dantrolene binding site in sarcoplasmic reticulum. *J Med Chem*. 1999; 42: 1872–80.

[69] Sutko JL, Airey JA. Ryanodine receptor Ca^{2+} release channels: does diversity in form equal diversity in function? *Physiol Rev*. 1996; 76: 1027–71.

[70] Ward A, Chaffman MO, Sorkin EM. Dantrolene. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in malignant hyperthermia, the neuroleptic malignant syndrome and an update of its use in muscle spasticity. *Drugs*. 1986; 32: 130–68.

[71] Paul-Pletzer K, Yamamoto T, Bhat MB, et al: Identification of a dantrolene-binding sequence on the skeletal muscle ryanodine receptor. *J Biol Chem*. 2002; 277: 34918-34923.

[72] Szentesi P, Collet C, Sarkozi S, et al. Effects of dantrolene on steps of excitation-contraction coupling in mammalian skeletal muscle fibers. *J Gen Physiol*. 2001; 118: 355–75.

[73] Zhou J, Allen PD, Pessad IN et al. Neuromuscular Disorders and Malignant Hyperthermia, in: Miller RD - *Miller's Anesthesia*, 7th Ed, Philadelphia, Churchill Livingstone Elsevier, 2009; 1171-1195.

[74] Brandom BW, Larach MG, Chen MS, et al. Complications Associated with the Administration of Dantrolene 1987 to 2006: A Report from the North American Malignant Hyperthermia Registry of the Malignant Hyperthermia Association of the United States. *Anesth Analg*. 2011; 112(5): 1115-23.

[75] Harrison GG. Dantrolene — dynamics and kinetics. *Br J Anaesth*. 1988; 60: 279-286.

[76] Krause T, Gerbershagen MU, Fiege M, et al. Dantrolene: a review of its pharmacology, therapeutic use and new developments. *Anaesthesia*. 2004; 59: 364-373.

[77] Ellis KO, Castellion AW, Honkomp LJ, et al. Dantrolene, a direct acting skeletal muscle relaxant. *J Pharm Sci*. 62 (6): 948–51.

[78] Ellis RH, Simpson P, Tatum P, et al. The cardiovascular effects of dantrolene sodium in dogs. *Anaesthesia*. 1975; 30: 318-322.

[79] Meyler WJ, Wesseling H, Agoston S. The effects of dantrolene sodium on cardiac and skeletal muscle in rats. *Eur J Pharmacol*. 1976; 39: 127-131.

[80] Hatae J, Ohba M, Kawata H. Effects of dantrolene sodium on the excitation-contraction coupling of the mammalian and amphibian cardiac muscle. *J Mol Cell Cardiol*. 1980; 12: 857-67.

[81] Honerjäger P, Alischewski N. Inotropic and electrophysiological effects of dantrolene on guinea pig papillary muscle. *Naunyn Schmiedebergs Arch Pharmacol*. 1983; 322: 237–244.

[82] Koehntop DE, Beebe DS, Belani KG. The safety of dantrolene in a patient with a severe cardiomyopathy requiring a heart transplant. *Anesth Analg*. 1987; 85: 229–30.

[83] Joynt RL. Dantrolene sodium: long-term effects in patients with muscle spasticity. *Arch Phys Med Rehabil*. 1976; 57(5): 212-7.

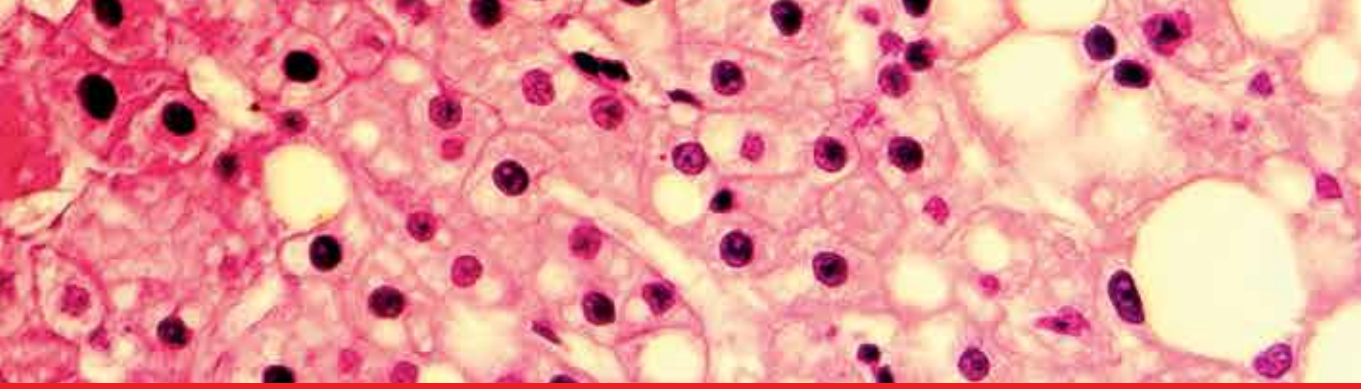
[84] Walgren JL, Mitchell MD, Thompson DC. Role of metabolism in drug-induced idiosyncratic hepatotoxicity. *Crit Rev Toxicol*. 2005; 35(4): 325-61.

[85] Durham JA, Gandolfi AJ, Bentley JB. Hepatotoxicological evaluation of dantrolene sodium. *Drug Chem Toxicol*. 1984; 7(1): 23-40.

[86] Sorensen EM, Acosta D. Comparison of dantrolene sodium with erythromycin estolate using primary cultures of rat hepatocytes. *Drug Chem Toxicol*. 1985; 8: 219-37.
[87] Inan S, Wei H. The cytoprotective effects of dantrolene: a ryanodine receptor antagonist. *Anesth Analg*. 2010; 111(6): 1400-10.
[88] Ogburn R, Myers R, Burdick G. Hepatitis associated with dantrolene sodium. *Ann Intern Med*. 84: 53-54.
[89] Schneider R, Mitchell D. Dantrolene hepatitis. *JAMA*. 1976; 235(15): 1590-1.
[90] Wilkinson SP, Portmann B, Williams R. Hepatitis from dantrolene sodium. *Gut*. 1979; 20: 33-36.
[91] Abernathy CO, Utili R, Zimmerman HJ, Ezekiel M. The effects of dantrolene sodium on excretory function in the isolated perfused rat liver. *Toxicol Appl Pharmacol*. 1978; 44(3): 441-52.
[92] Fitzgibbons DC. Malignant hyperthermia following preoperative oral administration of dantrolene. *Anesthesiology*. 1981; 54: 73-75.
[93] Donegan JH, Danegan WL, Cohen EB. Massive hepatic necrosis associated with dantrolene therapy. *Dig Dis Sci*. 1978; 23(Suppl.): 48-52.
[94] Lundia SR, Uden DL, Hanson RF. Dantrolene-associated hepatitis. *Drug Intell Clin Pharm*. 1977; 11: 278-280.
[95] Cornette M, Gillard C, Borlee-Hermans G. Hépatite toxique mortelle associée al'usage du dantrolene. *Acta Neurol Belg*. 1980; 80: 336-347.
[96] Shih TH, Chen KH, Pao YY, et al. Low-dose dantrolene is effective in treating hyperthermia and hypercapnia, and seems not to affect recovery of the allograft after liver transplantation: case report. *Transplant Proc*. 2010; 42(3): 858-60.
[97] Della Rocca G, De Flaviis A, Costa MG, Chiarandini P, Pompei L, Venettoni S. Liver transplant quality and safety plan in anesthesia and intensive care medicine. *Transplant Proc*. 2010; 42(6): 2229-32.
[98] Claxton BA, Cross MH, Hopkins PM. No response to trigger agents in a malignant hyperthermia-susceptible patient. *Br J Anaesth*. 2002; 88: 870-3.
[99] Iaizzo PA, Kehler CH, Carr RJ, Sessler DI, Belani KG. Prior hypothermia attenuates malignant hyperthermia in susceptible swine. *Anesth Analg*. 1996; 82: 803-9.
[100] Kunst G, Graf BM, Schreiner R, Martin E, Fink RH. Differential effects of sevoflurane, isoflurane, and halothane on Ca2+ release from the sarcoplasmic reticulum of skeletal muscle. *Anesthesiology*. 1999; 91: 179-86.
[101] Urwyler A, Deufel T, McCarthy T, West S. Guidelines for molecular genetic detection of susceptibility to malignant hyperthermia. *Br J Anaesth*. 2001; 86: 283-7.
[102] Steadman RH. Anesthesia for liver transplant surgery. *Anesthesiol Clin North Am*. 2004; 22(4): 687-711.
[103] Liu H, Lee SS. Acute-on-chronic liver failure: the heart and systemic hemodynamics. *Curr Opin Crit Care*. 2011; 17(2): 190-4.
[104] Vincent JL, Gustot T. Sepsis and cirrhosis: many similarities. *Acta Gastroenterol Belg*. 2010; 73(4): 472-8.
[105] Liu LL, Niemann CU. Intraoperative management of liver transplant patients. *Transplant Rev (Orlando)*. 2011; 25(3): 124-9.
[106] Wu J, Zhu SM, He HL, Weng XC, Huang SQ, Chen YZ. Plasma propofol concentrations during orthotopic liver transplantation. *Acta Anaesthesiol Scand.* 2005; 49(6): 804-10.

[107] Konopka KH, van Wijhe M. Opioid-induced hyperalgesia: pain hurts? *Br J Anaesth*. 2010; 105(5): 555-7.
[108] Yudkowitz FS, Chietero M. Anesthetic issues in pediatric liver transplantation. *Pediatr Transplant*. 2005; 9(5): 666-72.
[109] Verne GN, Soldevia-Pico C, Robinson ME, Spicer KM, Reuben A. Autonomic dysfunction and gastroparesis in cirrhosis. *J Clin Gastroenterol*. 2004; 38(1): 72-6.
[110] Gao L, Ramzan I, Baker B. Rocuronium plasma concentrations during three phases of liver transplantation: relationship with early postoperative graft liver function. *Br J Anaesth*. 2002; 88(6): 764-70.
[111] Wang CH, Chen CL, Cheng KW, et al. Bispectral index monitoring in healthy, cirrhotic, and end-stage liver disease patients undergoing hepatic operation. *Transpl Proc*. 2008; 40: 2489-91.
[112] Toprak Hİ, Sener A, Gedik E, Uçar M, Karahan K, Aydogan MS, Ersoy MÖ. Bispectral index monitoring to guide end-tidal isoflurane concentration at three phases of operation in patients with end-stage liver disease undergoing orthotopic liver transplantation. *Transplant Proc*. 2011; 43(3): 892-5.
[113] Tao KM, Yang LQ, Liu YT, Tao Y, Song JC, Wu FX, Yu WF. Volatile anesthetics might be more beneficial than propofol for postoperative liver function in cirrhotic patients receiving hepatectomy. *Med Hypotheses*. 2010; 75(6): 555-7.
[114] Lv X, Yang L, Tao K, Liu Y, Yang T, Chen G, Yu W, Lv H, Wu F. Isoflurane preconditioning at clinically relevant doses induce protective effects of heme oxygenase-1 on hepatic ischemia reperfusion in rats. *BMC Gastroenterol*. 2011; 11: 3.
[115] Hopkins PM. Malignant hyperthermia: pharmacology of triggering. *Br J Anaesth.* 2011; 107(1): 48-56.
[116] Hannaman MJ, Hevesi ZG. Anesthesia care for liver transplantation. *Transplant Rev (Orlando)*. 2011; 25(1): 36-43.
[117] Capacchione JF, Radimer MC, Sagel JS, Kraus GP, Sambuughin N, Muldoon SM. Trauma, systemic inflammatory response syndrome, dietary supplements, illicit steroid use and a questionable malignant hyperthermia reaction. *Anesth Analg*. 2009; 108(3): 900-3.
[118] Nissen P, Frederiksen HJ, Secher NH. Intraoperative hemodynamic monitoring during liver transplantation: goals and devices. *Minerva Gastroenterol Dietol*. 2010; 56(3): 261-77.
[119] Ali SZ, Taguchi A, Rosenberg H. Malignant hyperthermia. *Best Pract Res Clin Anaesthesiol*. 2003; 17(4): 519-33.
[120] Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA, Kaplan RF, Muldoon SM, Nelson TE, Ording H, Rosenberg H, Waud BE, Wedel DJ: A clinical grading scale to predict malignant hyperthermia susceptibility. *Anesthesiology*. 1994; 80: 771-9.
[121] Allen GC, Larach MG, Kunselman AR. The sensitivity and specificity of the caffeine-halothane contracture test: a report from the North American Malignant Hyperthermia Registry. The North American Malignant Hyperthermia Registry of MHAUS. *Anesthesiology*. 1998; 88: 579-88.
[122] Litman RS, Rosenberg H. Malignant hyperthermia: update on susceptibility testing. *JAMA*. 2005; 293: 2918-24.

[123] Rosenberg H, Antognini JF, Muldoon S. Testing for malignant hyperthermia. *Anesthesiology*. 2002; 96: 232-7.
[124] Krivosic-Horber R. Malignant hyperthermia. Treatment of the acute episode. *Acta Anaesthesiol Belg*. 1990 ; 41: 83-86.
[125] Krause T, Gerbershagen MU, Fiege M, Weisshorn R, Wappler F. Dantrolene: a review of its pharmacology, therapeutic use and new developments. *Anaesthesia*. 2004; 59: 364-73.
[126] Rosenberg H, Davis M, James D, Pollock N, Stowell K. Malignant hyperthermia. *Orphanet J Rare Dis*. 2007; 2: 21-34.
[127] Brandom BW. Recognition and treatment of Malignant Hyperthermia. ASA Refresher Courses in *Anesthesiology*. 2005; 33: 23-9.
[128] Gronert GA. Malignant hyperthermia. *Anesthesiology*. 1980; 53(5): 395–423.
[129] Baker KR, Landriscina D, Kartchner H, Mirkes DM. The Icarus effect: the influence of diluent warming on dantrolene sodium mixing time. *AANA J*. 2007; 75: 101-6.
[130] Clavien PA, Harvey PR, Strasberg SM: Preservation and reperfusion injuries in liver allografts: an overview and synthesis of current studies. *Transplantation*. 1992; 53: 957.
[131] Plattner O, Kurz A, Sessler DI, Ikeda T, Christensen R, Marder D, et al. Efficacy of intraoperative cooling methods. *Anesthesiology*. 1997; 87: 1089-95.
[132] Malignant Hyperthermia Association of the United States (2011). Medical Professionals' FAQs. [Online]. Available: *http://medical.mhaus.org/index.cfm/fuseaction/Content.Display/PagePK/MedicalFAQs.cfm* (July 23, 2011)
[133] Saltzman LS, Kates RA, Corke BC, Norfleet EA, Health KR. Hyperkalemia and cardiovascular collapse after verapamil and dantrolene administration in the swine. *Anesth Analg*. 1984; 63: 473-8.
[134] Hopkins PM. Recrudescence of malignant hyperthermia. *Anesthesiology*. 2007; 106: 893-4.
[135] Hopkins PM. Malignant hyperthermia: advances in clinical management and diagnosis. *Br J Anaesth*. 2000; 85: 118-28.
[136] Kim TW, Nemergut ME. Preparation of modern anesthesia workstations for malignant hyperthermia-susceptible patients: a review of past and present practice. *Anesthesiology*. 2011; 114(1): 205-12.



Edited by Hesham Abdeldayem and Naglaa Allam

This book covers a wide spectrum of topics including history of liver transplantation, ischemia-reperfusion injury, immunology of liver transplantation, viral hepatitis and liver transplantation, other indications for liver transplantation, prognostic factors and perioperative period.

The authors of the chapters are experts in their respective fields. They are proponents covering different aspects of liver transplantation and come from many centers across the world. The interdisciplinary approach and the authority of the contributors resulted in a valuable reference to anyone interested in developing a global view in liver transplantation including medical students, residents, fellows, nurses, and practicing physicians and surgeons as well as researchers in the field of liver transplantation.

ISBN 978-953-51-6800-3

Photo by Dr_Microbe / iStock

IntechOpen