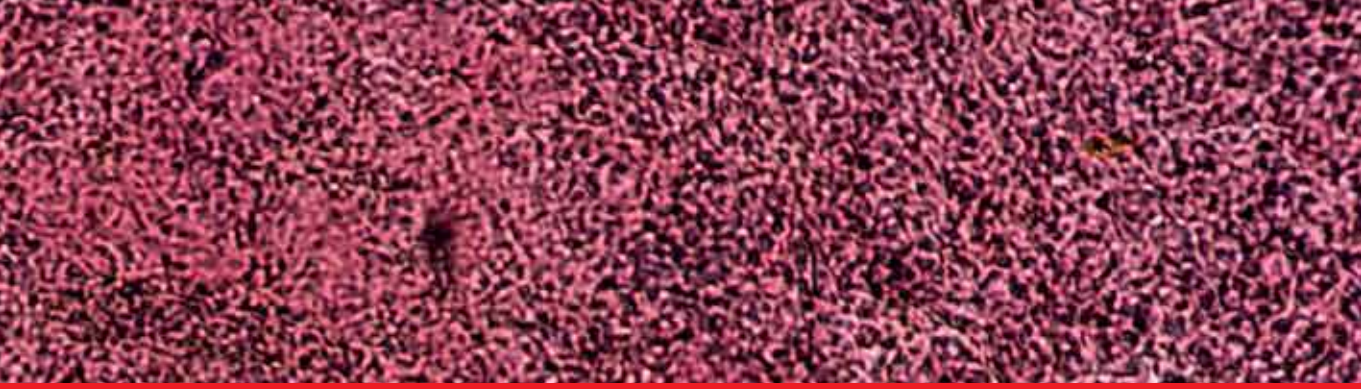

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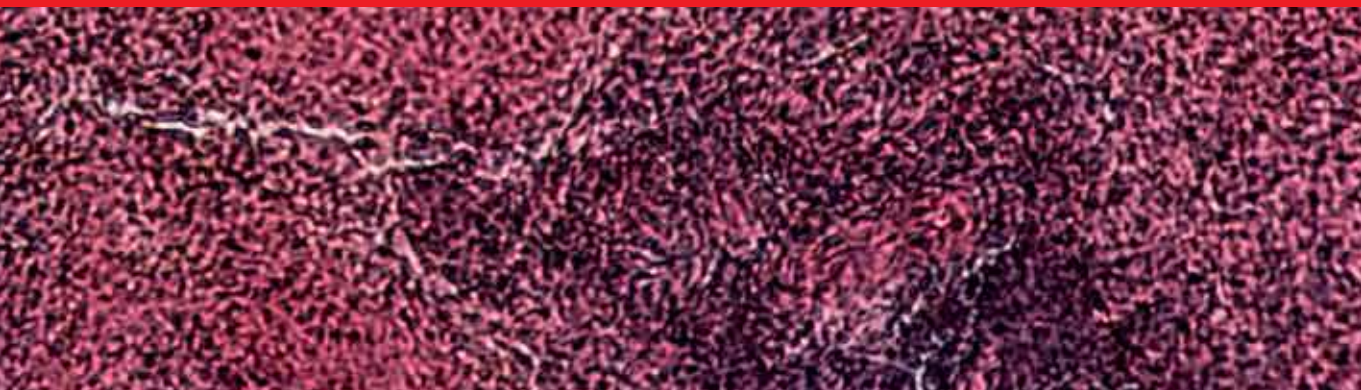

LIVER RESEARCH AND CLINICAL MANAGEMENT

Edited by **Luis Rodrigo**

Liver Research and Clinical Management
http://dx.doi.org/10.5772/intechopen.70390
Edited by Luis Rodrigo

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First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

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

Liver Research and Clinical Management
Edited by Luis Rodrigo

p. cm.

Print ISBN 978-1-78923-090-1

Online ISBN 978-1-78923-091-8

eBook (PDF) ISBN 978-1-83881-472-4

Meet the editor

Dr. Luis Rodrigo, MD, is actually an Emeritus Professor of Medicine at the University of Oviedo (Spain). He has been the chief of Gastroenterology Service at the HUCA Hospital in Oviedo, for more than 40 years. He obtained the PhD degree in 1975 and has developed a long teaching and research career. He has published a total of 572 scientific papers, 290 written in English and the rest in Spanish. He has participated as the main investigator in a total of 45 clinical trials and has directed 40 doctoral theses. He has contributed actively to the formation of around 100 specialists in gastroenterology working in his hospital and other hospitals in Spain and abroad. He has written around 35 chapters in books of several subjects and has been the editor of 21 books in his specialty and related diseases.

Contents

Preface

It is my pleasure and a great honor for me to present you this new book from the area of medicine, hepatology section entitled *Liver Research and Clinical Management* that could be described as the application of basic and experimental as well as clinical research and its application to the treatment of human liver diseases in the daily clinic. It is what has been applied in other words to the transfer of knowledge from the bench to the bed, applied to liver diseases, that have been occurring in recent decades.

This book is divided into several sections that treat problems related to different diseases including topics on "nonalcoholic fatty liver disease" that is an increasingly common problem especially in developed countries like a large epidemic disease, mainly in relation to the obesity and bad food habits of the population. In another section, the authors describe the important role of the "imaging evaluation of the liver" in the diagnosis and control of different problems related to the liver and the biliary tract.

Other sections are devoted to "hepatic trauma" that appears as a consequence of the open or close accidents around or inside this important organ, the way of early detection, and surgical repair. "Hepatocellular carcinoma" is the most common primary tumor of the liver that appears commonly associated to liver cirrhosis of long duration, whose diagnosis is sometimes difficult, and the several ways of treatment are described.

Liver transplantation is commonly used as the therapy of choice for patients who have a liver advanced insufficiency or end-liver disease. There is a long experience all over the world with this procedure that provides and restores a good quality of life on the receptor that needs a long-term immunosuppression to avoid the rejection. Usually, the liver donor came from a cadaver, but the shortage of donations has introduced the possibility of living donors of the liver as another important source for providing livers for transplantation.

Another possibility of restoring the liver function is to use the "gene therapy" that is advancing a lot in recent years but needs to continue improving its results. The "psychosocial aspect evaluation" of all these therapies and the impact on the patients and their families are clearly emphasized.

Finally, I want to thank all the authors for their excellent contributions and the InTech editorial team and especially Ms. Romina Skomersic, for her continuous collaboration and kind support in the total book preparation and her help and easily resolving all my needs during all the editorial processes.

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Section 1

Non-Alcoholic Fatty Liver Disease

Chapter 1

Diagnosis and Characterization of Non-Alcoholic Fatty Liver Disease

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72668

Abstract

Non-alcoholic fatty liver disease (NAFLD) can develop cirrhosis and even hepatocellular carcinoma, resulting in a high liver-related morbidity and mortality, being important to know those risk factors for disease progression, among which the presence of diabetes stands out. In addition, it is a disease with multisystemic behavior, becoming an independent risk factor for cardiovascular disease and extrahepatic tumors. Hence, early diagnosis and multidisciplinary management of NAFLD are really important. In this chapter, we will expose the different diagnostic and follow-up tools available for this disease, and with them we will make an algorithm according to the recommendations and the current evidence.

Keywords: NAFLD, biomarkers, transient elastography, multisystemic disease

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver damage whose distinctive feature is the accumulation of intrahepatic fat, especially triglycerides, which cannot be attributed to secondary causes such as alcohol and certain drugs. NAFLD is nowadays considered to be the most common cause of chronic liver disease in western countries, showing a prevalence of around 30% in the general population [1]. Within NAFLD, two histological subtypes can be distinguished: (a) non-alcoholic fatty liver (NAFL), which includes patients with simple steatosis with or without mild inflammation and (b) non-alcoholic steatohepatitis (NASH), characterized by the presence of hepatic inflammation and hepatocyte injury (ballooning) with or without fibrosis [2, 3]. NAFL is a generally benign condition, and NASH is the progressive subtype that can lead to cirrhosis and hepatocellular carcinoma

(HCC) [4]. However, several studies with paired liver biopsies have demonstrated that both patients with NASH and those with NAFL have the potential to develop a progressive hepatic disease, and in this risk of progression there are some key factors such as diabetes mellitus [5, 6]. In general, patients with NAFLD have a higher long-term mortality than the general population, cardiovascular disease (CVD) being the principal cause of death, followed by different types of cancer [7–9] and liver-related complications, as well as the cardiovascular risk caused by the different factors of the metabolic syndrome, very frequent in this type of subjects; NAFLD is itself an independent risk factor for CVD [10]. Liver-related mortality is increased up to 10-fold in patients with NAFLD. In this sense, it should be emphasized that cirrhosis and HCC are the fifth most prevalent cause of mortality in the world. Therefore, given the hepatic and cardiovascular morbi-mortality generated by NAFLD, the early identification of these patients is important to provide suitable management that can lower the mortality for all causes.

2. Screening and diagnostic criteria

The mechanisms leading to the development and progression of NAFLD are not completely known, but it is widely accepted that the initial events are dependent on the development of obesity and insulin resistance (IR) [11]. For this reason, NAFLD has a strong association with the factors constituting the metabolic syndrome, the prevalence in this group of patients being considerably heightened. This relation is especially close in morbid obesity, where NAFLD is present in more than 90% of the cases, this condition taking the form of steatohepatitis in a third of the cases, while in up to 5–10% of the subjects, the liver disease has progressed to cirrhosis [12, 13]. The association between NAFLD and IR or diabetes mellitus type 2 (DM2) has also been clearly established [14]. It has been demonstrated that DM2 is associated with a greater hepatic content of triglycerides independently of the body mass index (BMI) [15, 16]. Thus, the prevalence of NAFLD in DM2 patients can reach up to 70% [11, 17, 18]. Moreover, both prediabetes (glucose intolerance and altered glucose when fasting) and DM2 are related to the severity of liver damage, the presence of steatohepatitis, fibrosis and even HCC [1, 19, 20]. Overall, 80% of the NAFLD cases present with some of the cardiovascular risk factors that constitute the metabolic syndrome (IR, obesity, dyslipidemia and arterial hypertension), and its prevalence directly increases the number of these factors that are present [21].

As a consequence of its high prevalence, especially in subjects with the abovementioned risk factors, its prognostic implications, and given that NAFLD is generally an asymptomatic disease, some authors recommend the implantation of an NAFLD-screening programme within the risk population [22, 23]. However, this topic is at present controversial given the great load on the national health systems that could be caused by these screening programmes and the lack of efficacious treatments currently available. In fact, the principal associations for the study of liver diseases (American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL)) in their guidelines for clinical practice do not recommend this screening in any population [24] or they recommend it, with an A2 level of evidence, only for patients with DM2 independently of the levels of hepatic

enzymes [25]. To attempt to answer this question, valid cost-utility studies are necessary in screening programmes. There is no discussion, however, about the need to act when faced with a patient suspected of having NAFLD and not to underestimate its discovery due to the limited clinical and analytical repercussion manifested at first. In our opinion, patients with NAFLD and suspected to have advanced disease must be evaluated in specialist units for their correct characterization in case of a prompt availability of specific treatment.

2.1. Clinical and analytical manifestations

A diagnosis of NAFLD is very often reached through a casual analytical discovery during a health examination after an alteration in tests of liver function, or an alteration in hepatic morphology detected through an image study done with another objective, given that it is generally an asymptomatic disease. In the cases in which the patient reports symptoms, they are usually mild and unspecific, asthenia and abdominal problems being frequent, especially in the right hypochondria. The physical exploration may be normal or detect a soft, painless hepatomegaly, although occasionally it is difficult to evaluate as these patients very often present with central-type obesity, and in the patients with advanced fibrosis and cirrhosis, we may find signs of portal hypertension such as ascites, splenomegaly or jaundice [26].

Analytically, most of the patients present tests with normal or discretely altered liver function, with a predominance of ALT (alanine aminotransferase) compared to AST (aspartate aminotransferase). On specific occasions, a discrete elevation can be appreciated in the markers of cholestasis, especially GGT (gamma-glutamyl transpeptidase), which has been related to obesity and IR [27]. Another frequent analytical discovery is the elevation of the levels of ferritin in blood and of the transferrin saturation index without having demonstrated a corresponding increase in the deposits of hepatic iron [28]. Something similar occurs with the presence of elevated autoantibodies, which appear quite frequently in NAFLD and are considered an epiphenomenon [29].

2.2. Diagnosis of steatosis

Hepatic steatosis is defined histologically as the deposit of fat ≥5% of the hepatocytes and is classified in four grades depending on the percentage of hepatocytes with steatotic vacuoles. The normal liver (S0) contains fat in less than 5% of the hepatocytes while grade 1 steatosis (S1) corresponds to less than 33% of the steatotic hepatocytes. In grade 2 and 3 steatosis, fat is present in at least 33 or 66% of the hepatocytes, respectively.

The presence of risk factors such as DM2, metabolic syndrome and obesity with the elevation of the hepatic enzymes, especially ALT, increases the possibility of fatty liver presenting. Nevertheless, although the ALT is a useful test, it is not valid for predicting the presence of this disease, or even the risk of progression, given that it can occur with normal hepatic enzymes [30]. In fact, in patients with DM2 and normal levels of ALT, a high prevalence of NAFL and NASH has been reported [31].

In clinical practice, ultrasound scan is a first-rate image technique if NAFLD is suspected due to its wide availability, low cost and safety [32]. The sensitivity of this technique is 93%

when the steatosis is greater than 33%; however, this sensitivity decreases considerably when the steatosis affects less than 30% of the hepatocytes [33, 34]. Steatosis can also be diagnosed through computerized tomography (CT), but its cost and the patient's exposure to radiation make its systematic use in long-term follow-up unadvisable in this pathology; moreover, its sensitivity does not improve substantially if the steatosis is mild [32]. Magnetic resonance imaging (MRI), including spectroscopy, can diagnose content levels of hepatic fat >5% and it is reliable to determine changes (≥0.5%) in the grade of steatosis after weight loss. Although its use has widened in many studies, its use in clinical practice is limited by its cost and duration [35, 36].

The recently developed CAP (controlled attenuation parameter), an application of transient elastography (TE), which will be discussed later, available in the latest generation devices, enables the immediate and easy quantification of steatosis. CAP measures the degree of attenuation of the ultrasound wave transmitted through the liver, which is proportional to the amount of hepatic fat, and is less influenced by the sampling error than the liver biopsy, since it explores a liver volume approximately 100 times greater. Its values oscillate between 100 and 400 dB/m and it is possible to measure the liver stiffness used for the evaluation of fibrosis simultaneously. The studies published to date indicate that CAP is capable of diagnosing steatosis in chronic liver diseases of diverse causes even in mild stages (>10%) and has a good correlation with the degree of steatosis [37–44]. These studies show different cut-offs of CAP for the different grades of steatosis, but all of them demonstrate that the cut-offs do not differ among the different causes of liver diseases, in contrast with what happens with transient elastography [40]. In this sense, a recent meta-analysis including 2735 patients has established a series of cut-offs for the different grades of steatosis: 248 dB/m for S1, 268 dB/m for S2 and 280 dB/m for S3, with a sensitivity of 69, 77 and 88%, respectively, and a specificity of 82, 81 and 78%, respectively. In this meta-analysis, etiology, BMI and diabetes showed a significant influence on the value of the CAP, so the authors suggested the cut-offs established but subtracting 10 dB/m from the value of the CAP for the patients with NAFLD, 10 dB/m for diabetics, and subtracting/adding 4.4 dB/m for each unit of BMI above/below 25 kg/m^2 in the interval of 20–30 kg/m^2 [45].

Another image technique is magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF), which is based on chemical shift-based water fat separation methods. MRI-PDFF has shown good correlation with histology-determined steatosis grade in NAFLD patients, so it could be used for follow-up and treatment-response evaluation [46].

Lastly, within the non-invasive diagnosis of steatosis, various serological tests of biomarkers have been developed to predict the existence of hepatic fat (**Table 1**) [47–51]. However, all these biomarkers may be influenced by inflammation and fibrosis, and given that they do not provide great advantages compared to image techniques and routine analysis, their use in clinical practice is not widespread; even so, the Fatty Liver Index (FLI) that uses easily available parameters could be considered, although CAP has demonstrated better performance than this test for the diagnosis of grade 2–3 steatosis [44]. Nevertheless, FLI has been associated independently to liver-related mortality, as well as to the mortality rates due to

Indices	Formula	Cut-offs	Sensitivity (%)	Specificity (%)
Hepatic	8 × ALT/AST ratio + BMI	30 (low cut-off)	93	40
Steatosis	(+2, if DM; +2, if female)	36 (high cut-off)	45	93
Index (HSI)				
Fatty liver	exp(n)/1 + exp (n) × 100	10 (low cut-off)	95	29
Index (FLI)	(n) = 0.953 × ln(TG) + 0.139 × BMI + 0.718 × ln(GGT) + 0.053 × waist circumference – 15.745	60 (high cut-off)	44	91
SteatoTest	Proprietary formula (a2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, bilirubin, ALT, cholesterol, triglycerides, glucose, BMI, age, gender)	0.3 (low cut-off)	90	54
		0.7 (high cut-off)	46	88
NAFLD liver	−2.89 + 1.18 × metabolic	−0.640	86	71
Fat score	Syndrome (yes: 1, no: 0) + 0.45 × Type 2 diabetes (yes: 2, no: 0) + 0.15 × insulin + 0.04 × AST − 0.94 × AST/ALT			
Lipid	LAP (men) = Waist	20 (low cut-off)	99	16
accumulation	circumference – 65	80 (high cut-off)	43	94
product (LAP)	LAP (women) = Waist circumference − 58			

Table 1. Indices for diagnosis of steatosis.

cardiovascular disease and cancer, but it seems that these associations are interfered by the risk conferred by the state of insulin resistance [52].

When steatosis is suspected in the aforementioned non-invasive methods, the liver biopsy is still the gold-standard method to conclusively diagnose NAFLD and the only one capable of distinguishing between NAFL and NASH, thus enabling the classification of the disease

according to the grade of activity (inflammation and hepatocyte injury) and the stage of fibrosis, the best predictors of the disease progression [53, 54]. The advantages and, especially, the drawbacks of the liver biopsy are dealt with later.

2.3. Initial diagnosis of NAFLD

From what has been mentioned so far, we can specify a series of characteristics that indicate a patient with NAFLD: (1) radiological evidence of steatosis or CAP >248 dB/m ± Abnormal liver blood test, (2) the presence of insulin resistance or another component of the metabolic syndrome, (3) consumption of alcohol of <30 g/d in men and <20 g/d in women and (4) exclusion from other causes of chronic liver disease (viral hepatitis, cholestatic diseases, autoimmune hepatitis, hemochromatosis, α1 antitrypsin deficiency, Wilson's disease, drug-induced liver injury and celiac disease) [24, 25]. Once the initial diagnosis of NAFLD has been made, our next step is to evaluate the stage of disease and the necessity of carrying out a liver biopsy.

3. Diagnosis of steatohepatitis

A key element in the diagnosis of NAFLD is the differentiation of NASH from NAFL and the staging of the liver fibrosis, given that patients with NASH and advanced fibrosis are those at the greatest risk of developing hepatic complications and cardiovascular disease [54–56].

3.1. Liver biopsy

As it was mentioned earlier, the chosen method to evaluate the grade of histological lesion is still the liver biopsy. However, liver biopsy has well-known limitations and cannot be proposed for all patients, given the high prevalence of NAFLD worldwide. Liver biopsy is invasive and is not without complications. Besides, there are other drawbacks: (1) sampling error, since a typical liver biopsy samples only 1/50,000 of all liver tissues, and histological lesions of NASH are unevenly distributed throughout the liver parenchyma [57]; (2) inter- and intra-observer variability, as observed by Gawrieh et al. although there was a high agreement ratio in the assessment of steatosis grading and fibrosis staging between pathologists, the agreement was suboptimal for lobular inflammation and hepatocellular ballooning [58]; and (3) the existence of different criteria for the definition of NASH. The Non-alcoholic Steatohepatitis Clinical Research Network (NASH-CRN) proposed the system termed the NAS scoring system in order to classify NAFLD according to severities of fatty change, inflammation and hepatocellular ballooning [3]. NAS is markedly reproducible and is useful for assessing therapeutic effects in Clinical trials, but it is incapable of diagnosing NASH in patients with burned-out NASH, in whom fatty changes and inflammatory cell infiltration resolving in fibrosis have progressed [59].

Given these limitations, non-invasive methods have been developed for the diagnosis of NASH and fibrosis as a first option to examine NAFLD patients and to help determine which require a liver biopsy. The ideal test should be economical, reproducible and capable of diagnosing the whole spectrum of lesions, including within NAFLD, and even reflecting the

changes produced on initiating specific treatment. Nowadays, we do not have a test available that has these characteristics, so these non-invasive methods are based on diverse complementary approaches: clinical factors, genetics, serological markers, image tests and transient elastography [60, 61].

3.2. Risk factors associated with non-alcoholic steatohepatitis and progressive disease

The best predictor of the evolution of NAFLD is the presence of necroinflammation and fibrosis in liver biopsy; however, there are more and more studies reporting no insignificant rates of progression of simple steatosis [5, 6, 62]. A first study that analyzed patients with NAFLD and paired biopsies demonstrated that even patients with simple steatosis can progress to NASH and advanced fibrosis, especially in the presence of metabolic risk factors [6]. Therefore, there is a series of non-modifiable and modifiable factors in patients associated with a greater risk of development of NASH and more progressive disease.

Various transversal studies have demonstrated that the disease is more severe in older patients, although this phenomenon could be due to the sum of pathogenic factors and a greater duration of the liver disease itself and the associated diseases [8, 63, 64]. In fact, the longitudinal studies have not managed to demonstrate that age is a factor that aggravates the disease per se [65]. The association between sex and fibrosis progression is controversial; two transversal studies show that men and post-menopause women have a greater risk of fibrosis in comparison with pre-menopause women; moreover, precocious menopause is associated with a greater risk of fibrosis [66–69]. Other non-modifiable factors are genetic; dozens of genes with multiple polymorphisms associated with NAFLD have been discovered thanks to genome-wide association studies (*GWAS*), but the number of strongly validated genes in large independent cohorts is limited to two, *patatin-like phospholipase domain containing 3* (PNPLA3) and *transmembrane 6 superfamily member 2* (TM6SF2) [70]. The presence of the single nucleotide polymorphisms (SNPs) rs738409 and rs58542926 of the genes PNPLA3 and TM6SF2, respectively, has been associated with a greater risk of NAFLD, as well as a more severe disease [71–76]. Recently, an SNP of IL28b (also implicated in the response to interferon in chronic hepatitis C patients (VHC)) has been associated with an increment in fibrosis in NAFLD patients [77]. Moreover, in a control-case study carried out by our working group, we have observed that the presence of the variants rs1421085 and rs1558902 of the fat mass and obesity-associated (FTO) gene confer a high risk of liver inflammation particularly in patients of normal weight with NAFLD (unpublished).

On the other hand, NAFLD tends to be more severe in patients with various factors of the metabolic syndrome, particularly DM2 and obesity. In fact, the reduction in weight and good glycemic control are associated with an improvement in inflammation and liver fibrosis [11, 78, 79]. However, it is known that NASH can also be present in slim subjects although it is unknown whether the natural history of the disease in these slim subjects is similar to that present in obese subjects. As for arterial hypertension, it is arguable whether its treatment improves the histology of NASH [5, 80]. Another factor of the metabolic syndrome, frequent in NAFLD patients, is dyslipidemia, fundamentally in the form of hypertriglyceridemia and atherogenic dyslipidemia [64, 81]; but moreover, a recent study has related the very low-density lipoprotein (VLDL) profile with the NAFLD severity,

observing that a decrease in small VLDL particle concentration is associated with more advanced fibrosis [82]. Vitamin D deficiency is also frequent among NAFLD patients, and its levels have been correlated negatively with the severity of steatosis, inflammation and fibrosis [83, 84].

Another possible factor associated with NAFLD progression is the alcohol consumption, a controversial aspect as despite there being a limit above which the consumption of alcohol would define alcoholic steatohepatitis (≥ 60 g/d in women and ≥80 g/d in men), it is not clear that we are confronting a pathology different to NASH given that the pathogeny of these entities presents a great similarity. Moreover, the quantification of alcohol consumption is quite subjective, imprecise, habitually underestimated and not contrasted with objective determinations through biomarkers. At present, there is no agreement on the impact of light-moderate consumption of alcohol on NAFLD given that the literature available about this topic shows contradictory results relate to NAFLD progression [85, 86]. Nevertheless, it seems that all the relevant studies are in favor of a possible benefit from the moderate alcohol consumption, defined as the consumption of up to one drink a day for women and two drinks a day for men [87]. While the consumption of large doses of alcohol leads to the development of insulin resistance and to the infiltration of macrophages into the adipose tissue [88], moderate consumption has been associated with an improvement in the sensitivity to insulin and high concentrations of adiponectin [89–91]. Various studies suggest a significant association between the moderate consumption of alcohol and the less histological severity of NAFLD [92, 93]. As for the development of HCC, only one prospective study exists that evaluates the consumption of alcohol with the risk of HCC in NAFLD, finding a greater risk of this tumor with moderate use of alcohol; however, this study is carried out in patients with cirrhosis due to established NASH, without evaluating the impact of alcohol on patients with a less severe disease [94].

3.3. Non-invasive diagnosis of non-alcoholic steatohepatitis

There is still no available image test in clinical practice capable of differentiating NAFL from NASH, so various biomarkers have been evaluated to predict the existence of NASH, which are related to pathogenesis pathways of the disease (apoptosis/cellular death, inflammation and oxidative stress).

The most studied serum biomarker associated with the presence of NASH is cytokeratin 18 fragments (CK18-F), a product of the degradation resulting from the apoptosis of hepatocytes mediated by caspase 3 [95], which is measured using enzyme-linked immunosorbent assay (ELISA). Various studies have demonstrated a significant increase in CK18-F in NASH patients in comparison with NAFL patients, and a positive correlation with fibrosis and the histological components of NASH [96, 97]. However, the sensitivity and specificity of this test are quite low, around 60% [98]. Oxidized low-density lipoprotein (LDL), thiobarbituric acid reactive substances (TBARS) and malonaldehyde have been used as markers of oxidative stress, but the results are contradictory [99, 100]. Among the markers of inflammation studied include leptin, protein C reactive, interleukin 6, hyaluronic acid, adiponectin and tumor necrosis factor α (TNFα). All of them have been evaluated in short series or pilot studies in heterogeneous groups of patients with contradictory results [101].

With the aim of improving the diagnostic value of the biomarkers, predictive models have been developed that combine some of these serum biomarkers with analytical parameters and clinical variables, but they have not been adequately validated, so up to now, they are not recommendable in clinical practice [102–107] (**Table 2**).

3.3.1. Emerging fields

Emerging fields in the search for non-invasive biomarkers of NAFLD are proteomics, metabolomics and epigenetics.

Proteomics provides essential information about the biologically active entity named protein. Thanks to proteomic analysis, key changes in serum protein expression levels have been demonstrated between control subjects and patients with different stages of fatty liver [108].

In the last years, studies about the use of metabolomic to discover biomarkers of progression of NAFLD have received great interest, and not only in this liver disease [109–111]. In fact, a Spanish group has developed the so-called OWL Liver Test that consists in the determination

Model	Variables	Sensitivity (%)	Specificity (%)
HAIR score	Hypertension, ALT, insulin resistance	80	89
NASHTest	Age, gender, weight, height, cholesterol, triglycerides, α2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, ALT, AST, bilirubin	88	50
NASH score	PNPLA3 genotype, insulin, AST	75	74
Nice model	Ck18, ALT, metabolic syndrome	84	86
NAFLD diagnostic panel	Diabetes, gender, BMI, triglycerides, M30, M65-M30	91	47
OxNASH	Age, BMI, AST, 13-Hydroxyoctadecadienoic acid, linoleic acid	81	–

Table 2. Predictive models for non-alcoholic steatohepatitis.

of more than 500 serum metabolites through liquid chromatography coupled with mass spectrometry (LC–MS) in NAFLD patients obtaining a metabolomic profile that enables the differentiation between NAFL and NASH with good specificity and sensitivity [112]. Moreover, the same group thanks to the study of metabolomic profiles at the serum level observed two different subtypes of NAFLD according to the involvement of the methionine metabolism, subtype M and subtype no M, distinguishing those patients that could benefit from therapy with SAMe (S-adenosyl methionine) [113].

Recently, studies in rodents suggest that epigenetic events, inheritable events not caused by changes in DNA sequence, may influence susceptibility to NASH. The three most commonly described epigenetic mechanisms are DNA (CpG) methylation, post-translational histone modifications and microRNAs (miRNAs). Several miRNAs have been identified in serum/plasma of NAFLD patients that show diagnostic potential for distinguishing NAFL from NASH and advanced fibrosis [114].

4. Diagnosis of hepatic fibrosis

The stage of fibrosis ranges from absent (F0) to cirrhosis (F4), with stages F2–F4 considered to be clinically significant and stages F3–F4 considered to be advanced fibrosis. Apart from liver biopsy, there are two broad categories of non-invasive markers used to determine the stage of liver fibrosis: serum and radiological markers. This stratification based on markers of fibrosis is more tractable than those used for NASH and so it is currently used to identify patients who are at risk of disease progression.

4.1. Serum biomarkers

There are two large groups of predictive models of advanced fibrosis: 'simple bedside models', which use a combination of routine blood tests and clinical variables, and 'complex models', which use serum markers of fibrosis (measures of extracellular matrix deposition and turnover).

Although several of these predictive models of advanced fibrosis have been evaluated (**Table 3**) [61, 66, 115], two of the tests have been more widely studied and have easily available parameters, *Fibrosis-4 index* (FIB-4) and *NAFLD Fibrosis Score* (NFS). FIB-4 is based on age, levels of AST, ALT and platelet count. Values of this index below −1.30 enable the exclusion of the presence of advanced fibrosis with a sensitivity of 74% and a specificity of 71%, while values above 2.67 indicate advanced fibrosis with a sensitivity and specificity of 33 and 98%, respectively [115]. NFS is another formula developed and validated for the detection of advanced fibrosis that includes age, BMI, presence of diabetes or hyperglycemia, platelet count, albumin and AST/ALT ratio (http://nafldscore.com/). In a meta-analysis of 13 studies with more than 3000 patients, a value of NFS < −1.455 had a sensitivity of 90% and a specificity of 60% to exclude advanced fibrosis, while a value of >0.676 identified the presence of it with a sensitivity of 67% and a specificity of 97% [116].

Model	Variables	Cut-offs	Sensitivity (%)	Specificity (%)
FIB-4	Age, AST and ALT	1.30 (low cut-off)	74	71
	levels, platelets	2.67 (high cut-off)	33	98
NAFLD	Age, hyperglycemia,	−1.455 (low cut-off)	90	60
fibrosis score	BMV, AST/ALT ratio,	0.675 (high cut-off)	67	97
	albumin, platelet			
AST to	AST, platelet	1	27	89
platelet ratio				
index (APRI)				
AST/ALT ratio	AST, ALT	0.8 (low cut-off)	90	60
		1 (high cut-off)	67	97
BAAT score	BMI, age, ALT, serum	2	71	80
	triglycerides			
BARD score	BMI, AST/ALT ratio,	2	89	44
	diabetes			
Enhanced	Age, hyaluronic acid,	8.5 (low cut-off)	80	90
liver fibrosis	TIMP-1, PIIINP	11.3 (high cut-off)		
(ELF) test				
FibroTest	α2-macroglobulin,	0.3 (low cut-off)	77	77
	haptoglobin, GGT,	0.7 (high cut-off)	15	90
	bilirubin,			
	apolipoprotein			
Hepascore	Age, gender, bilirubin,	0.44	75	84
	hyaluronic acid, α2-			
	macroglobulin			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; TIMP-1, tissue inhibitor of metalloproteinase *1*; PIIINP, procollagen III amino-terminal peptide; GGT, gamma glutamyl transferase.

Table 3. Predictive models for significant and advanced fibrosis in NAFLD patients.

The principal drawback of all these biomarkers is that none of them is specific of the liver and their results can be influenced by co-morbidities of patients, so a critical interpretation of the result is necessary.

4.2. Imaging methods to measure fibrosis

With respect to image techniques, transient elastography (FibroScan®) is the most widely used technique in the diagnosis of liver fibrosis, not only in NAFLD but also in different chronic

liver diseases [117]. TE measures the propagation velocity of low-frequency waves (50 Hz) through the hepatic parenchyma using ultrasounds and is expressed in kilo Pascal (kPa); the higher the propagation velocity, the greater the stiffness of the tissue. The advantages provided by this technique are its speed, the immediacy of the results and the ease of handling. However, proper results require careful interpretation of data, based on at least 10 successful measurements, a success rate above 60% and an interquartile range (IQR) of <30% of the median value. A limitation of TE in NAFLD is the high rate of technical failure due to the attenuation of the elastic wave by interposition of adipose tissue secondary to the central obesity, very frequent in these patients. Although an XL probe has been developed, which enables greater penetration of the wave, this difficulty is often insurmountable [118, 119]. Moreover, this technique has been initially validated in patients with chronic infection by VHC [120], while the studies focusing on evaluating its use in NAFLD are smaller and have often used different cut-offs [42, 118, 121–129] (**Table 4**). According to the results of several studies, the cut-offs with M probe accepted for NAFLD patients are 7.0 kPa for significant fibrosis (≥F2), 8.7 kPa for advanced fibrosis (≥F3) and 10.3 kPa for cirrhosis (F4) [124, 126, 128]. When using the XL probe, these cut-offs differ as the measure of liver stiffness with this probe is less than that with the M probe in the same patient; in this case, 6.2, 7.2 and 7.9 kPa are the cut-offs for significant fibrosis, advanced fibrosis and cirrhosis, respectively [119, 130, 131].

Another liver elasticity-based imaging technique is ARFI (*acoustic radiation force impulse imaging*). Although for the time being there are few studies that have evaluated its utility in NAFLD patients, its great advantage is that it can be easily connected to traditional ultrasound scan enabling the positioning of the zone of interest under visual control [132, 133]. Another method suitable for studying the elastic properties of the hepatic parenchyma is magnetic resonance elastography (MRE). MRE can be more reliable than TE to diagnose advanced fibrosis; moreover, it has the advantage of being able to evaluate the whole hepatic parenchyma even in obese patients, but the technique is expensive and not widely available [134, 135]. Magnetic resonance imaging is more widely available and is the basis of new software called DEMILI (*Detection of Metabolic-Induced Liver Injury*), which through computerized optical analysis of its images determines a series of optical biomarkers enabling the detection of the presence of NASH (NASHMRI) and predicting significant fibrosis (FibroMRI) in NAFLD patients. For the detection of NASH, a cut-off has been established with NASHMRI of >0.5, presenting a sensitivity and specificity of 87 and 60%, respectively. In the case of FibroMRI, the cut-off is also >0.5 for the prediction of significant fibrosis with a sensitivity of 77% and a specificity of 80% [136]. Given that this technique enables the analysis of the total volume of the liver, as well as its use in the diagnosis of NASH and significant fibrosis, it enables the potential effects of a therapy to be monitored.

A recently developed technique is multiparametric magnetic resonance (MR) that includes T_1 mapping for fibrosis/inflammation imaging, T_2* mapping for liver iron quantification and proton magnetic resonance spectroscopy (^{1}H-MRS) for liver fat quantification. In a recent study, it has demonstrated good correlation with disease severity in NAFLD patients, showing excellent accuracy in quantifying both the inflammatory and fibrotic components of NAFLD [137].

A summary of the approach to the management and characterization of NAFLD patients is shown in **Figure 1**.

Study	Patients, *n*	Probe	Fibrosis Stage	Cut-off (kPa)	Sensitivity (%)	Specificity (%)
Yoneda et al. [122]	67	M	$F \geq 2$	6.65	82	91
			$F \geq 3$	8.0	87	84
			$F = 4$	17.0	100	98
Yoneda et al. [125]	97	M	$F \geq 2$	6.65	74	97
			$F \geq 3$	9.8	85	81
			$F = 4$	17.5	100	97
Nobili et al. [129]	52	M	$F \geq 2$	7.4	100	92
			$F \geq 3$	10.2	100	100
			$F = 4$	–		
Wong et al. [128]	246	M	$F \geq 2$	7.0	79	76
			$F \geq 3$	8.7	84	83
			$F = 4$	10.3	92	88
Lupsor et al. [124]	72	M	$F \geq 2$	6.8	67	84
			$F \geq 3$	10.4	100	97
			$F = 4$	–		
Petta et al. [118]	169	M	$F \geq 2$	7.25	69	70
			$F \geq 3$	8.75	76	78
			$F = 4$	–		
Kumar et al. [126]	205	M	$F \geq 2$	7.0	78	79
			$F \geq 3$	9.0	85	88
			$F = 4$	11.8	90	88
Pathik et al. [121]	110	M	$F \geq 2$	9.1	–	–
			$F \geq 3$	12.0	90	80
			$F = 4$	20.0	90	80
Cassinotto et al. [123]	291	M	$F \geq 2$	6.2	90	–
			$F \geq 3$	8.2	90	–
			$F = 4$	9.5	90	–
Imajo et al. [42]	142	M	$F \geq 2$	11.0	62	100
			$F \geq 3$	11.4	86	84
			$F = 4$	14.0	100	76

Table 4. Comparative studies of FibroScan with liver biopsy in the detection of fibrosis in NAFLD.

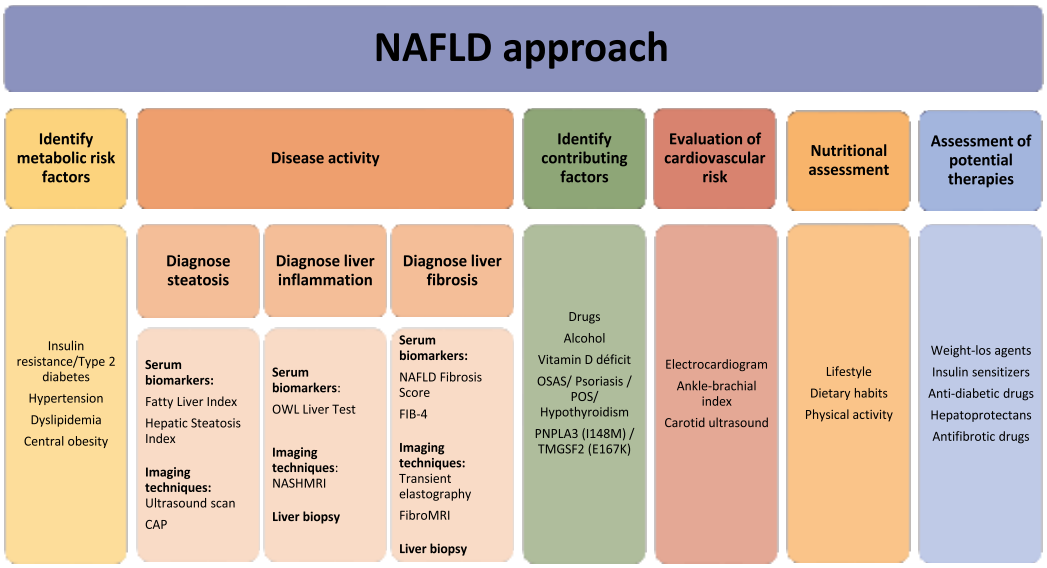

Figure 1. Practical approach to the management of patient with NAFLD.

5. Monitoring disease progression

A recent meta-analysis of 11 studies that evaluated the progression of NAFLD through the use of paired liver biopsies revealed that patients with NAFL and NASH presented a progression of fibrosis of 33.6% and an improvement of it of 22.3%, the rate of fibrosis progression being greater in patients with NASH than with NAFL (progression in a stage of 7.1 years compared to 14.3 years, respectively) [5]. However, there is a lack of homogenization in the speed of fibrosis progression in all these studies with paired biopsy, which is mostly due to the presence of characteristics of the metabolic syndrome in the patients [5–7, 62, 65, 138–144]. For this reason, due to the lack of studies that provide complete data about the differential progression of the disease in patients with different stages of NAFLD, there is no guide on the frequency of follow-up of these patients or on the means available to monitor the progression. Nevertheless, once the diagnosis of NAFLD is made, the follow-up will depend on the presence of metabolic risk factors and the severity of the hepatic disease, which will be determined by the presence of NASH and, especially, the stage of fibrosis.

The principal metabolic factor of the risk of NAFLD progression is DM2 [1, 19, 145]. Patients with DM2 have a more severe grade of NAFLD than the patients without DM2, with rates of NASH up to 80% and of advanced fibrosis of 30–40% [146, 147]. These data confirm the need for closer monitoring in these patients. Bazick et al. [147] developed a clinical model to detect NASH and advanced fibrosis in patients with NAFLD and DM2 with a sensitivity and specificity of 57 and 90%, respectively. This model includes easily accessible parameters such as BMI, circumference at the waist, HbA1c, insulin resistance, ALT, AST, albumin and ferritin, for NASH; and age, BMI, waist/hip ratio, arterial hypertension, ALT/AST ratio, alkaline

phosphatase, bilirubin, globulin, albumin, serum insulin, hematocrit, INR and platelets, to predict advanced fibrosis. However, further studies are still necessary to externally validate this model. Other metabolic factors described with more evidence for the disease progression are central obesity, arterial hypertension and high levels of LDL cholesterol [7, 148–150]. No study shows cost-efficacy in the monitoring of the progression in these at-risk patients, but we recommend carrying out NFS and/or FIB-4 every 2 or 3 years in these patients with non-significant fibrosis, and if NASH and/or significant fibrosis is presented in the initial diagnosis, the follow-up will not differ from the rest of the patients.

The other factor having the greatest effect on the disease progression is liver fibrosis [151]. In general, in a period of 15 years, 13% of the patients with stage F2 and 25% of those presenting with F3 will develop cirrhosis [6, 7, 62]. These patients with significant fibrosis should be considered for pharmacological treatment, besides lifestyle modifications (diet and exercise). Moreover, NAFLD patients may develop HCC even in the absence of cirrhosis [152], given that it is the continuous hepatocyte injury that leads to a compensatory proliferation, key driver of the development of HCC [153]. Therefore, patients with NASH and significant fibrosis, which is indicative of important cellular damage, are also at risk of developing this liver tumor.

With all this information, we recommend recalculating NFS and/or FIB-4 every 4–5 years for patients with NAFL without risk factors or if the patient develops DM2; in patients with NASH without significant fibrosis, we recommend an annual follow-up with a calculation of NFS and/or FIB-4 and carrying out TE and ultrasound, and in patients with significant fibrosis, a 6-monthly follow-up is recommended with special interest in screening for HCC. The management and follow-up of the patients with advanced fibrosis/cirrhosis due to NASH does not differ from the rest of etiologies [25].

Another important question is the evaluation of the response to the therapy provided. The non-invasive methods available currently have not been reliable or have not been validated to document efficacy of the treatments, so liver biopsy is still necessary to determine this efficacy, especially in a clinical trial setting.

6. Screening of associated diseases

In recent years, several studies have confirmed that the morbimortality associated with NAFLD is not limited only to hepatic injury, yet it is a disease with multisystemic behavior with affectation of different organs.

6.1. Insulin resistance and metabolic syndrome

As was previously mentioned, the concurrent characteristics of metabolic syndrome increase the risk of developing NAFLD, and a recent study of the HepaMet group relates the severity of NAFLD with the number of factors of the metabolic syndrome present (publication pending). However, the presence of NAFLD in itself also increases the risk of developing complications such as dyslipidemia and insulin resistance [154–156]. In this sense, the diagnosis and

quantification of hepatic fat can be useful in the prediction of future development of diabetes and other cardiovascular risk factors [56].

Insulin resistance is a key in the physiopathology of NAFLD, associated with the increase in the deposit of fat and fibrosis, and it substantially increases the risk of developing DM2, which indicates that NAFLD can precede the development of diabetes. Moreover, and as it was mentioned earlier, several studies have demonstrated that, especially in patients with insulin resistance and/or diabetes, liver fibrosis can progress even when a baseline hepatic histology described only simple steatosis without hepatocellular damage [62, 141]. All in all, in daily clinical practice the use of screening tools is necessary to detect the presence of diabetes (fasting blood glucose levels, HbA1c or, if available, the oral glucose tolerance test) or insulin resistance. The reference technique for the diagnosis of IR in non-diabetic patients is the hyperinsulinemic-euglycemic clamp test, although this procedure is expensive and complicated, so it is not routinely used in daily clinical practice [157]. In these cases, the calculation of HOMA-IR (*homeostatic model assessment*) is an acceptable alternative to evaluate the IR, although there is no agreement on the threshold that defines insulin resistance using this formula [158]. Nevertheless, HOMA-IR can help us during the follow-up to identify patients at risk of fibrosis progression [6, 62]. The next question once the patients with IR are identified is whether it is necessary to treat them pharmacologically or not; and, whether in diabetic patients is necessary to intensify the anti-diabetic treatment to avoid liver disease progression or not. As expected, several insulin-sensitizing agents have demonstrated an improvement in the hepatic histology [159–161], even in patients without DM2 [162, 163], given that both entities share multiple physiopathological mechanisms, so this treatment can be considered in patients with NASH and/or multiple factors of progression in which a decrease of IR cannot be achieved with diet and exercise, although the EASL and AASLD guidelines do not contemplate it. Given that IR plays an essential role in NAFLD progression but not the only one, we do not believe that it is necessary to treat DM2 differently/intensely in patients with NAFLD, independently of the grade, provided that the IR is controlled.

6.2. Cardiovascular disease

Cardiovascular disease is quantitatively the main cause of death in NAFLD patients. Besides the risk itself of the characteristics of the metabolic syndrome, multiple pathogenic conditions of NAFLD contribute to the development of cardiovascular disease. In fact, patients with NAFLD often present elevation in the markers implicated in the development of atherosclerosis, such as CD36 in its soluble form (sCD36), a membrane receptor responsible for, among other things, the transport of fatty acids [164]. The spectrum of CVD in NAFLD includes atherosclerotic coronary heart disease, heart failure and cardiac arrhythmias. This necessitates the study of probable CVD, especially subclinical atherosclerosis, in all these patients [10]. There are little data to define the optimal means of screening NAFLD patients with CVD, but it is important to be aware that there are different techniques for the detection of subclinical atherosclerosis that are bloodless and some of which are very easily performed. Among these, the measurement of ankle-brachial index and carotid ultrasound are assessments especially useful for patients with intermediate cardiovascular risk, situation affecting a very important part of the population with NAFLD [165].

6.3. Extrahepatic cancer

The second most prevalent cause of death among patients with NAFLD is cancer, both gastrointestinal (colon, esophagus, stomach and pancreas) and extraintestinal (kidney and breast), which leads to the suspicion that this liver disease might promote the development of neoplasms.

The association of insulin resistance/diabetes, obesity and metabolic syndrome with an increase in the risk of a large number of cancers is well established [166–171]. These three characteristics are closely related to NAFLD and contribute significantly to the risk of developing HCC; nevertheless, various recent studies indicate that NAFLD can be an additional and independent risk factor for extrahepatic cancers [172, 173], especially colorectal cancer (CRC) [127, 174]. In several studies, colorectal lesions, particularly tubular adenomas and carcinomas, were significantly more prevalent in NAFLD patients, regardless of age, sex and manifestations of metabolic syndrome; even the presence of NASH has been related to a greater risk in comparison with those with NAFL [174, 175]. This rise in the risk of CRC in NAFLD can be explained by the increase in insulin and pro-inflammatory cytokines and the alteration of the adipokines metabolism predominantly leptin versus adiponectin that exists in these patients and which promotes cellular proliferation, inhibition of apoptosis and angiogenesis [176, 177].

Although these data clearly suggest more rigorous screening programmes for CRC in NAFLD patients, there are no well-designed prospective studies enabling the verification of a causal relation between NAFLD and CRC or studies that evaluate the usefulness of earlier screening in this liver disease, so no guidelines make a distinction with respect to CRC screening in these patients.

6.4. Other associated diseases

There is increasing interest in the possible contribution of NAFLD to the development and progression of chronic kidney disease (CKD) [178–181]. A recent meta-analysis has revealed that the presence and severity of NAFLD are associated with an increase in the risk and severity of CKD [181]. However, it is difficult to establish NAFLD as an independent risk factor of CKD given the close relation between NAFLD and other known risk factors of CKD such as obesity and IR. Obstructive sleep apnea syndrome (OSAS) is strongly associated with NAFLD independently of other traditional factors; it is a consequence of the decrease in the lipid metabolism provoked by intermittent hypoxia [182–185]. Other described diseases associated with NAFLD include osteoporosis [186], psoriasis [187], polycystic syndrome [188] and other endocrinopathies such as hypothyroidism [189], hypopituitarism [190] and hypogonadism [191]. Until now, there is no evidence for screening of all these pathologies for the mere fact that the subject presents NAFLD, so all that needs to be studied is the presence of them if the patient has clinical manifestations related to them. Moreover, a recent study by our group has demonstrated that psychotic patients with specific pharmacological treatment have a high risk of developing NAFLD in the first years, so its early detection will enable better prevention of cardiovascular events, which are so increased in this population [192].

7. Diagnostic algorithm and follow-up

While working on the different sections of this chapter, we have detailed the fundamental elements for the development of a diagnostic algorithm and a follow-up procedure for NAFLD (**Figure 2**). This algorithm is based on clinical evidence available in the current literature with respect to the topic and on different guidelines issued by the principal international associations for the study of the liver (EASL and AASLD). In the case of monitoring and follow-up of these patients where the existing evidence is not relevant in certain aspects, our recommendations are based on the experience of our clinical group in different high-quality studies in this field.

Once the initial diagnosis of NAFLD is made, our posterior attitude will depend on the result of the non-invasive liver fibrosis methods. In general, current image techniques are quite reliable to distinguish between advanced fibrosis (≥*F*3) and mild fibrosis or null (*F*0–*F*1), but they are insufficient to identify those patients with significant fibrosis (≥*F*2). Therefore, in clinical practice, we recommend the combination of elastographic techniques with serum markers, more specifically TE and NFS due to their wide accessibility and ease of application. When these two parameters generate doubt about the grade of fibrosis or indicate possible significant fibrosis, liver biopsy is necessary. Depending on the result, we determine the posterior follow-up as can be seen in the algorithm (**Figure 2**). The presence of metabolic risk factors influences not only the therapeutic management but also the follow-up. If liver biopsy is

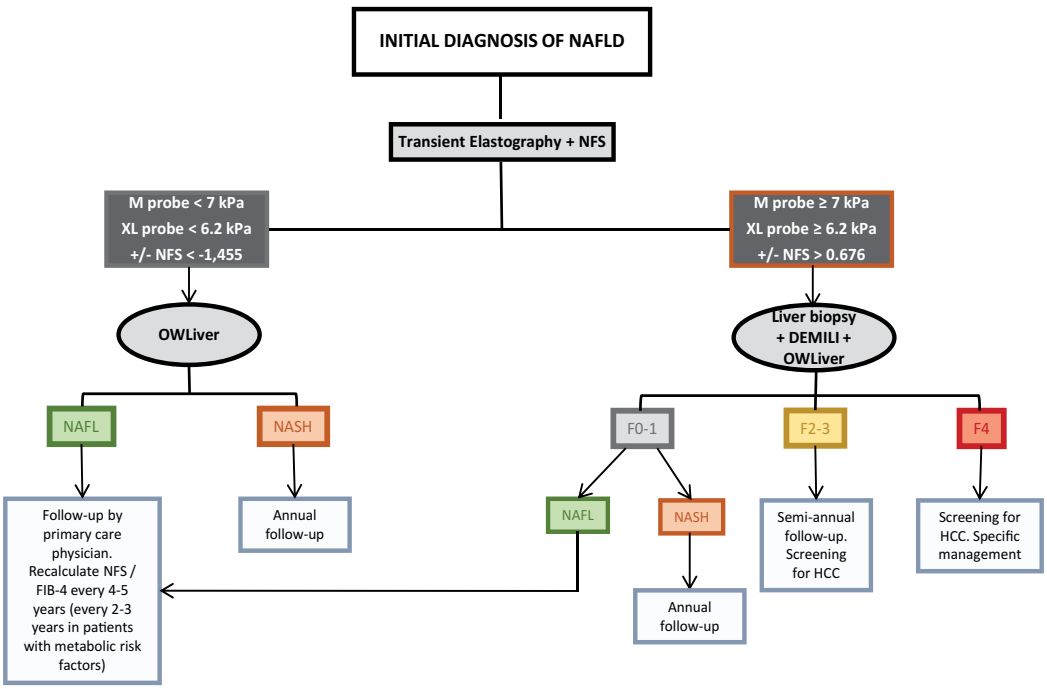

Figure 2. Clinical algorithm for the diagnosis of NAFLD and monitoring disease progression.

not to be performed on the patient with NAFLD, due to advanced age, to the absence of significant fibrosis in the non-invasive methods or to contraindication, we could evaluate the performance of the OWL Liver Test to help identify those patients with NASH who require a closer follow-up. If the patient does not present improvement in laboratory parameters even in imaging tests, we should evaluate to repeat liver biopsy 5 years after the last one, or even before if progression of the disease is suspected.

8. Conclusions

NAFLD is currently the primary cause of chronic liver disease in the western world and its growth is a consequence of its close relation to obesity and metabolic syndrome. One of the great challenges in this disease is to diagnose and classify it correctly, given that the characteristics defining NAFLD are the common denominator of many liver diseases. Its correct characterization is important as in spite of presenting a generally benign and slowly developing evolution from the hepatic viewpoint; the fatty liver can progress towards more severe forms with the development of inflammation, fibrosis, cirrhosis and HCC, thus conferring morbimortality. However, its potential morbimortality is not limited to this organ, but goes beyond; NAFLD is being considered a mediator of systemic diseases. Therefore, the early identification of these patients would help to improve its prognosis through an individualized intervention depending on the stage of liver disease, on the metabolic risk factors present and on the cardiovascular risk, which translates into the need for a systemic approach to the disease with multidisciplinary management including primary care physician, endocrinologists, nutritionists, psychologists and hepatologists.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this chapter.

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Chapter 2

Non-Alcoholic Fatty Liver Disease, Diabetes Mellitus, and Zinc/Zinc Transporters: Is there a Connection?

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73735

Abstract

Immune response and metabolic regulation are closely connected with each other in such a way that dysfunction could lead to a variety of metabolic diseases such as obesity, diabetes mellitus (Dm), lipid metabolism disorders, and fatty liver disorders. Combined with uncritical "sugar-based" overeating and malnutrition, these multisystem metabolic diseases expand into a global epidemic. There are correlations between a fatty liver disease and diabetic metabolism state. A fatty liver leads to insulin resistance and thus to the development of a type 2 Dm; insulin resistance in turn augments the fatty liver. Zinc is a trace element of fundamental importance for a variety of biological processes. The liver is the main organ of the zinc metabolism. Metallothionein and zinc transporters are the key regulators of cellular zinc homeostasis. Molecular studies support the assumption of a correlation between zinc and Dm. Zinc is essential for the synthesis, secretion, and storage of insulin. ZnT8 is a significant autoantigen for type 1 Dm. Genetic polymorphisms in the ZnT8 gene are associated with an increased risk of developing type 2 Dm. Cellular zinc restriction induces the release of stress, particularly in the endoplasmic reticulum (ER). ER stress alone or coupled with cellular stress, as well as chronic inflammation, are central to the development of insulin resistance and type 2 Dm. The present insights into the context of a non-alcoholic fatty liver disease (NAFLD) and a type 2 Dm indicate that zinc and zinc transporters at the cellular level in various forms and in interactions with other mediators both in the regulation of physiological processes and in the formation of pathological processes, such as the cellular and ER stress, as well as chronic inflammation, and the development of metabolic disorders are involved.

Keywords: NAFLD, HCC, insulin resistance, diabetes mellitus, zinc, zinc transporter

1. Main text

Non-alcoholic fatty liver diseases (NAFLD) comprise a broad spectrum of liver diseases that go from non-alcoholic fatty liver (NAFL) to non-alcoholic fatty liver hepatitis (NASH), secondary fatty liver to fatty liver cirrhosis [1–4]. NAFLD represent 11–46% of all chronic liver diseases in the world [1]. NAFLD-induced liver changes look similar to those of alcoholic liver damage [4]. The accumulation of triglycerides and free fatty acids in the hepatocytes and an increased lipogenesis are typical features of an NAFLD. NAFLD is in part also causally associated with other diseases (e.g., metabolic multisystemic diseases such as obesity, type 2 diabetes mellitus (Dm), dyslipidemia, and hypertension [5, 6]. These diseases show a strongly increasing prevalence in particular in the Western and Asian industrialized states [4]. Due to the central role of the liver in the glucose metabolism, fatty acids and amino acids, there is a close interaction between the fatty liver and development of a diabetic metabolic state. On the one hand, the fatty liver will lead to insulin resistance and development of type 2 Dm; on the other hand, insulin resistance compounds the fatty liver [1, 7, 8]. Insulin resistance results in a reduction of glucose intake of the liver and other organs at concurrently increased hepatic glucose production. For this reason, modulation of the hepatic glucose metabolism is a target for antidiabetic treatment [9]. It is not clear yet whether the fatty liver is a cause or consequence of insulin resistance [8]. To differentiate NAFLD from alcoholic fatty liver disease or a mixed form, a daily alcohol limit of 10 g/day in women and 20 g/day in men is assumed. NASH, which can occur in up to 30% of the patients with NAFLD, is characterized by the presence of a mixed-cell infiltration in the hepatic lobules and a cell swelling of the hepatocytes (ballooning). NASH has a multifactorial genesis where genetic as well as environmental factors (e.g., excessive fat accumulation, mitochondrial dysfunction, influence of endotoxins and proinflammatory cytokines) contribute to chronic inflammation of the hepatocytes [1, 5, 6]. NASH as such is deemed a risk factor for the development of cirrhosis and hepatocellular carcinoma (HCC) [1, 4, 10]. Liver biopsy with subsequent histopathological evaluation is the diagnostic gold standard for differentiation between NASH and NAFLD [2, 6, 11]. At this time, NASH is the second most frequent underlying liver disease in the USA in patients to receive a liver transplant for HCC [12]. In a large, population-based study, Younoussi et al. [11] examined the prevalence and incidence of HCC in 2004–2009. Chronic hepatitis C-infection, at 54.9%, was the most frequent cause, and NAFLD was the third most frequent one at 14.1%. The authors explain the annual increase of the HCC incidence around the world with increased HCC screening, as well as with the increase of NAFLD [4, 11]. It is forecast that NAFLD will be the main cause of HCC development in approximately 20 years, after successful eradication of chronic hepatitis C infection, reduction of hepatitis B infection, and concurrent global increase of overnutrition [11, 13]. In particular, type 2 Dm is considered an independent risk factor of HCC [14]. Although liver cirrhosis is a precancerous condition and more than 90% of liver carcinomas develop based on cirrhosis, HCC can, similar to hepatitis B, develop without cirrhotic changes to the liver in NASH as well [15–17].

Zinc is an essential trace element that can be found in all tissues and that is of fundamental relevance for many biological processes, including the division, growth, and differentiation

of cells [18–20]. Regulation of zinc homeostasis involves many proteins such as metallothioneins, zinc transporters, and specific permeable channels [21, 22]. Metallothioneins are important for the resorption and storage of zinc.

There are two major protein families that mammalian zinc transporters belong to [23, 24]. The first group of transporters are ZIP (Zrt/-like proteins), which are responsible for transporting zinc into the cytosol from either extracellular space or from intracellular compartments. There are 14 ZIP transporters, designated as solute family SLC39A1-A14 [23, 24]. The second group of 10 transporters are ZnT (zinc transporters), which designated as SLC30A1-A10 [23, 24]. They generally transport zinc out the cytosol into extracellular space or intracellular organelles such as zincosomes. Zincosomes are vesicles that can sequester high levels of zinc [25].

The liver is essential for zinc homeostasis, with zinc deficits leading to the impairment of many hepatic functions. On the other hand, liver diseases are often associated with zinc deficits [26, 27]. The scope of zinc deficit is not determined as much by the genesis (alcohol, viruses, etc.), but rather by the severity of liver damage, fibrosis or cirrhosis, with or without metabolic and/or portal decompensation, or the presence of a HCC [28]. Although a connection between zinc and the development of Dm has been discussed for years, only molecular studies of the last few years have supported this hypothesis [29]. Zinc increases the insulin effect in peripheral tissues and is indispensable for synthesis, secretion, and storage of insulin in the pancreatic ß-cells. It stabilizes the insulin structure, protects against insulin degradation, and is secreted together with insulin, proinsulin, and C-peptide in the early phase of glucose-stimulated insulin secretion; it has an insulin mimetic effect [30]. Type 2 Dm is usually associated with decreased plasma or serum zinc concentrations, whereas type 1 Dm plasma or serum zinc mostly elevates [30]. This is interpreted that at the beginning of type 1 Dm, a destruction of ß-cells takes places, and with decreased zinc concentration later, when the hyperzincuria outweighs the zinc release from ß-cells [30]. **Table 1** shows the causes of zinc deficiency in liver cirrhosis and diabetes mellitus.

Liver cirrhosis [28]	**Diabetes mellitus [30]**
Inadequate intake	Inadequate intake
Changes in protein and amino	Polyuria, hyperzincuria
acid metabolism	Osmotic diuresis
Diminished hepatic extraction	Increased intestinal secretion
Portosystemic shunts	Decreased intestinal absorption
Alcohol-induced impaired absorption	
Cytokines, IL-1, IL-6	Inflammation, cytokines, IL-1, IL-6
Endotoxins	Acidosis
Catabolism	

Table 1. Causes of zinc deficiency in liver cirrhosis and diabetes mellitus.

Current studies on the function of zinc transporters show that genetic variations of ZIP or ZnT genes, as well as changes to the expression and activity of the zinc transporters, are involved in the pathogenesis of various diseases [31–33]. Pancreatic ß-cells express various zinc transporters (e.g., ZnT3, ZnT5, ZnT8), which are required to ensure zinc homeostasis [29, 34, 35]. Examinations by Yi et al. [32] show that reduced expression of ZnT8 impairs biosynthesis and release of insulin and ß-cell functions. For example, hypoglycemia releases glucagon from pancreatic a-cells as regulated by the activity of ZnT8 [36]. Impaired ß-cell function leads to an absolute or relative deficit of insulin, which subsequently causes type 1 or type 2 Dm. The functional relevance of the ZnT8-function for glucose regulation is supported by association of the auto-antibodies against ZnT8 (ZnT8A) with diabetes; these auto-antibodies have an increased prevalence (in type 1: 60%, type 2: 6–24%) as compared to healthy persons (8%; [33]). Genetic polymorphisms in the SLC30A81 ZnT8-gene are associated with an increased risk of developing Dm type 2 [29, 37–40]. Genetic variants of the ZnT8 protein (e.g., rs13266634) lead to different hepatic insulin "clearance" rates that regulate the peripheral insulin concentration [41]. Individuals with the above risk allele have an impaired insulin metabolization and storage. This is also associated with reduced effectiveness of zinc substitution [33]. Reduced function of ZnT8 and the resulting reduced zinc content in islet cells are a genetic predisposition of such persons for an impaired glucose regulation and type 2 Dm [40, 42]. Predictive examinations of these gene versions that are sensible for clinical relevance in the meaning of diagnostic risk stratification (e.g., at positive family history for Dm, metabolic syndrome, NAFLD) will require further studies [43]. Modulation of ZnT8 activities also provides a new potential therapeutic point of attack for Dm and NAFLD [38]. In addition to ZnT8, the "influx transporter" ZIP14 plays a functionally relevant role in hepatic zinc regulation [24, 44]. According to the examinations by Aydemir et al. [9], ZIP14-mediated zinc transport is involved in regulation of the insulin receptor activity and maintenance of the glucose homeostasis in the hepatocyte. They also observed that there was an increase of ZIP14 and an increase of controlled zinc transport during glucose absorption on the cell surface. In the course of this, zinc is relocated to various locations in the hepatocyte through sequential translocations, from the membrane surface to the earlier and late endosomes. The authors conclude from this that ZIP14 may have a relevance analogue to that of ZnT8 regarding the diagnosis and treatment of type 2 Dm and NAFLD. Current findings by Kim et al. [45] showed the relevance of zinc trafficking and the functional ZIP14 activity for adaptation to endoplasmic reticulum (ER) stress connected to metabolic diseases. According to Zhang [46], zinc restriction in the cells triggers ER stress, which highlights the relevance of zinc for maintaining a normal ER function. There are epidemiological, clinical, and experimental indications that cellular stress (impaired biological processes in the cell) and excessive inflammation are causatively connected to various metabolic conditions, for example, obesity, type 1 and 2 Dm and arteriosclerosis [47–49]. Öczan et al. [50] found that ER stress plays a central role in peripheral insulin resistance and type 2 Dm on a molecular, cellular, and organismal level. The conditions triggering ER include glucose and food withdrawal, viral infections, lipids, increased synthesis of secretory proteins as well as mutated or incorrectly designed proteins [50].

In an excellent review for expression of ZIP 9 transporters in ß-cells of the pancreas, Lawson et al. [51] showed how complex, overlapping, and interlocking zinc transporters work. They

identified ZIP6, ZIP7, ZIP9, ZIP13, and ZIP14 in humans and rodents and ZIP1 in rodents as potentially biologically relevant for the zinc effects in the ß-cells of the pancreas.

Inflammation is the first reaction of the immune system to infection or other damage to cells and tissues to protect the human or animal organism. Prolonged or chronic inflammations are harmful and release inflammatory substrates such as pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha), free radicals, hormones and other small molecules, leading to impairment and damage of the physiological cellular processes [47]. Zinc and zinc transporters are involved in the development of ER stress and impairment of the protein synthesis, the unfold protein response (UPR; 45). UPR could be triggered in yeasts and in some mammals by zinc restriction UPR [45, 52, 53]. In the liver, impaired apoptosis leads to dysregulation of the lipid metabolism, causing hepatic steatosis [54]. Kim et al. [45] were able to show that the ZIP14-mediated zinc transport is critical for the prevention of prolonged apoptotic cell death and steatosis during ER stress, that is, for hepatocellular adaptation to ER stress.

Hashimoto et al. [55] found in patients with chronic hepatitis C that zinc deficiency promotes the insulin resistance by exacerbating iron overload in the liver and induces hepatic steatosis by facilitating lipid peroxidation.

Both type 2 Dm and NAFLD are consequences of chronic inflammation and cellular and ER stress. Although the processes that occur during this have not been fully determined yet, it seems highly likely that zinc and zinc transporters play an essential role in the pathogenesis of such diseases.

Zinc supplementation has been investigated as a potential adjunct therapy in the management of Dm; however, the outcomes of such interventions are conflicting [56, 57]. Capdor et al. [57] found a modest reduction in glucose concentration and tendency for a decrease in HbA1c following zinc supplementation and suggested that zinc may contribute to the management of hyperglycemia in individuals with chronic metabolic disease. Ruz et al. [58] remarked that studies available to date on zinc supplementation in type 2 Dm suggest that zinc supplementation is only effective in patients with initially reduced zinc concentrations.

Pia et al. [59] studied the protective effects of zinc supplementation on diabetic liver injury in a rat model of type 2 Dm. They found that zinc supplementation improved liver conditions in type 2 Dm rat models through multiple pathways, in which GRP78 linked ER stress and LC3-II-linked autophagy are ameliorated to some degree. The results of a systematic review by Barbosa de Carvalho et al. [60] about the role of zinc in patients with type 2 Dm confirming the role of zinc in controlling circulating glucose concentration through maintenance of insulin homeostasis. Based on these positive findings, the authors concluded that adequate dietetic ingestion and/or zinc supplementation are essential in the control of type 2 Dm. Lastly, Islam et al. [61] reported in a double-blind randomized placebo controlled pilot study an improving of glucose handling in pre-diabetes by zinc supplementation.

According to the long-term experiences with zinc supplementation in patients with chronic liver diseases, in particular in case of decompensated liver cirrhosis, administration of zinc leads to an increase and often normalization of the zinc levels, with the duration depending

on the scope of zinc deficit in the serum [28]. Zinc supplementation improved in patients with liver cirrhosis and hepatic encephalopathy with and without Dm neurologic symptoms and signs of malnutrition [62–64]. Zinc administration increased glucose disposal entirely due to noninsulin-mediated glucose uptake without any systematic effect on insulin secretion and sensitivity [65]. Ruz et al. [58] recommended to further determine the role of zinc in type 2 Dm and therapeutic effectiveness of supplementation by long-term studies under observation of factors such as stage of disease, comorbidities, that is, also NAFLD, duration and type of medication (zinc preparation), and, finally, also examining the genetic variations in SLC30A8 as well due to the heterogeneity and complexity with multiple influences on the disease (**Figure 1**).

Figure 1. Schematic illustration of the organs, cells, substrates and main mediators involved in the development of insulin resistance linking NAFLD and type 2 Dm.

2. Concluding remarks

The data and findings that are available to date, and certainly not comprehensive, on the interrelation of fatty liver disease and type 2 diabetes mellitus show that zinc and zinc transporters on a cellular level are involved in the regulation of physiological processes as well as the development of pathological processes such as cellular stress, ER stress and not least chronic inflammation in diverse manners and interactions with other mediators, and therefore also in the development of such metabolic diseases.

Due to high complexity of the diseases, there are no simple solutions, that is, normalization of one "pathway" is not enough to recover functional homeostasis (controlling the diseases, health) of the integrated processes. Sole zinc substitution is surely ineffective in most cases, but may promise success in combination with other substrates.

Acknowledgements

I thank Anne Grüngreiff, my daughter, for excellent technical assistance.

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Section 2

Imaging Evaluation of the Liver

Chapter 3

Imaging Evaluation of Liver Tumors in Pediatric Patients

Chengzhan Zhu, Bingzi Dong and Qian Dong

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73855

Abstract

Imaging plays crucial roles in the management of pediatric patients with suspected liver malignant tumors. Three-dimensional (3D) imaging could significantly improve the resection rate of pediatric tumors and increase the safety of the surgery. With the development of medical imaging, 3D reconstruction technology, the innovation of liver surgery and the proposal of precise hepatectomy, the intrahepatic vascular anatomy of the liver and liver segmentectomy based on that vascular anatomy have become well developed. With the analysis of 3D digital liver, we proposed a new type of liver classification system: Dong's digital liver classification system. And we measured the normal total liver volume from neonate to aging making a reference for surgeons all around the world. And the Human Digital Liver Database was established by the Affiliated Hospital of Qingdao University and Hisense Company, aiming to collect digital liver from neonates, children, adults, and the elderly, from normal livers, livers with cancer, and simulated livers resected using Hisense CAS. Then we showed one case report of patient with giant liver tumor. With the application of Hisense CAS and our data, we successfully removed the tumor. We believe that the new techniques in imaging will help surgeons to accomplish better operations.

Keywords: three-dimensional imaging, liver tumor, digital liver classification, total liver volume, Human Digital Liver Database

1. Introduction

Liver tumors constitute 1–4% of all solid tumors in children, of which 40% are benign. They mainly include hemangioma, liver hamartoma, and liver cell adenoma. Malignant tumors mainly include hepatoblastoma (HB), hepatocellular carcinoma (HCC), malignant liver

mesothelioma, and rhabdomyosarcoma [1]. For most hepatic malignancies, hepatectomy or liver transplantation is optimal for cure. Resectability can be limited by multifocality, bilobar involvement, vascular thrombus or vascular invasion, extension to hepatic hilum, and distant metastasis [2]. If the tumor cannot be resected at initial imaging evaluation, the child is usually first treated with chemotherapy and/or radiation, and then re-imaged. For this reason, proper imaging evaluation of the liver is necessary which will shorten the surgical waiting duration and increase the success of the resection. In the cases where liver resection has high morbidity and high incidence, liver transplantation is recommended.

Imaging plays crucial roles in the management of pediatric patients with suspected liver tumors. MR imaging is recommended for children than computed tomography (CT) because of less radiation [3, 4]. However, CT could clearly show the liver anatomy and be helpful in staging, which is widely used in preoperative evaluation in the pediatric patients [3, 5]. Moreover, if the CT or MR imaging indicates a malignant mass, CT of the chest should be performed to assess the presence of lung metastasis [6].

In our experience, three-dimensional imaging can significantly improve the resection rate of pediatric tumors and increase the safety of the surgery [7]. In our center, we prefer CT scans for preoperative evaluation of pediatric liver tumors. However, it is very important to avoid non-contrast and multiphase images, and use low-dose CT scan in pediatric patients. CT phase of portal venous are very useful for evaluation of primary malignant liver tumors in children.

2. Common malignant liver tumors in pediatric patients

2.1. Hepatoblastoma (HB)

HB comprises 1% of all pediatric malignancies. HB most often occurs in infants and young children between 6 months and 4 years old. The median age of occurrence is 18 months. After 5 years of age, it becomes rare but histologically more aggressive in children over 8 years old. It occurs equally in males and females [8]. Based on radiological imaging, preoperative staging system (Pretreatment Extent of Disease or PRETEXT) which define extent of liver parenchyma involvement is an important guideline for treatment selection [9]. The new international surgical guidelines, which are being developed for the upcoming Pediatric Hepatic International Tumor Trial, will recommend primary surgical resection at diagnosis for PRETEXT I and II tumors of which the radiographic margin on the middle hepatic vein is wide [10].

As staging and treatment are mainly dependent on imaging, high-quality radiographic imaging has come to be of vital importance. For imaging assessment, both contrast-enhanced CT and MRI are recommended. Non-contrast CT typically shows a relatively well-defined, heterogeneous mass, slightly hypodense compared with liver tissue, with or without calcifications. On contrast-enhanced CT (**Figure 1**), the tumor reveals a heterogeneous enhancement, which may be hyperdense relative to liver parenchyma in the early arterial postcontrast phase and usually appears iso- or hypodense on delayed images (11). Invasion of the portal vein and its subsequent thrombosis must be evaluated in all suspected cases of hepatoblastoma. The tumor thrombus can even spread along IVC and encroach in the lumen of right atrium.

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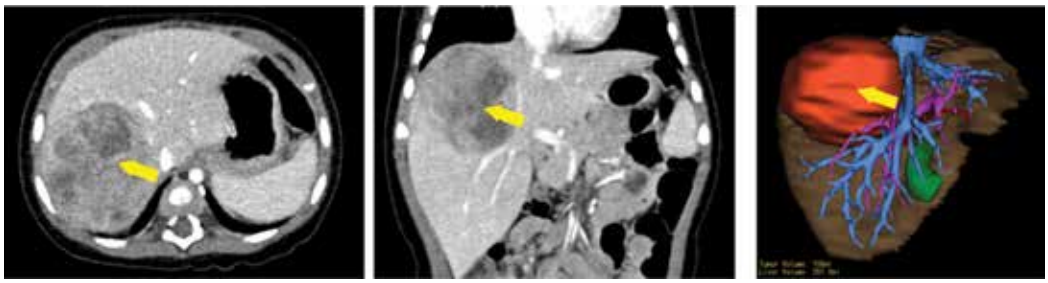

Figure 1. CT and three-dimensional reconstructed liver of PRETEXT II hepatoblastoma resectable at diagnosis (white arrow).

Metastasis may be seen in lymph nodes and lung parenchyma; it is rare in the brain and bones [11]. Twenty percent of HBs present with metastasis and most of them are in the lungs; therefore CT chest is necessary for staging.

2.2. Hepatocellular carcinoma (HCC)

The incidence of HCC in children was 0.5–1.0 cases per million children [12]. Different from HB, the median age of occurrence in children with HCC is 10 to 11.2 years [3]. The male to female ratio is 2:1 in young children, but it increases with age. Unlike adults, in whom HCC usually accompanies underlying liver disease, only 20–35% of children with HCC children have underlying liver disease [13]. HCC in children is now considered a distinct tumor family consisting of adult type HCC and variants, fibrolamellar HCC, and transitional liver cell tumor [14]. HCC is usually multifocal and may present with a variable number and distribution of tumor nodules. Recognizing HCC lesions smaller than 1.0 cm is still difficult.

In fibrolamellar HCCs, tumor cells are circumscribed by bundles of acellular collagen. This form is seen more frequently in adolescents than in adults and has better prognosis. HCCs are highly variable and show non-characteristic features on CT imaging: the tumors may be homogeneous or heterogeneous, solitary or multifocal, well- or ill-defined. On unenhanced CT images, HCCs typically appear isodense or slightly hypodense relative to liver parenchyma. On enhanced CT, they show early arterial contrast enhancement and rapid washout. HCCs are often inconspicuous on delayed scans. HCC sometimes invades the vasculature in the liver, and even the inferior vena cava may be seen [11]. The diagnosis of underlying cirrhosis may help during differential diagnosis, but it is rare in children. Three-dimensional CT image (**Figure 2**) analysis techniques are now available to estimate tumor volume and provide detailed information regarding the intrahepatic anatomy that resembles the actual intraoperative findings [15]. CT volumetry may permit calculation of resected tumor volume and anticipated size of the remnant liver in planning resection [16]. Plain CT of the chest should be performed to rule out the lung metastases. As for HB, tumor staging is an important consideration in determining the plan of treatment and prognosis. The PRETEXT staging system is recommended because it is currently the only staging system that allows surgical planning [9]. HCC is relatively chemoresistant. Complete resection or liver transplantation of localized tumor is the best option. In the SIOPEL-1 report, the overall resection rate was 36% and the 5 y OS and EFS was 28 and 17% respectively [13]. For liver transplantation, patient survival was

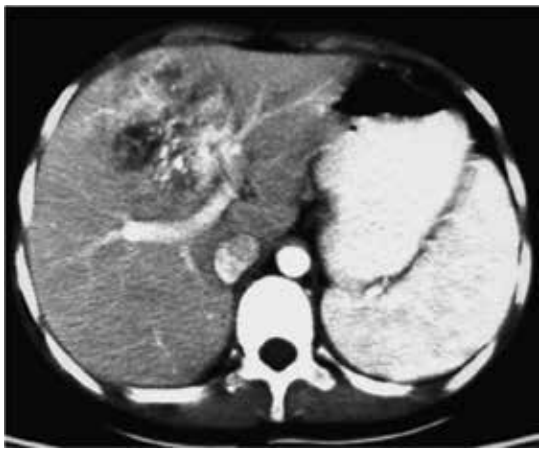

Figure 2. CT of hepatocellular carcinoma in pediatric patient.

63% at 5 years and 58% at 10 years in a study of orthotopic liver transplantation in 41 HCC children <18 years. Recurrence was the primary cause of death in 86% [17]. The outcomes of liver transplantation in HCC are not as good as that for HB.

2.3. Pediatric hepatic sarcomas

Pediatric hepatic sarcomas include undifferentiated embryonal sarcoma (UES), biliary rhabdomyosarcoma, and angiosarcoma [5]. UES is a rare malignant neoplasm, and its the incidence is higher than the other two types of sarcoma. UES was recently shown to share genetic features with mesenchymal harmatoma. Diagnosis of UES is usually between 6 and 10 years but some studies report presentation in young teenagers [18]. The tumor appears on ultrasound as a hetero-echoic mass, and a hypodense multicystic lesion on CT scan or MRI (**Figure 3**), usually exceeding 10 cm in size, with a predominance for involving the right hepatic lobe [19].

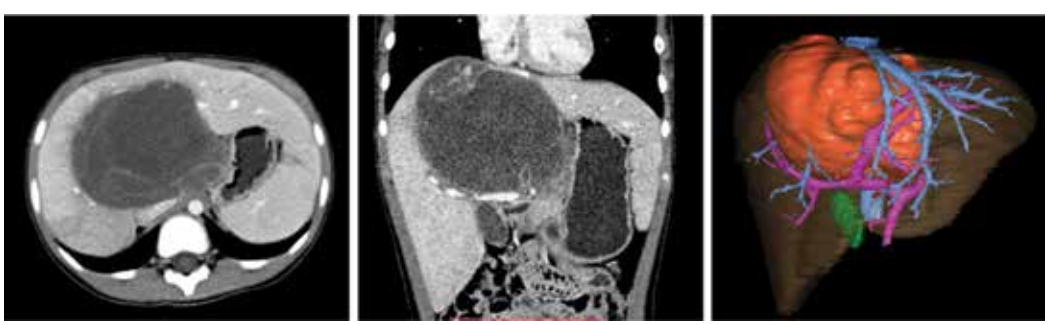

Figure 3. CT and three-dimensional reconstructed liver of undifferentiated embryonal sarcoma.

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3. Value of CT scan in guiding the surgical treatment

The objective of surgery is to achieve complete resection of the tumor, both macro- and microscopically, which is paramount for cure of malignant liver cancers. The liver resection strategy is based on pre-operative understanding of liver segmentation, vascular occlusion techniques, and experience in performing different types of hepatectomy, including extensive resection (left and right trisegmentectomies). Although abdominal CT should only be considered if MR imaging is not available or contraindicated, there are some limitations of MRI in some hospitals at developing countries. In our experience, MRI is the best available technique for diagnosing liver tumors, but its value is less clear in preoperatively evaluating the resectability of liver tumors especially in pediatric patients. The development and rapid clinical acceptance of single-detector helical CT during the last decade and, more recently, the introduction of multidetector CT (MDCT) have resulted in significant improvements in the study of the liver. MDCT makes it possible to precisely image the vascular anatomy, including the anomalous branches, feeding arteries, or drainage veins. Moreover, each image phase could be independently and simultaneously extracted or combined. In addition to technical advances, such as shorter scanning times, multiplanar imaging, and improved ability to perform multiphasic contrast-enhanced studies, newer and better intravenous contrast media and advances in post-acquisition data processing techniques have renewed researchers' enthusiasm for using hepatic CT scanning [11].

Furthermore, the software program for volumetry provides a proposed remnant liver volume and an optimal cut line of the liver. Various preoperative simulations can thus be considered. This volumetric analysis positively contributes to the safety of the procedure by assisting in the selection of the optimal operations. Preoperative evaluation of the relationship between the tumor and surrounding vasculature was simulated to perform liver resection with 3D software (**Figure 4**).

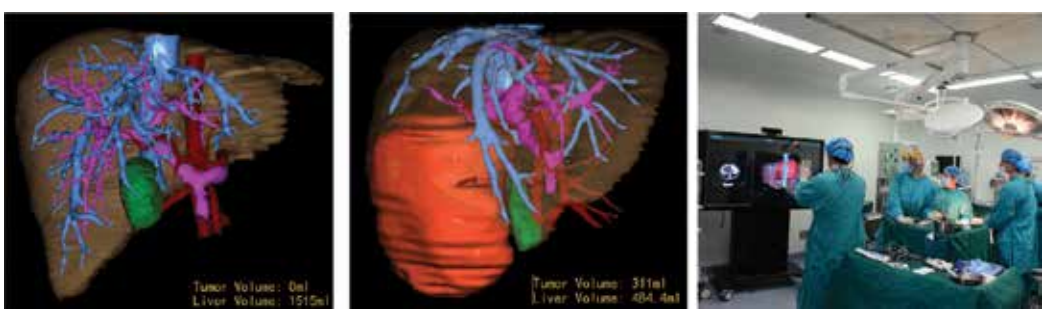

Figure 4. Three-dimensional reconstructed liver indicating total liver volume, liver tumor volume, and intraoperative navigation system.

4. 3D simulation software and Hisense Computer Assisted Surgery System (Hisense CAS)

With the development of three-dimensional simulation software, it is possible to achieve virtual hepatectomy, which can assist the surgeons planning the operation. The development of three-dimensional simulation software makes it possible to achieve virtual hepatectomy, which can assist surgeons to plan the operation, especially the complicated one. The history of 3D simulation software as it relates to hepatectomy can be divided into three stages: [1] successful 3D rendering of liver structures due to the introduction of multidetector row CT in the 1990s [20, 2] virtual hepatectomy depending on the reconstruction of the liver using 3D simulation software since 2000 [21, 3] the clinical practice and popularization of virtual hepatectomy using software packages since 2005, such as operation planning and operative navigation [22]. In some developed countries, such as Japan, virtual hepatectomy has routinely been performed in adult patients undergoing anatomic liver resection. It helps surgeons to plan the operative approach precisely, accurately position the lesion range, and be familiar with the operative route. Hisense Computer Assisted Surgery System (Hisense CAS) is a 3D simulation software package specifically developed for pediatric patients. It can provide precise and exquisite 3D visualization of pediatric liver structures using DICOM data from conventional CT. Considering that children have more refined anatomical structures, the accuracy of Hisense CAS was improved. Hepatectomy can be simulated on a personal computer, and the results can be shared with anyone in the cooperative team. Hisense CAS allows a surgeon to instantaneously manipulate the liver simulation in the operating room using a gesture-controlled display (**Figure 4**).

CT imaging can be performed using a 64-row-MDCT Scanner (Sensation64; Siemens, Erlangen, Germany) with the following parameters: kVp 120, mAs 100, slice collimation 0.625 mm, feed/rotation 12 mm, and rotation time 0.5 s. Patients received 2.0 ml/kg of an iodinated contrast agent (Ultravist; Bayer HealthCare LLC, Germany) to delineate the hepatic vasculature, which was administered intravenously using an automated injector system (CT 9000; Liebel-Flarsheim, Cincinnati, OH) at a rate of 2.0 ml/s. Automated bolus tracking with bolus detection on the level of the ascending aorta assured accurate timing of the arterial phase. For display of the portal and hepatic venous anatomy, third and fourth CT image sets were acquired at 10 and 40 s after the arterial imaging [23].

Four steps are required for transferring the CT DICOM file into 3D digital liver using Hisense CAS: [1] upload the primary CT DICOM data into the Hisense CAS; [2] auto or semi-automatically reconstruct the liver structures (liver parenchyma, portal vein, hepatic veins, and tumors) in a 3D context by extraction of neighboring voxels with a similar CT density, and automatically calculate the total liver volume and tumor volume; [3] virtual liver resection using the software (automatically calculating the remnant liver volume); and [4] assessment of the optimal surgical procedures based on the virtual hepatectomy. The surgical team could communicate and discuss the surgical liver anatomy with radiologists or pediatricians based on 3D reconstruction, such as the tumor locations, the appearance of the vessel branches, or approach of liver resection. Various virtual surgical strategies could be explored in the Hisense CAS. Finally, the surgical team could develop the optimal plan of operation [7].

5. Dong's digital liver classification

With the development of medical imaging, 3D reconstruction technology, the innovation of liver surgery and the proposal of precision hepatectomy, the intrahepatic vascular anatomy of the liver and liver segmentectomy based on that vascular anatomy have become well developed. With the analysis of 3D digital liver, we proposed a new type of liver classification system: Dong's digital liver classification system. Professor Dong Qian of the Affiliated Hospital of Qingdao University analyzed the anatomy of thousands of digital human livers from newborns to the elderly to build a new system of liver classification based on intrahepatic vascular anatomy [24].

1260 cases of normal human liver were rendered into 3D digital livers using their DICOM files. Based on the anatomical variation of the portal branches supplying liver segments, we built our Dong's digital liver classification system.

We divided the digital liver into four groups based on the type of segmentation and the variations in portal vein anatomy. Type A livers are similar to Couinaud or Cho's segmentation, containing eight segments (**Figure 5**). Type B livers have nine segments because there are three subdivisions of right-anterior portal vein (**Figure 6**). The defining characteristic of Type C is the variation in the right-posterior portal vein, which is arcuate-shaped (**Figure 7**). Type C-a livers have arcuate-shaped right-posterior portal veins and right-anterior portal veins like those in Type A livers. Type C-b livers have arcuate-shaped right-posterior portal veins and right-anterior portal veins like those in Type B livers. Type D livers have anomalous portal vein variations, which require three-dimensional simulation and individualized liver resection plan (**Figure 8**).

Type A: Similar to Couinaud [25] or Cho's segmentation [26], containing eight segments (**Figure 5**).

Segment I (3–6 P1 branches): Caudate lobe. There are 3–6 small branches (P1) originating from the back of right and left portal vein, surrounded by 5–8 tiny short hepatic veins.

Segments II and III: The left portal vein divides into the third-grade portal vein (P2 and P3) and perfuses the upper and lower outer sides of the left liver, which contains segments II and III.

Segment IV: Portal veins divided from the left portal vein perfuse the inner part of the left liver.

Segments V and VIII: The right portal vein divides into the right anterior and posterior branches, and then the anterior trunk further divides into several branches. (**Figure 5**).

Segments VI and VII: The right posterior portal vein further divides into right anterior (P6) and posterior branches (P7). The anterior branches perfuse segment VI, the lower outer area of the right liver.

Type B: Nine segments due to three subdivisions of right-anterior portal vein (**Figure 6**).

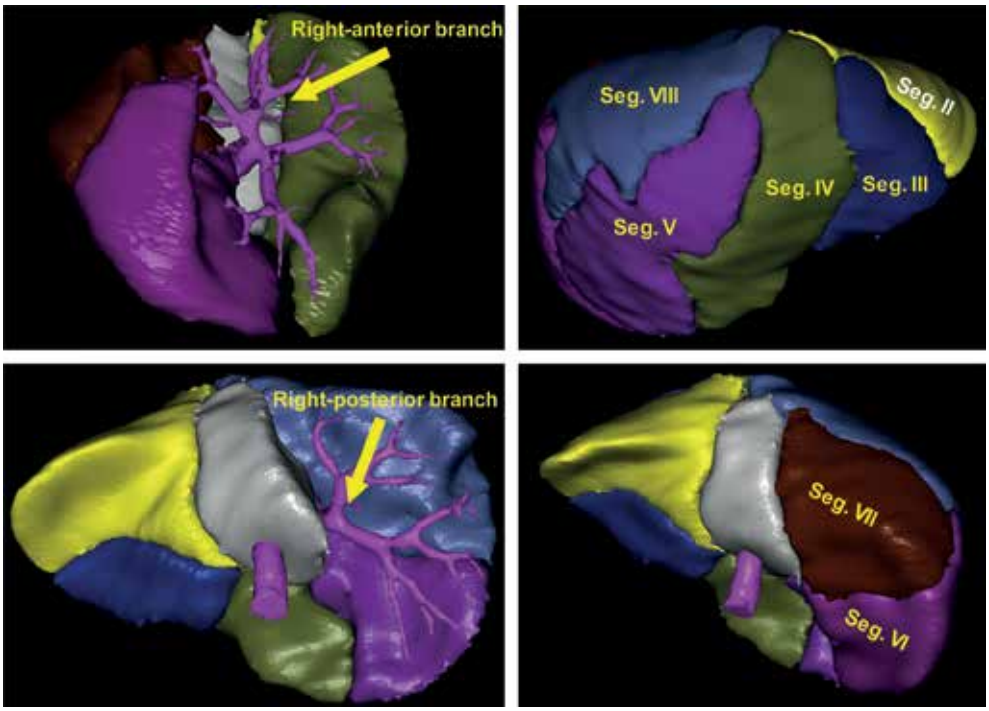

Figure 5. Right-anterior and right-posterior part of liver anatomy indicating Type A segmentation of Dong's digital liver classification system, similar to Couinaud or Cho's segmentation.

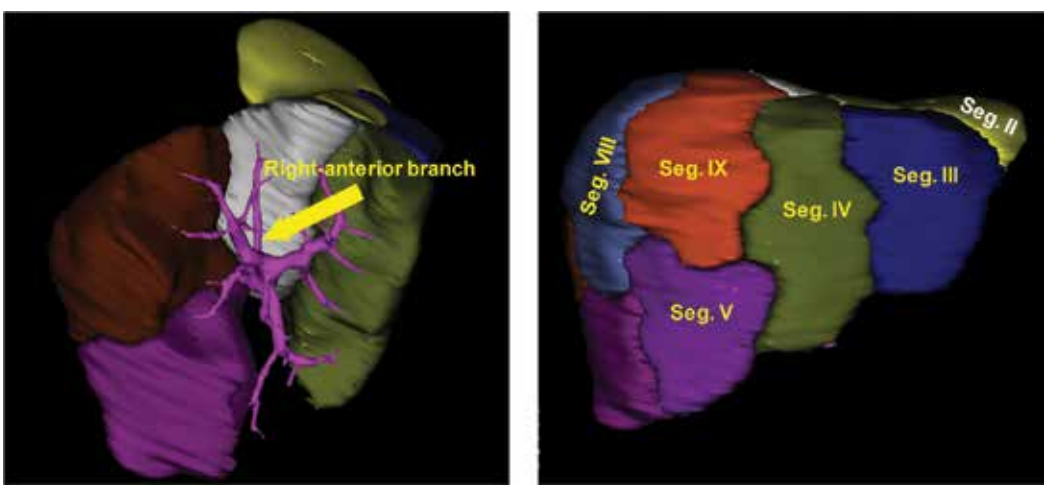

Figure 6. Right-anterior part of liver anatomy indicating Type B segmentation of Dong's digital liver classification system.

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Type C: The right posterior portal vein does not divide into main branches as in Type A or B livers, whose portal veins separate into 5–11 branches from an arcuate trunk. During precise hepatectomy, it is difficult to resect only segments VI or VII as in Type A and Type B. The prevalence of Type C livers is not high, but it makes a considerable difference in precise surgery. Cases in which the right posterior portal vein is arcuate type and the right anterior portal vein separates into only P8 and P5 are defined as Type C-a (**Figure 7**). When the right posterior portal vein is arcuate type and the right anterior portal vein separate to P5, P8, and P9, we define it as Type C-b.

Type D: This is a catchall category, appearing in about 12.43% of all livers. It includes all variations that cannot be classified into any of the previous three types (**Figure 8**).

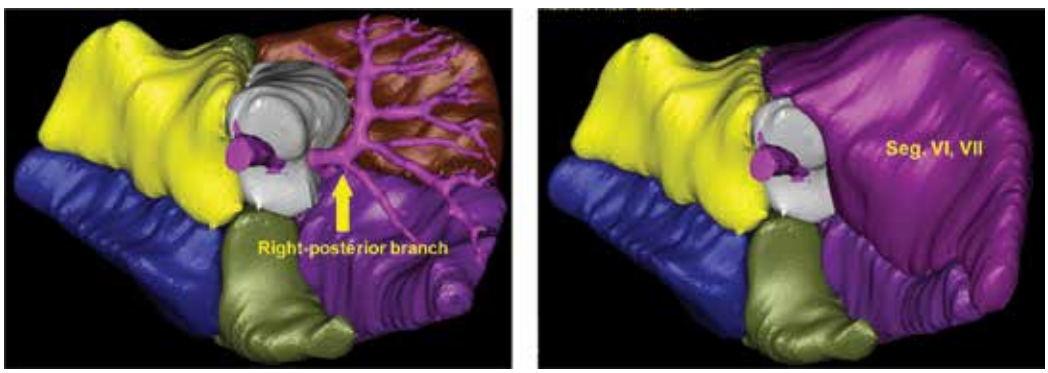

Figure 7. Type C livers have arcuate-shaped right-posterior portal veins.

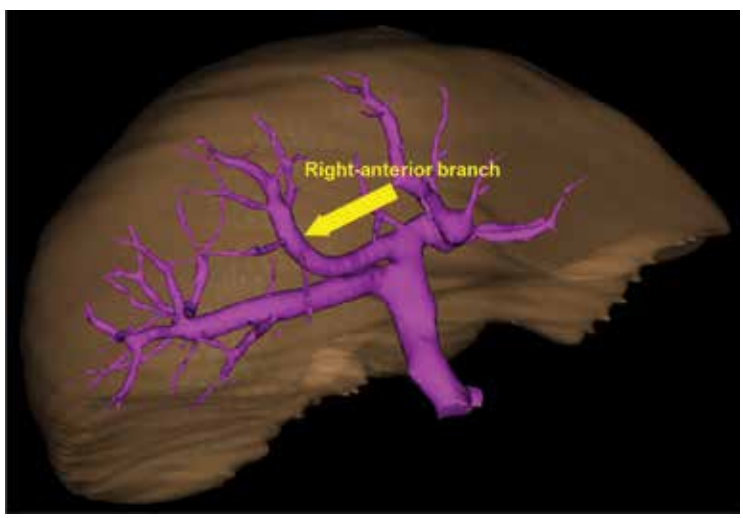

Figure 8. Rare variation of portal vein which is classified into Type D segmentation.

6. Measurement of liver volume from neonates to the elderly

Total liver volume, the basic unit of liver function, is an important factor to evaluate the resectability of liver cancer. There have been many studies of the total liver volume and necessary remnant liver volume in adult patients but only a few reports regarding liver volume in children. Because measured total liver volume has been proposed as the golden standard of liver volume for preoperative surgical plan, we tried to summarize the average total liver volume of Chinese patients of different ages, from neonates to the elderly.

Age	N	Liver volume (cm^3)
<1 Month	28	140.0339 ± 50.0707
1–3 Months	26	191.1462 ± 38.9132
4–6 Months	31	261.5065 ± 70.9437
7–9 Months	22	273.1917 ± 50.0732
10–12 Months	33	305.4692 ± 36.3323
1–2 Years	56	374.3617 ± 65.8447
2–3 Years	66	440.8111 ± 71.4779
3–4 Years	58	500.0037 ± 103.2837
4–5 Years	49	549.4533 ± 84.6325
5–6 Years	33	639.4677 ± 126.7067
6–7 Years	44	722.0357 ± 140.8796
7–8 Years	44	824.6372 ± 137.9766
8–9 Years	32	844.4633 ± 93.6353
9–10 Years	37	935.8571 ± 189.1018
10–11 Years	29	985.0464 ± 121.0802
11–12 Years	27	1048.9250 ± 167.5279
12–13 Years	29	1118.4593 ± 155.2817
13–14 Years	22	1125.0250 ± 147.9899
14–18 Years	30	1323.8862 ± 226.3454
18–30 Years	42	1361.8682 ± 205.3783
30–40 Years	74	1381.1037 ± 300.3834
40–50 Years	139	1423.7647 ± 216.9305
50–60 Years	197	1343.2768 ± 246.6878
60–70 Years	181	1284.4183 ± 190.7129
70–80 Years	106	1263.1282 ± 170.2464
80–100 Years	21	1089.3429 ± 199.0259
Total	1456	

Table 1. Standard liver volume range (X ± S, cm^3).

Upper abdominal CT films from 1456 children (enhanced CT 837, plain CT 619) aged 1 day to 100 years were selected. None had any history of liver disease, and CT had been performed for other clinical purposes. The patients were divided into 26 groups by age (**Table 1**).

7. Human Digital Liver Database

The Human Digital Liver Database (HDLD) was established by the Affiliated Hospital of Qingdao University and Hisense Company, aiming to collect digital liver from neonates, children, adults, and the elderly, from normal livers, livers with cancer, and simulated livers resected using Hisense CAS. The link of the HDLD is http://www.hdldb.net, which now is only available in Chinese (the English version is being translated now). The HDLD will show the digital liver in image and video form. All visitors could study the updated clinical cases at any angle of reconstructed 3D digital liver, including the vascular system, anatomical differences in the liver, and the correlation between vascular and liver tumors. The HDLD will also provide the intra-operation video comparing to the preoperative surgical plan, to help doctors and medical students better understand the anatomy and surgical procedure of pediatric liver resection, especially for patients with giant liver tumors (**Figure 9**).

7.1. Normal children and adult digital liver database

Vascular anatomical variation and total liver volume are two of the more important factors that surgeons consider when making surgical plans. We have collected thousands of CT scan data from across the nation. We would like to establish a digital liver database showing the reconstructed digital liver and separate these digital livers into different groups according to the anatomical variations in liver vasculature and liver volume (**Figure 9**). The Dong's Digital Liver Classification was established based on our collection of digital livers. We believe that a normal digital liver database may serve as an important reference for surgeons all around world.

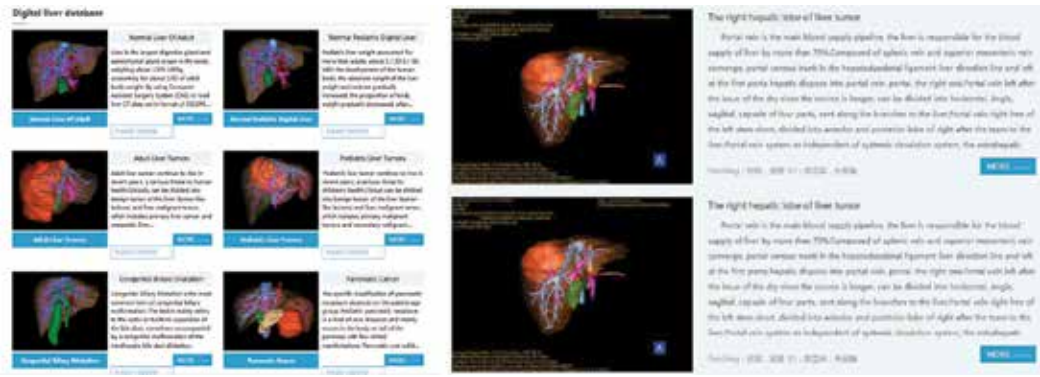

Figure 9. The Human Digital Liver Database.

7.2. Liver tumor database and simulated liver surgery

The liver tumor database shows the reconstructed digital liver image and simulated liver resection according to the surgeon's preoperative plan, and the intraoperative video of clinical cases, aiming to share the experience gained by staff at our center freely around the world. With the help of Hisense CAS, the successful surgical resection of liver tumors in pediatric patients has improved in our center. With the 3D simulation, we have found that we can clearly understand the anatomical variation in intrahepatic vasculature, the correlation of vasculature with liver tumors, and calculate the remnant liver volume of the simulated liver easily. In the database, we would like to show some difficult cases, such as those with very large liver tumors and those with vascular variation.

8. Clinical application of Hisense CAS for diagnosis and surgical plans in children with large liver tumors

An 11-month-old male infant was referred with abdominal distension and loss of appetite for the past 2 months [27]. Upon examination, a firm, non-tender mass with a smooth surface was evident arising from the right lobe of the liver, which filled the abdominal cavity. Serum ALT, AST, GGT, ALP, and Alb were normal. Both serum α-fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels were within normal ranges. Ultrasound (US) revealed a well-defined, multicystic mass involving the liver. Enhanced CT images similarly showed a giant cystic mass with minimally enhanced septation and peripheral solid components (**Figure 10**).

The DICOM data obtained from the CT images were uploaded to 3D simulation software, the Hisense Computer Assisted Surgery System (Hisense, China) to simulate the liver. The relationship between HMH and the intrahepatic vasculature was revealed in a 3D context (**Figure 11**). The right hepatic vein (RHV), the middle hepatic vein (MHV), and the left hepatic vein (LHV) were confluent with a common trunk. The hepatic veins (HVs), the portal veins (PVs), and the inferior vena cava (IVC) were displaced, with no obvious infiltration or encasement. The volume of both the functional liver and the HMH was automatically calculated. The positional relationship between the vessels and HMH could be confirmed from any angle instantaneously in the computer. Various virtual hepatectomies were performed to predict the risk and the difficulty of the actual hepatectomy. Finally, an optimal surgical

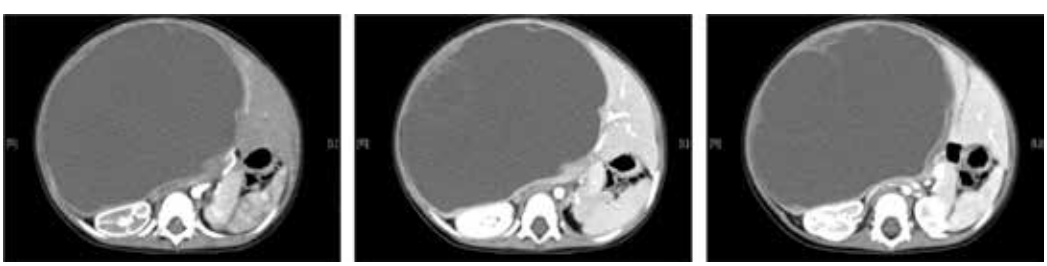

Figure 10. Preoperative enhanced CT scan.

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plan was developed using 3D simulation software to safeguard RHV. The enucleation of HMH for the case was performed after adequate preoperative preparation.

After laparotomy, the fluid was aspirated using a 20 G needle from the cystic components of HMH to reduce its volume, thereby facilitating surgical resection. The resection line at the rim of HMH, which was indicated by virtual hepatectomy was made using the electrotome. The hepatic portal occlusion was used to reduce the risk of bleeding. The hepatic parenchyma was dissected using the CUSA system. The intrahepatic vessels were dissected to be safeguarded or else ligated and divided, a matter that had been assessed by the virtual hepatectomy. After 20 min, the HMH was removed with surrounding rim of normal liver tissue. The right hepatic vein was successfully safeguarded. The remnant liver volume was about 210 ml, which approximately equaled the automatically calculated remnant liver volume (230.1 ml). There was no anatomical discrepancy between the operation and the 3D simulation. The convalescence was uneventful. Histopathology confirmed the diagnosis of mesenchymal hamartoma (**Figure 12**).

In summary, three-dimensional (3D) imaging could significantly improve the resection rate of pediatric tumors and increase the safety of the surgery. Dong's digital liver classification system and human digital liver classification system will be useful for surgeons all around the world.

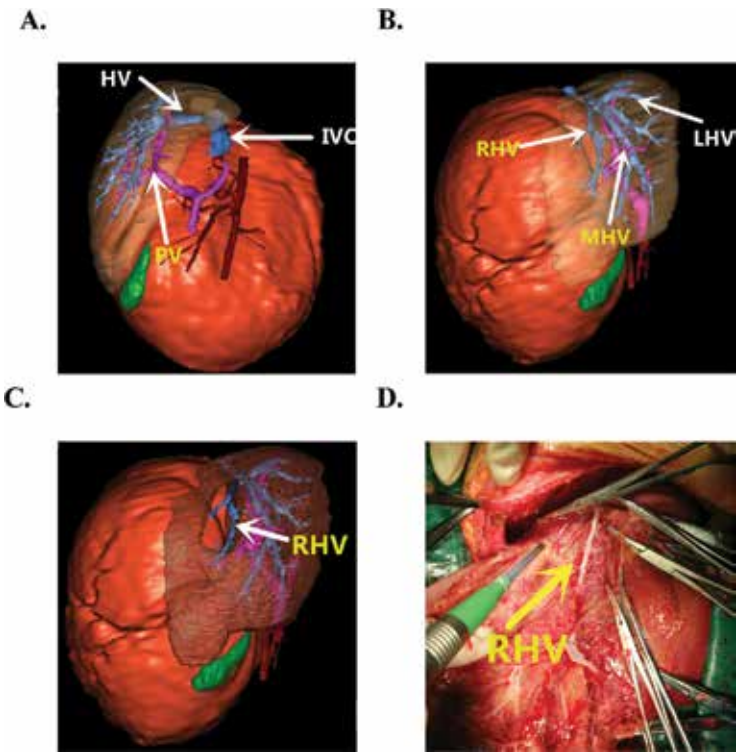

Figure 11. Comparison of 3D simulation and intraoperative liver anatomy.

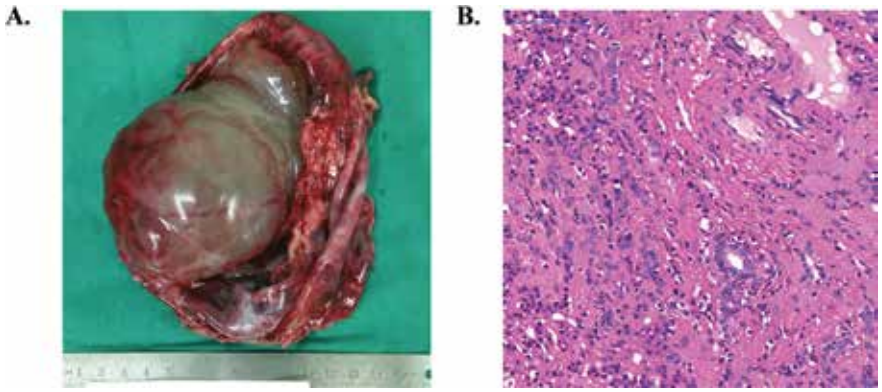

Figure 12. Resected HMH tumor and pathology.

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Chapter 4

Intraoperative Ultrasound of the Liver: Actual Status and Indications

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73856

Abstract

Intraoperative liver ultrasound represents an essential component in the hepatobiliary surgery arsenal, having an essential role in describing liver lesions, their topography, and loco-regional extension. It also has an important role in establishing surgical strategy, in modulating the surgeon decisions, and thus in preventing postoperative complications. This chapter tries to make a synthetic review of principal indications for using ultrasound in liver surgical treatment, underlining the liver's lesions characteristics and advantages brought by this method. Also, we wanted to underline the importance that ultrasound has for guiding the surgeon in interventional intraoperative techniques or in any anatomical liver resection. The role of enhanced contrast intraoperative ultrasound is put in front by the better diagnostic results obtained for both primary and metastatic tumors of the liver.

Keywords: intraoperative ultrasound, liver tumors, contrast enhanced ultrasound

1. Introduction: brief history

The main advantage of the ultrasound imaging method is the real-time visualization of the anatomy and structure of the liver lesions, allowing for the adaptation of the therapeutic decision during surgery.

The concept of intraoperative ultrasound (IOUS) was first introduced in the 60s and was used to evaluate renal lithiasis when doing nephrolithotomy. Due to the limitations of A-mode ultrasonography (difficulty in interpreting images), IOUS began to be more applicable in the surgical sphere later, in the early 80s [1], when high-frequency real-time B mode-ultrasound was introduced [2]. The use of IOUS in hepato-bilio-pancreatic surgery was emphasized for

the first time in the literature in the mid-80s [3]; later, it became an exploratory technique routinely performed in specialized centers for staging liver disease and guiding surgical procedures on patients diagnosed with hepatocarcinoma on cirrhotic liver [4–7]. Studies in the 90s showed that the information provided by IOUS may modify the initial therapeutic plan in up to 53% of cases [8, 9].

Although first reports related to laparoscopic transducers used in A-mode date back to early 1964, the laparoscopic IOUS technique has been developed relatively recent [10].

2. General aspects

Currently, there is a wide range of equipment for IOUS, probes of different types and shapes, adapted according to the type and localization of the lesion. Standard transductors for transabdominal ultrasound can also be used, but there may be some limitations on image resolution and on the large size of the transducer that do not offer optimal maneuverability [1]. Conventional transducers can be used at the beginning of the liver examination to obtain an overview of the organ anatomy [1, 11]. The transducers used in IOUS usually operate at high frequency: 7.5–10 MHz [12]. There are different shapes: linear T-shaped probes, interdigital probes, microconvex probes and more recently, T-shaped probes with trapezoidal scanning window [13]. In case of liver surgery, the ideal transducer should be a small one that can be easily manipulated in narrow spaces, with a special design to allow the probe to be held in the palm between two fingers, thus allowing the operator to have permanent contact with the surface of the liver, without omitting to scan some areas [11, 14] (**Figure 1**).

When necessary, IOUS can also be used in laparoscopic surgery, with special transducers suitable for this type of approach. Transducers used during laparoscopic surgery are either linear or curved, mounted at the end of a long, thin articulated arm, with a design that allows insertion and manipulation inside the trocar (**Figure 2**) [15].

Figure 1. Scanning the liver surface with a intraoperative mini-convex probe, 1–13 MHz, 65°, Hitachi Aloka Medical, Ltd., Japan (intraoperative aspect, from the personal archive of the authors).

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Figure 2. Intraoperative laparoscopic ultrasound of the liver. HCC on cirrhotic liver. L44LA intraoperative probe, 13–2 MHz, 36 mm, Hitachi Aloka Medical, Ltd., Japan (intraoperative aspect, from the personal archive of the authors).

The possibility of performing intra-operative contrast ultrasound (CE-IOUS) is an important factor in choosing the ultrasound equipment. Nowadays, the most commonly used contrast agents are SonoVue (Gaseous sulfur hexafluoride, Bracco, Milan Italy) and Sonazoid (Gaseous perflutane, GE Healthcare, Norway/DaiichiSankyo, Japan) [11, 16–19].

In order to ensure a good examination, the ultrasound machine should be positioned in front of the main operator, the patient (the organ to be examined) being located between the surgeon and the monitor (a collinearity between operator, organ and monitor) in order to view simultaneously the ultrasound monitor and the surgical field. The ultrasound monitor should have size and resolution large enough to allow optimal remote viewing. Examination must always begin with the inspection and palpation of the liver and of the entire peritoneal cavity. These steps should not be avoided in favor of IOUS [20]. Mobilization of the liver begins with the sectioning of suspensory ligaments, thus creating enough space to manipulate the ultrasound transducer. Worth mentioning some of the artifacts that may appear on the examination of the VIIIth and IVa liver segments after the sectioning of the cavo-hepatic adhesions. Therefore, in the case of suspected lesions located in these areas (adjacent to the cavo-hepatic region), dissection at this level should be performed only after ultrasound exploration.

3. IOUS of the liver: benign tumors

Benign tumors can develop on a normal or steatotic liver, may be solitary or multiple, with increased echogenity (hemangiomas, focal nodular hyperplasia) or anechogenic, with posterior acoustic strengthening (serous cysts) and distinct contours (hydatid cysts), with no vascularization or characteristic circulatory pattern; may have a mass effect on liver structures or even adjacent organs. A characteristic for benign tumors is the fact that they have elastic consistency and do not invade vascular elements [20, 21].

Hemangiomas are benign tumors, mostly asymptomatic, incidentally discovered. These tumors can present themselves under various echographic aspects; most commonly, are well-defined, round, hyperecogenic, homogeneous, usually small (<3 cm), and may present the posterior acoustic strengthening effect [22]. As hemangiomas grow in size, they can change their echogenicity,

from homogeneous to heterogeneous, with their edges becoming irregular. These features make them more difficult to differentiate from malignant tumor formations. When surgery is indicated, IOUS has the role to localize and visualize the relationships of the hemangioma with the intrahepatic structures. The surgeon can trace the hepatic resection line outside the hemangioma, minimizing hemorrhagic risk, and preserving the healthy hepatic parenchyma to its full potential. The CE-IOUS can be useful, capturing the contrast agent by the hemangioma being most of the time characteristic. Differentiation from malignant tumor formation becomes difficult for arterial hemangiomas or for those with arterio-venous shunts [21].

For *focal nodular hyperplasia*, the central location of a fibrous scar is characteristic. This tumor appears as a well-defined lesions with variable size, usually unique, of solid consistency and inhomogeneous structure. Rarely, the central scar can be distinguished when using simple ultrasound, without contrast agent. When using CE-IOUS, in the arterial phase, there is a central filling followed by a complete capture in the venous phase. At this stage, the center of the tumor becomes hypoechoic. In the late phase, the tumor remains isogenic together with the hepatic parenchyma, which strengthens the diagnosis of benign lesion [21].

Hepatic adenoma appears ecographically as a well-defined solid tumor lesion; it may have an inhomogeneous structure in the presence of intratumoral hemorrhage. Doppler ultrasound does not detect a vascular signal. When using CE-IOUS, in the arterial phase, there is a centripetal and inconsistent capture; in the venous phase, a moderate washout may be noted. In the late phase, the appearance is isoechoic or hyperechoic [21].

Differentiation between focal nodular hyperplasia and hepatic adenoma is important for establishing the therapeutic indication, surgery being indicated for large adenomas, due to the risk of rupture and hemorrhage as well as due to its malignant potential.

Because sometimes it is difficult to make a benign-malignant US differentiation, intraoperatory, when the situation imposes, might by necessary to make a bioptic puncture for establishing a correct diagnosis [20]. IOUS has an important guiding role, especially in the case of lesions located in the depth of the liver parenchyma, hard to reach when palpating.

Simple hepatic cysts (biliary cysts) are benign tumors with no malignant potential, usually asymptomatic, that can be easily diagnosed with ultrasound imaging. They are described by ultrasound as well-defined lesions with very thin walls, no Doppler signal, anechoic, with transonic content due to the liquid composition. Simple hepatic cysts have therapeutic indications only when they become symptomatic, often due to symptoms related to the mass effect they have on neighboring structures.

Percutaneous ultrasound guided treatment with cyst evacuation is often possible, but is followed by an increased risk of relapse, with the rebound of collection. In this idea, the laparoscopic surgical resection of the cystic dome is indicated. This technique is easy if the lesions are located superficially, in segments II, III, IVB, V, VI (after Couinaud) [23]. The lesions localized intraparenchymatous can be approached safely only when using IOUS [24].

Depending on the evolutionary stage, *hydatid cysts* may appear as single or multiple lesions, anechoic, with membranes and sediment inside, with thin or calcified walls. They may be multilocular or may contain multiple fluid compartments (daughter vesicles). IOUS helps the surgeon in finding the cysts and in some situations it can detect bile duct communication. These lesions

can compress the intrahepatic vessels with mass effect, signs of invasion, or embedding of these structures being absent [21]. IOUS has the same indications as in the case of simple cysts, being a real help for the surgeon, for establishing surgical tactics and for checking the radicality of the treatment (the content of the remaining cavity, residual content, multilocular abscesses, etc.) [25].

4. IOUS of the liver: malignant tumor

IOUS finds its usefulness in liver surgery for both primary and secondary malignant lesions facilitating the detection, characterization of lesions and guiding the surgical procedure [26, 27]. Most studies have evaluated the role of IOUS for treatment of hepatocarcinomas and hepatic metastases due to colorectal cancer, these pathologies being considered the most common liver malignant lesions. Intraoperative detection and local treatment of these lesions may have a major impact in choosing surgical strategy [28, 29].

4.1. Hepatocarcinoma (HCC)

HCC is the most common primary malignancy in the liver, and is frequently associated with cirrhosis [30, 31].

Ecographically, this tumor has the appearance of a solid tumor with irregular contours, heterogeneous, uni-, or multilocular ("encephaloid form"). Typically, it invades the liver vessels, primarily the portal branches, but also the suprahepatic veins. Doppler screening usually highlights a high-speed arterial flow. Vessel distribution is irregular, disordered. CE-US shows hypercaptation in the arterial phase with a specific "washout" of contrast substance in the venous phase. In the late phase, the tumor appears as hypoechoic. This behavior is usually described in tumor nodules larger than 2 cm [21].

In the case of HCC, IOUS is superior in detecting lesions measuring less than 1 cm, preoperative MRI having a lower sensitivity and specificity for these lesions [11, 32]. It has also been shown in several studies that CE-IOUS can modify in 19–29% of the cases the initial treatment plan [33, 34]. CE-IOUS finds its usefulness especially in cirrhotic patients when it comes to differential diagnosis between malignant lesions and regenerative nodules [29, 35]. It has been demonstrated that neoangiogenesis of tumor nodules is a specific criteria for distinguishing hepatocarcinomas from dysplastic or regenerative nodules [35].

CE-IOUS has a sensitivity of 100%, a specificity between 69 and 100% and can modify the surgical strategy in up to 79% of patients [36–38], most frequently by detecting new lesions. The literature emphasizes that the filling pattern of the contrast agent in nodules found by IOUS can guide surgical resection [36]. It has also been shown that the vascular pattern of HCC visualized by using CE-IOUS has been associated with the expression of some genetic profiles, suggesting that CE-IOUS images can be used as an indicator for predicting prognosis of patients [39].

During hepatic resection, which is the standard treatment for HCC, particular attention should be paid in preserving as much hepatic parenchyma as we can, the remaining hepatic volume being an important prognostic factor for the short outcome [37, 39, 40]. Thus, local resection of the tumor formation or its ablation under IOUS guidance may be chosen to minimize the

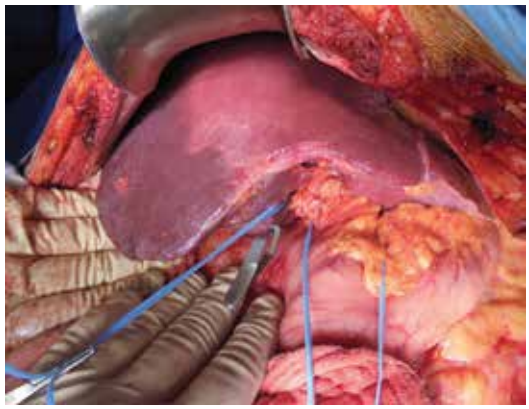

Figure 3. Anatomical resection: ischemic delimitation of sixth and seventh liver segments (intraoperative aspect, from the personal archive of the authors).

volume of resected liver parenchyma, respecting the oncological resection margin. Also, in order to minimize the risk of postoperative complications (hemorrhage, necrosis of the liver parenchyma) and remote relapse (by satellite micrometastases, specific for HCC), the use of IOUS is vital in guiding anatomical resections. These involve the ultrasound identification of vascular pedicles corresponding to the affected hepatic segments and through various associated maneuvers (digital compression, injection of contrast agents) an exact delimitation of the targeted resection area can be obtained (**Figure 3**). More details will be given in the following rows, in the sub-section dedicated to the role of IOUS in guiding hepatic resections.

4.2. Hepatic metastases

Despite significant advances in preoperative staging diagnostic procedures (conventional CE-US, multi-sliced CT, CE-MRI, and PET-CT), studies have shown that 10–30% of the patients with colo-rectal cancer remain with undiagnosed hepatic metastases during primary tumor surgery [41–46].

In this respect, IOUS and CE-IOUS have a special role in completing the diagnosis, in addition to the liver's palpation technique. IOUS is considered the "gold standard" in open surgery for colorectal cancer since 1980, being able to detect liver metastases that cannot be palpated intraoperatively and that have not been visualized with preoperative imaging techniques [8, 47–50].

Liver metastases have a non-characteristic echographic appearance, being circumscribed lesions with imprecise or halo delineation, with a homogeneous or heterogeneous pattern. They may be solitary (usually liver metastases from colonic neoplasms) or multiple. Their echogenicity is variable. When they are large, they can compress the bile ducts (which may appear to be dilated) and the liver vessels. As for their vascularization, they may be hypovascular (in gastric, colon, pancreatic, or ovarian cancers) with hypoechoic pattern in arterial phase and similar in the venous and late phases or hypervascular (neuroendocrine tumors, malignant melanomas, sarcomas, renal tumors, breast, or thyroids), with a hyperechoic appearance during the arterial phase, with wash out during the venous phase and hypoechoic pattern at about 30 s after the injection of the contrast substance [51].

Several studies in the literature have shown that after the surgical treatment of the primary tumor, the ultrasound of metastasis after colorectal cancer can be correlated with prognosis. Thus, Gruenberger et al. [52] demonstrated that in patients with hyperechoic ultrasound liver metastases, survival is longer than in those with the hypoechoic aspect of the lesions. This suggests that the role of IOUS is more than a diagnostic one and can be useful in establishing prognosis [53].

The CE-IOUS applied for colorectal liver metastases has an 96% accuracy, in contrast to 74 and 79%, percentages associated with pre-operative CT and MRI [34, 54]. The fact that undetected preoperative liver metastases represent the main cause of recurrent neoplasia [55] highlights the important role that IOUS has in the management of patients diagnosed with colorectal cancer. This is why routine IOUS is recommended in these patients [56].

Chemotherapy is an important, standardized element in regard with the adjuvant and neo-adjuvant therapy in colorectal cancer patients. [57, 58] Regarding hepatic metastases, good results of cytostatic treatment mean either stagnation or regression of these lesions [59, 60]. A particular situation is when liver metastases are no longer visible in CT and/or MRI performed after chemotherapy. Literature indicates that the complete, real response is found in up to 66% of cases [61, 62]. For the rest of the cases (34%), chemotherapy can affect the echogenity of the metastases making them difficult to be identified with preoperative imaging (CT, MRI, even IOUS) [13, 33]. In these situations, CE-IOUS allows the surgeon to check areas where hepatic lesions have been described before chemotherapy [11]. The role of this technique is highlighted in many studies that have shown that only be confirmation given by the CE-IOUS in regard with the lack of lesions can be associated with a complete therapeutic response [59, 62].

Resection or ablation of all lesions is the gold standard in the treatment of colorectal liver metastases [63]. Even in patients with unresectable metastases, local ablation or combination between ablation and surgical resection of the lesions has been shown to be able to locally control the disease [64]. It is obvious that IOUS plays a major role in liver surgery for the detection and localization of metastatic lesions [28].

5. The role of laparoscopic approach

The laparoscopic approach and minimally invasive surgery have more and more indications and thus the role of IOUS in laparoscopic surgery has become increasingly important. Of course, laparoscopic surgery has some disadvantages in assessing the liver because the surgeon loses the advantage of palpating the structures and lesions. IOUS manages to compensate for most of these laparoscopic minuses by providing intraoperative high utility imaging with greater sensitivity in detecting liver lesions than most preoperative imaging techniques [65–69]. Intraoperative laparoscopic ultrasound (LIOUS) has a sensitivity and specificity similar to that in open surgery [69]. Several authors have suggested routine use of LIOUS in laparoscopic colorectal surgery [70] and prior to planned laparotomies for liver resections [71]. In cases where hepatic disease is known, with the help of LIOUS data, around 64% of cases could be exempted from laparotomy [71, 72].

The success of the laparoscopic approach depends primarily on the location of the lesions [73, 74]. Guiding surgical maneuvers by the use of LIOUS is possible especially in superficial tumors on the left lobe or on the anterior segments of the right lobe (hepatic segments II, III,

Figure 4. Laparoscopic ultrasound guided radiofrequency ablation of HCC on cirrhotic liver (intraoperative aspect, from the personal archive of the authors).

IVb, V, and VI). Direct visualization and LIOUS should be used to compensate for the impossibility of liver palpation in laparoscopic surgery [75, 76]. In the case of laparoscopically treated malignant lesions, it is important to mark by IOUS imaging the oncological resection margins, this way ensuring their tracing by minimally invasive approach. Furthermore, the completion of the treatment is possible using ablative techniques (radiofrequency, microwave). The laparoscopic approach finds its indications especially for higher-risk cirrhotic patients (altered hepatic markers, clotting disorders) with subcapsular neoplastic lesions (**Figure 4**).

With the evolution of technology and the experience of surgical teams, laparoscopic approaches to hepatectomies have become more and more used in centers of excellence. Several studies have shown that laparoscopic hepatectomy is a safe procedure and could have advantages over open surgery, translated by reduced blood loss and a shorter hospitalization stays [77, 78]. As for LIOUS, it should guarantee the same performance as the ultrasound used in conventional liver surgery. Although, LIOUS has been introduced since 1981, few studies have addressed this subject. Although reported to be a safe and accurate method [79], it is currently not routinely used in laparoscopic surgery [80], although the reliability of LIOUS in the staging of liver disease has been demonstrated to be similar to conventional IOUS [81]. Moreover, although many articles mention LIOUS as an important technique, few scientific papers described this technique [82–85].

6. Ultrasound-guided techniques

It has been demonstrated that making biopsies under IOUS guidance, laparoscopic or "classic," have a high diagnostic accuracy and are considered safe procedures with possible impact on surgical management [86, 87]. For example, liver metastases detected intra-operatively and confirmed by histopathological examination as having pancreatic origin could be a contraindication for pancreatic radical surgery [58].

In terms of non-excisional treatment of hepatic tumor formations, this can also be achieved by ablative techniques, such as ethanol injection [88], RFA (coagulation necrosis induced by high-frequency alternating currents-thermal energy) [89] and MWA (same as RFA, although

MWA uses different parts of the electromagnetic spectrum) [90]. Although the elective treatment is by percutaneous approach, there are situations when both classical or laparoscopic method are indicated.

Laparoscopic approach is particularly preferred on patients who are on the waiting list for liver transplantation or for those who cannot benefit from liver resection due to comorbidities, liver cirrhosis, or hepatic dysfunction due to chemotherapy, especially when percutaneous procedures are not possible [91–93]. Indications are subcapsular lesions located in the immediate vicinity of important structures (diaphragm, stomach, and gallbladder) or difficult to approach (caudal lobe). [84, 94–96]. Moreover, these ablation techniques can be combined with hepatic resections or can be performed serially after surgical resections, improving the oncological outcome and prognosis [97–99]. In the majority of cases treated by these procedures, IOUS is used as a guidance tool and for evaluation the efficacy of the treatment and appearance of complications [94].

Multiple studies have demonstrated that IOUS-guided ablations are a safe and an effective treatment option that provides excellent local control of both primary and secondary hepatic tumor lesions [64, 94, 100–102]. Recent studies have also reported that intraoperative RFA has a local recurrence rate equivalent to that obtained from low-grade HCC surgery [11, 96] and colorectal hepatic metastases [64, 100].

7. Guiding liver resections

Localization of liver lesions is related to portal branches and suprahepatic veins, which are used to define segmental boundaries. Without the use of IOUS, it would probably be impossible to define correctly, anatomically, the hepatic segments and often the limits of the tumors, especially due to the existence of multiple anatomical variants [13].

Hepatic resections are known to be the standard treatment for malignant liver tumor formations, being the only procedure that provides oncological radicality [58]. Preservation of hepatic parenchyma should be a goal of the surgical team, especially in patients with cirrhotic liver, whose liver function and prognostic could be influenced by extensive resection. In these situations, IOUS plays an essential role because it allows the evaluation of the intrahepatic tumors, facilitating a limited but oncological liver resection. Thus, in modern hepatic surgery, whether HCC or colorectal liver metastases, the use of IOUS allows the realization of the so-called "radical but conservative surgery." Thus, obtaining continuous information on the relationship between liver lesions and intrahepatic bilio-vascular structures, the surgeon can guide his resection line, respecting the Glisson pedicles, and suprahepatic veins, with the ultimate goal of preserving as much functional hepatic parenchyma as possible [11, 12, 103, 104].

IOUS is also a real help for anatomical resections. This technique involves the compression of segmental portal branches between the transducer and the operator's fingers, resulting in a transient ischemia of the target parenchyma. This area can be marked with the electrocautery, and then the resection is made along the demarcation line [105–110].

Starting from the use of IOUS, Torzilli introduces new types of resection, such as mini-mesohepatectomy, for tumor formations located at the confluence of the cave vein with superhepatic veins [11, 12, 111]. These resections are based on the ultrasound study of the relationship between the tumor and the suprahepatic veins and the analysis of the blood flow at this level after clamping the proposed vein for resection. Evidence of an inverse flow in the peripheral portion of the compressed vein or of a collateral shunt between the clamped vein and the other superhepatic vein or cava vein will allow the ligation and segregation of the tumor-affected suprahepatic vein and the achievement of a limited resection, while maintaining the principles of oncological radicality [11, 12].

Summarizing, the use of IOUS allows the extension of surgical indications for certain liver lesions that were either considered unresectable or required major surgery [104].

8. Future perspectives

IOUS is still characterized by several drawbacks: it cannot detect lesions smaller than 3 mm, its accuracy is dependent on the surgeon's skill and experience, the images are 2D and there is a "blind area "of about 1 cm below the surface of the liver, which is particularly problematic in the case of small hepatic metastases due to colorectal cancer that are mainly located on the surface of the liver. Of course, associating contrast agents has greatly improved IOUS accuracy; however, the disadvantage of visualization of the lesions for a too short period of time makes this technique to be of limited applicability in guiding hepatic resections that may last between 2 and 6 h [112].

Recently, a new fluorescent approach, using indocyanine green (ICG), has been proposed to improve the intraoperative detection of neoplastic lesions [113, 114]. ICG is a non-specific molecule that allows detection of tumor tissue, but with limited specificity. The main advantage of its use is its safety and its commercial availability as a contrast substance. The imaging technique of intraoperative fluorescence using ICG was initially used for the detection of sentinel lymph nodes in patients with gastric, colon, and breast cancer [115, 116]. Several studies have shown that malign liver tumors show strong fluorescence when preoperative ICG administration is made [117, 118]. This technique is based on the fact that ICG binds to plasma proteins and together emit light with a peak wavelength of approximately 830 nm when illuminated with infrared light [119].

Initially, ICG-fluorescence imaging was limited to open surgery alone. After year 2010, as laparoscopic and robotic imaging systems with fluorescence have developed, ICG-fluorescence imaging has been extended to minimally invasive abdominal surgery, especially for the visualization of extrahepatic biliary tract anatomy (during laparoscopic/robotic cholecystectomies) [120], an approach known as fluorescence cholangiography [121]. In 2014, the use of ICG-fluorescence imaging was reported for the identification of subcapsular hepatic tumors before liver transection [122]. A new laparoscopic imaging system is starting to be used, this system overlapping pseudo-color fluorescence images with white color-light images in real-time (fusion ICG-fluorescence imaging) with the proposal to identify segmental hepatic margins and localization of liver tumors [123]. Thus, ICG has the ability to "label" bile ducts

[121, 124–126], hepatic tumors [118, 127–130], edges of liver segments [117, 131–133], this being due both to ICG fluorescence [134], and to its property to be excreted into the bile [135]. Due to the property of being eliminated for more than 6 h after intravenous injection [126, 135], ICG-fluorescence imaging can also be used to identify small biliary fistulas after hepatectomy [136].

As for ICG-fluorescence imaging sensitivity in detecting liver metastases, it varies between 69 and 100%. However, sensitivity is limited because the examination does not have the ability to detect hepatic lesions at a depth greater than 8 mm in the hepatic parenchyma. It has also been shown that this method can detect new metastatic lesions in up to 43% of cases [137]. In fact, it has been reported that ICG-fluorescence imaging can detect superficial lesions of up to 2 mm in both HCC and metastases liver disease due to colorectal cancers [127, 129].

Currently, a combination of a fluorophore, such as ICG, with an anti-tumor antibody is evaluated in preclinical studies. These new molecules could present a major advantage in the future for clinical applications that would allow the detection of tumor lesions with a higher TBR (tumor-to-background ratio between the intensity of fluorescence in tumor tissue and normal surrounding tissue). Recently, Harlaar et al. reported the first clinical trial using IRD-800CW-labeled bevacizumab for the detection of peritoneal metastases of colorectal origin [138].

9. Key points

- The IOUS has applications in both open or laparoscopic abdominal surgery.
- For benign hepatic tumors, IOUS has the role to localize and to visualize the relationships with the intrahepatic structures.
- For intraoperative interventional maneuvers (biopsies, ablative techniques), IOUS guidance is mandatory.
- In the case of HCC, IOUS is superior in detecting lesions measuring less than 1 cm.
- In the case of HCC, CE-IOUS finds its usefulness especially in cirrhotic patients for the differential diagnosis between malignant lesions and regenerative nodules.
- IOUS is considered the “gold standard” in open surgery for colorectal cancer.
- CE-IOUS allows the surgeon to check areas where hepatic metastasis have been described before chemotherapy.
- IOUS is mandatory for anatomic resections and for limited but radical hepatectomy.

Acknowledgements

Bartoș Adrian is the coordinator of this chapter.

Conflict of interest

The authors have no conflict of interest.

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Hepatic Trauma

Chapter 5

Hepatic Trauma

Ali Ibrahim Yahya

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73162

Abstract

Liver is the second most common solid organ frequently injured by blunt trauma and could be the commonest organ injured by penetrating trauma. The injury can be mild and goes undetected or detected and treated conservatively. It can be severe where the liver wounds can bleed until death. Once the patients with liver injury are resuscitated, the degree of liver injury can be evaluated using ultrasound scan and computed tomography imaging. If the patient is stable, diagnostic peritoneal lavage is very helpful when the imaging facilities are not available. Non-operative treatment of liver trauma has been proven to be valuable in 80% of patients with grade I, II, III and IV (grade I—mild injury; grade II—moderate injury; grade III and IV—severe liver injury). Laparotomy is mandatory if the patient's condition is unstable. By using the explorative laparotomy technique, the grade of liver injury is assessed, and accordingly the procedure is performed including suturing, ligation of the bleeding vessel, segmental resection, perihepatic packing, and so on. Morbidity and mortality of liver injury can be minimized with early diagnosis and appropriate management.

Keywords: liver injury, non-operative treatment, grading of liver injury, perihepatic packing

1. Introduction to surgical anatomy of liver

The liver is situated at the right upper quadrant of the abdomen, extending from the 5th intercostal space at the mid-clavicular line to the 10th costal margin, with a length of about 13 cm which is called the liver span. It weighs 1500 g and is the largest intra-abdominal organ, receiving 1.5 L of blood flow per minute. It is surrounded by a membrane called Glisson's capsule. It has two lobes—right and left—separated by falciform ligament, two fissures anteriorly where the ligamentum teres is attached, posterior fissure where the ligamentum venosum is attached and the third fissure on the right lobe called porta hepatis where the hepatic triad enters the liver.

1.1. Surfaces of the liver

The liver has diaphragmatic surface which is related to chest cage. Visceral surfaces are related to the following structures: right kidney, right adrenal gland, gall bladder, duodenum and hepatic flexure.

Its surface is attached to the diaphragm by a falciform ligament, right and left triangular ligament and coronary ligament.

The liver is composed of eight segments. Each lobe is composed of four segments. Each segment has its own artery, vein and duct and can be resected separately without interfering with the other segments.

Cantlie line is an imaginary line that goes from the gall bladder fossa to inferior vena cava.

The liver is composed of hepatic plates. Each plate is composed of hepatocytes, sinusoids and Kupffer cells.

1.2. Vascular supply of liver

Hepatic artery comes from the coeliac axis and divides into the right and left. It supplies 25% of blood to the liver, and portal vein supplies 75% of blood to the liver tissues. The portal vein is formed by superior mesenteric vein and splenic vein behind the neck of the pancreas.

1.2.1. Hepatic veins

There are three large hepatic veins, which drain the hepatic parenchyma of the liver lobes into the inferior vena cava.

1.2.2. Nerve supply

Parasympathetic nerve from the right vagus via coelic plexus, left vagus to porta hepatis, and sympathetic nerve along the blood vessels.

Function of the liver:

1. Bile production and secretion.
2. Detoxification of toxins.
3. Protein synthesis.
4. Production of heparin, bile pigments.
5. Storage of glycogen.
6. Erythropoiesis in infants.

Epidemiology of liver trauma:

Liver is the second most common abdominal organ that can get injured by blunt trauma [1, 26] and is the most common cause of death in abdominal trauma—100% mortality if untreated

or missed from examination. Blunt abdominal trauma is more fatal than penetrating trauma. Before 1993, all liver injuries were treated through surgery. From 1998 onwards, non-operative treatment was introduced as the standard method of treatment for liver trauma with 80% of adult liver trauma treated conservatively and 97% of children also treated non-operatively [25].

Liver injury can be mild when the trauma affects less than 25% of one lobe, moderate when the trauma affects between 25 and 50% of the lobe, and severe when the trauma affects more than 50% of the lobe.

Why liver is prone to trauma?

The liver is prone to trauma for the following reasons:

1. Fixed position of the liver: The liver is an organ which is huge and fixed at the right upper quadrant of the abdomen.
2. Liver is an organ with friable parenchyma.
3. Liver has a thin capsule.

Liver trauma can be the following:

Subcapsular haematoma, laceration, contusion, liver avulsion, bile duct injury, and gall bladder injury. Eighty percent of liver trauma involves segments 6, 7 and 8.

2. Etiology of liver trauma

The liver can be injured commonly by the following:

1. Blunt trauma commonly due to road traffic accident and can follow fall down from height. Blunt liver trauma is 10 times more fatal than penetrating trauma [7]. Blunt abdominal trauma can sustain up to 1–8% of liver injury. Hepatic trauma forms 15–20% of abdominal trauma and 80% of blunt trauma.
2. Penetrating trauma caused by a bullet or by stabbing with a sharp instrument.
3. Iatrogenic trauma is very rare during surgery or during performance of percutaneous transhepatic cholangiography (PTC). Hepatic vein injury can occur during insertion of (transjugular portosystemic shunt (TIPS).

2.1. Diagnosis of liver trauma

2.1.1. A: Clinical picture of liver trauma

1. Liver injury can be obvious.
2. Liver injury can be easily predicted.
3. Liver injury can be difficult to predict.

Obvious liver trauma:

Liver injury can be positively diagnosed where the following points are clearly established:

1. The patient is in a state of shock where he or she was involved in a road traffic accident or hit by a bullet at the right upper quadrant of the abdomen.
2. The patient is with hypotension and pain at the right upper quadrant of the abdomen after a road traffic accident.
3. Hypotensive patient shows tenderness over the right side of chest with fractured ribs after the trauma.
4. Hypotensive patient with bruises at the right upper quadrant.

Liver trauma can be easily predicted with the following points borne in mind:

1. Drop in blood pressure in a patient with road traffic accident and with guarding and tenderness at the right side of the upper abdomen.
2. Penetrating wound at the right upper quadrant of the abdomen.

Liver trauma is difficult to predict:

1. Normal blood pressure with right upper abdominal pain with guarding and tenderness at the right upper quadrant

Clinical presentation of liver trauma:

1. Pain at the right upper quadrant.
2. Fracture of right lower ribs.
3. Shock

Grading of liver trauma: **American association of trauma**

Grade I: Subcapsular haematoma less than 10% of the surface area. Laceration less than 1 cm.

Grade II: Haematoma more than 10–50% surface area. Laceration from 1 to 3 cm.

Grade III: Haematoma more than 50%. Laceration more than 3 cm.

Grade IV: Ruptured haematoma and bleeding. Laceration of the liver from 25 to 75% of the lobe.

Grade V: More than 75% of liver laceration, retrohepatic vena cava injury or hepatic vein injuries.

Grade VI: Hepatic avulsion.

2.1.2. B: Investigations

2.1.2.1. Routine investigations

Routine examination includes full blood count, electrolytes, blood sugar, urea, hemoglobin may be normal where the injury is simple, or there may be low hemoglobin indicating blood loss where the injury is severe.

Liver function tests were not done at the admission time and may not be needed if the injury is simple; it could be done if the case showed severe liver trauma. Liver function includes bilirubin, and liver enzymes include glutamic pyruvate transaminase (GPT), glutamic oxaloacetate transainase (GOT) and alkaline phosphatase (ALK) phos.

Blood group is done routinely in all patients with hepatic trauma.

2.1.2.2. Imaging investigations

Ultrasound scan for liver trauma has 99% of specificity and 88% of sensitivity [19–21]. Fast ultrasound replaced peritoneal lavage. Looking to Morrison space if there is fluid in the space indicating bleeding. The use of contrast with ultrasound scan is more beneficial in liver trauma.

CT scan: This is done on a stable patient with oral and intravenous contrast. CT scan for liver injury has more than 90% of sensitivity and specificity (**Figure 1**). Useful for diagnosis of liver injury and follow-up of liver trauma, for any hemorrhage, bile accumulation or sepsis.

X-ray: X-ray chest may show fractured ribs at the site of the liver from 7th to 9th rib but is not specific and not done to look for liver injury.

DPL Diagnostic peritoneal lavage: This is an invasive procedure done on patients with trauma when there is intraperitoneal bleeding. It is useful and produces good results when performed by expert, but nowadays, it is replaced by ultrasound scan.

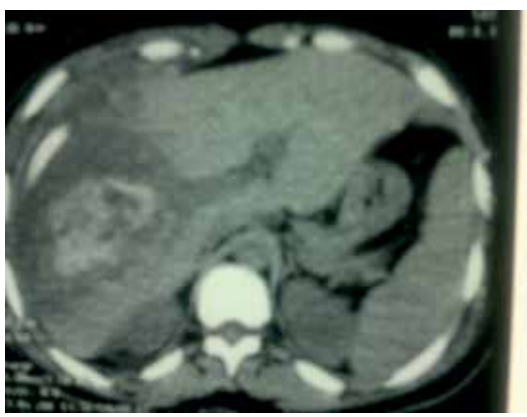

Figure 1. CT scan for a patient with massive liver trauma.

2.2. Treatment of liver trauma

Table 1 shows the number and types of liver trauma treated using different treatments in a busy general hospital.

Eighty percent of adults with liver trauma were treated conservatively, and 97% of those were children who were treated conservatively.

Healing of liver trauma: The liver has good capacity of healing once it is traumatized.

Mild liver trauma: Less than 25% of lobe damage takes 3 months to heal.

Moderate liver trauma: Between 25 and 50% takes 6 months to heal.

Severe injury: Liver injury, which encompasses more than 50% of lobe injured, takes 9 months to heal or more.

Patients with liver trauma blunt or penetrating, mild or severe once diagnosed or suspected should undergo resuscitation as usual traumatized patients, which include caring of respiration, putting good venous access for the fluids, treating emergency killing conditions like tension pneumothorax, fixing urinary catheter to know the output. After patient resuscitation, the grading of liver trauma is evaluated clinically and by imaging and the mode of treatment is planned which will include either [8–10].

1. Non-operative treatment.
2. Operative treatment.
3. Interventional radiology treatment of liver trauma.

Mode	Number of patients	Lobe	Grade	Procedure	Outcome
RTA	94	Left lobe and right lobe	Range from grade I to VI	1. Conservative treatment: 35 cases 2. Diagnostic laparoscopy: 12 suturing and insertion of drain 3. Laparotomy: 47 3-A. Repair of liver wounds: 30 3-B. Packing: 14 perihepatic packing 3-C. Resection: Three had segmental liver resection	13 died
Bullet	124	Left lobe Right lobe	Grade I and II had few patients, and most were grade III, IV	All underwent laparotomy, debridement, repair, omental packing, eight patients had perihepatic packing	18 died
Stab	13	Left lobe and right lobe	I and II	Conservative management	Nil

Table 1. Different types of hepatic trauma patients who were treated at Zliten teaching hospital.

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2.2.1. Conservative treatment of liver trauma

Blunt liver trauma can be mild, moderate or severe. Mild and moderate liver trauma can be managed conservatively without surgery [3, 11, 15, 18].

Conservative treatment includes the following:

1. Full assessment of patients.
2. Full assessment of the grade of liver injury by ultrasound and CT scan.
3. Correction of blood loss by giving blood.
4. Daily monitoring of patient.
5. Discharge of patient once he is fully stable and active.
6. Post-discharge follow-up by clinical assessment and imaging.

2.2.2. Non-operative treatment of liver injury

Non operative management was firstly conducted in children than started in adult, it is not indicated in elderly patients, choosing of the patients for non-operative management (NOM) depends on clinical condition of the patients and associated injury, less on grade of the liver of injury [2, 16].

2.2.3. Advantages of NOM

1. Less hospital stay.
2. Avoidance of unnecessary laparotomy.

An unstable patient can be defined as follows:

1. Systolic blood pressure less than 90 mmhg.
2. Pulse rate more than 120 beats per minute.
3. Altered consciousness level.
4. Altered breathing.
5. Cold clammy skin.

About 80% of blunt liver trauma can be treated conservatively, provided the patient is haemodynamically stable. It can be utilized even in grade IV.

Non-operative treatment can be performed for the following reasons:

1. Patients who are haemodynamically stable with no signs of peritonism.
2. Operative management should be available when needed.

3. Imaging facilities should be available to follow the treatments, which can lead to 100% success rate.

Liver trauma at Zliten University Hospital over a period of 9 years from 2009 to 2017—Patients: 231, deaths: 31, patients who underwent conservative treatment: 48 (**Table 1**).

Most of our patients with liver trauma during war, the time where the weapon is scattered in many regions of the country; none of our patients with hepatic trauma having had gun shot wounds left for conservative treatment, and all patients underwent surgery. This number affected our conservative management in hepatic trauma. Our rate of conservative treatment for patients with hepatic trauma was approximately 50%.

2.2.4. Complications of NOM

Complications of NOM can be diagnosed by clinical examination including blood tests, ultrasound scan and CT scan. Complications may reach up to 7% in grade III and V.

1. Bile collection may reach up to 20%—biliary peritonitis. Haemobilia: Bile leak is treated with endoscopic retrograde cholangiopancreatography most of our patients with liver trauma were during war. If fluid collection is significant, it can be drained percutaneously, laparoscopically or open surgery. **Figure 2** shows the CT of a child with hepatic trauma managed conservatively with the development of bilioma). **Figure 3** shows bilioma collection that was treated by laparotomy.

   Nagano-classified bile leak:

   Type A: Minor bile leak, small radicle from the liver surface—resolved spontaneously.

   Type B: Bile leak from a major duct on the liver surface not tied.

   Type C: Injury of duct branch from the main duct at the hilum.

   Type D: Main bile duct transected.

2. Infection and abscess formation may reach 7% and can be treated conservatively when clinical manifestation is significant.

3. Liver necrosis can be diagnosed clinically with raised liver enzymes, coagulation abnormalities or bile leak.

4. Bleeding: Hepatic artery pseudo-aneurysm accounts to about 1–2% and can be either extrahepatic or intrahepatic—more cases of extrahepatic nature. Liver compartment syndrome due to compression of the liver by huge subcapsular haematoma may result in liver failure.

2.2.5. Surgical treatment of liver injury

1. Surgery is indicated in a patient who is unstable.

2. Surgical treatment is indicated if the patient was on conservative treatment and showed signs of deterioration, [8, 27–29].

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Surgical procedures (**Figure 4** shows different repair of liver trauma).

1. Simple suturing of liver tear.
2. Debridment of unhealthy liver tissue and suturing.
3. Resection of severely damaged segment.
4. Liver lobectomy or hepatectomy for severely damaged lobe.

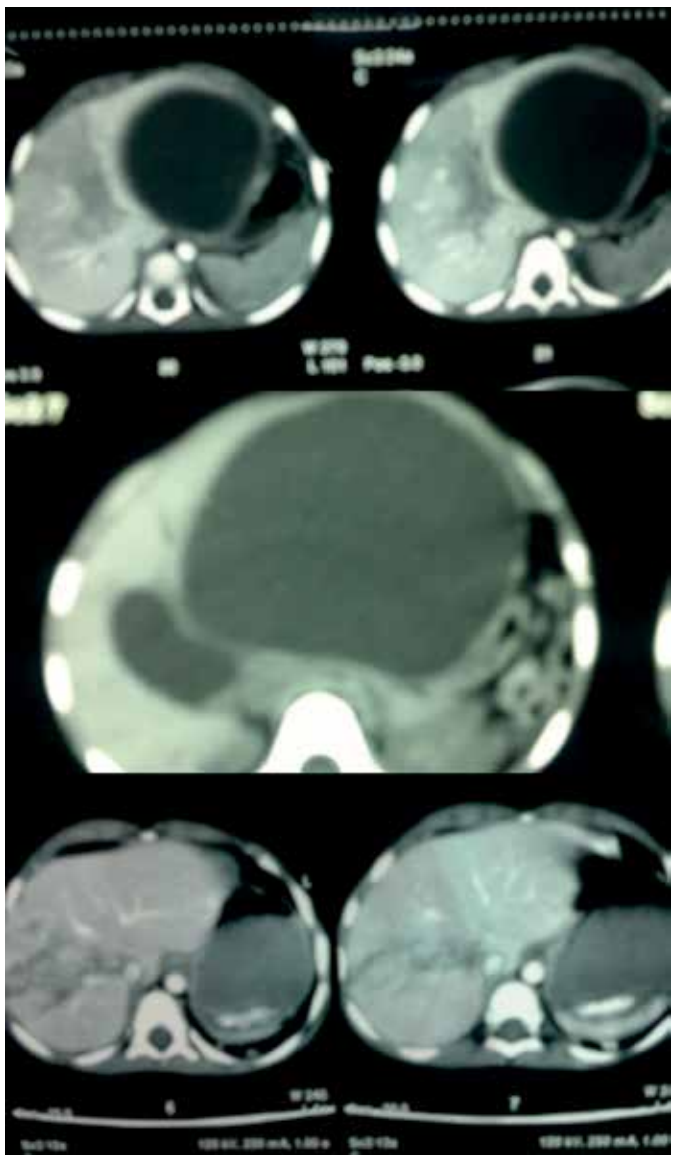

Figure 2. CT abdomen of a child who had liver trauma and was treated conservatively developed bile collection as a complication of hepatic trauma management.

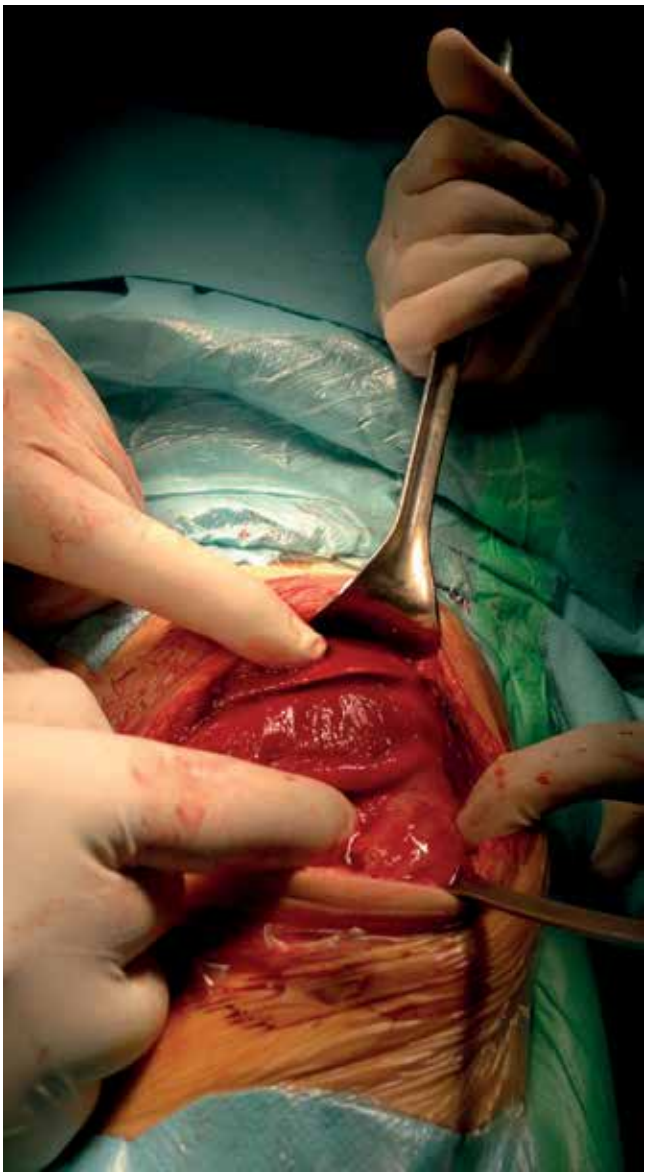

Figure 3. Child who had liver trauma managed conservatively and complicated by bile leak, presented with encysted bilioma. The child underwent laparotomy.[13].

5. Perihepatic packing for uncontrolled bleeding in unstable patients.
6. Arterial embolization which can be performed as the first option in patients who are planned for non-operative treatment or for those patient who developed bleeding after surgery [17].

Damage control in liver trauma:

Damage control is of three phases:

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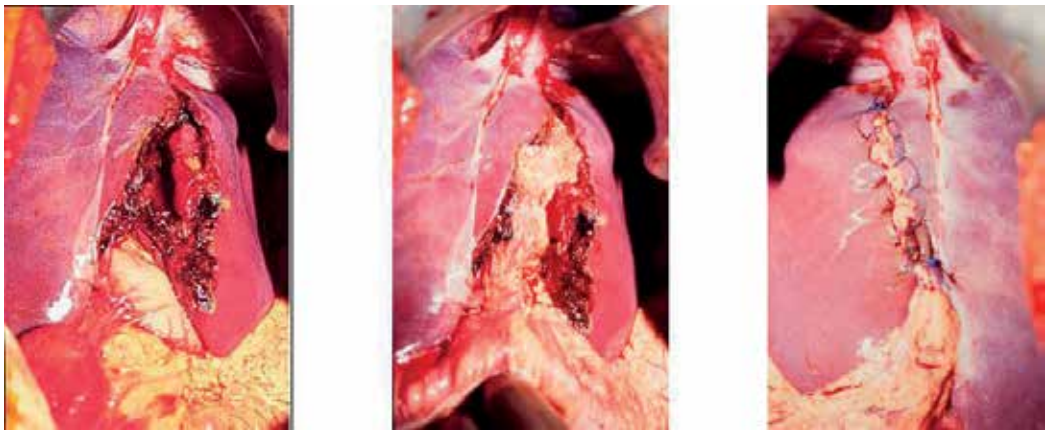

Figure 4. Liver trauma repaired and the wound packed with omentum (taken with permission from Prof. Ronald M. Stewart).

Phase I: Control of bleeding, closure of the abdomen.

Phase II: Intensive care unit resuscitation and overcome on acidosis, hypothermia, hypercoagulability.

Phase III: Re-exploration of the abdomen.

In 1983, Stone et al. proposed damage control for trauma patient [6, 14, 22–24]. Once patient had severe liver trauma, where the condition of the patient is deteriorating during surgery and the bleeding is continuous from the damaged liver, either the damage at the posterior aspect or whole of the liver, damage control is utilized in the form of packing the liver with abdominal gauze pack which are wrapped around the liver [4–6]. This technique is useful in the management of controlling the bleeding that occurs during surgery and liver resection. Packing is also useful to avoid the three killers of the patient during surgery which includes acidosis, hypercoagulability and hypothermia, which can cause cardiac arrest. To avoid the occurrence of these bad incidents, we should change to damage control. Usually six packs are placed around the liver to stop the bleeding. The abdomen left either open or closed depending on the patient's condition with the use of Bogota bag. Packing the liver with gauze packs can be complicated when patients need to go through full resuscitation in the ICU. For the correction of the three killers including acidosis, hyperthermia, hypercoagulability, usually it needs time for our patients 48–72 h to control sepsis with the use of antibiotics.

Complication of perihepatic packing:

The complication of perihepatic packing includes the following:

1. Compartment syndrome.
2. Respiratory embarrassment due to compression on the right dome of the diaphragm.
3. Abdominal sepsis if the packs were left longer than 3 days.

Other surgical procedure for liver trauma include

1. Laparoscopic assessment of liver trauma and suturing of liver tear [12].
2. Liver transplantation for severely damaged liver is difficult to perform because of availability of the liver and the experienced team.
3. Liver exclusion and extracorporeal circulation is seldom done for severe liver trauma.

Controlling of liver bleeding: Bleeding from the liver is controlled by the following procedures

1. Simple suturing.
2. Hepatorrhaphy and control of the arterial bleeding.
3. Use of omental pack and mattress sutures.
4. Selective hepatic artery ligation may control the bleeding.
5. Non-anatomical resection, anatomical resection, venovenous shunt, atriocaval shunts.

Mortality of blunt trauma is 27% and of penetrating trauma is 11%.

Overall, mortality of liver trauma is 10%, Grade III and IV mortality is 10% and V and VI are 75%.

There are many haemostatic materials used for liver trauma are very helpful for controlling the bleeding, which includes the following:

1. Surgical.
2. Spongostan.
3. Tacoceil.
4. Fibrin glue.

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Hepatocellular Carcinoma

Chapter 6

Molecular Mechanisms of Hepatocellular Carcinoma Related to Aflatoxins: An Update

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72883

Abstract

Hepatocellular carcinoma (hepatocarcinoma) is a major type of primary liver cancer and one of the most frequent human malignant neoplasms. Aflatoxins are I-type chemical carcinogen for hepatocarcinoma. Increasing evidence has shown that hepatocarcinoma induced by aflatoxins is the result of interaction between aflatoxins and hereditary factor. Aflatoxins can induce DNA damage including DNA strand break, adducts formation, oxidative DNA damage, and gene mutation and determine which susceptible individuals feature cancer. Inheritance such as alterations may result in the activation of proto-oncogenes and the inactivation of tumor suppressor genes and determine individual susceptibility to cancer. Interaction between aflatoxins and genetic susceptible factors commonly involve in almost all pathologic sequence of hepatocarcinoma: chronic liver injury, cirrhosis, atypical hyperplastic nodules, and hepatocarcinoma of early stages. In this review, we discuss the biogenesis, toxification, and epidemiology of aflatoxins and signal pathways of aflatoxin-induced hepatocarcinoma. We also discuss the roles of some important genes related to cell apoptosis, DNA repair, drug metabolism, and tumor metastasis in hepatocarcinogenesis related to aflatoxins.

Keywords: hepatocellular carcinoma, molecular mechanism, aflatoxin

1. Introduction

Hepatocellular carcinoma (also called hepatocarcinoma or liver carcinoma) is a major type of primary liver cancer and one of the most frequent human malignant neoplasms. This malignancy has been proved to correlate with aflatoxins, especially aflatoxin B1 (AFB1) [1–3].

Increasing evidence has exhibited that several mechanisms, including the toxic production from metabolism, the accumulation of DNA damage and genic mutation–induced aflatoxins, the decreasing DNA repair capacity, and dysregulation of signal pathways may play a central role in the tumorigenesis of aflatoxin-induced hepatocarcinoma [4–6]. In this review, we discuss the biogenesis, metabolism, and genic toxification of aflatoxins. We also discuss the molecular mechanisms of aflatoxin-induced hepatocarcinoma, involving in aflatoxin toxification, abnormal change of tumor relative genes, the interaction of aflatoxins and genetic factors, and signal pathway for tumorigenesis. The roles of some important genes related to cell apoptosis, DNA repair, drug metabolism, and tumor metastasis in hepatocarcinogenesis related to aflatoxins are further emphasized.

2. Aflatoxin biosynthesis, metabolism, and toxification

2.1. Aflatoxin biosynthesis

The biosynthesis of aflatoxins has been fully summarized in several previous reviews [7, 8]. In brief, aflatoxins are an important type of mycotoxins, which were the most early identified in the *Aspergillus flavus* (*A. flavus*) and regarded as causative agents of "turkey X" disease in the late 1950s and early 1960s. Thus, these toxins were named as "aflatoxins (namely *A. flavus* toxins)" according to their origin fungus [9]. Until now, 17 related aflatoxin isoforms and aflatoxin metabolites have been identified, and 4 of them often contaminated a number of agricultural commodities [10]. According to the amounts and fluorescent reactions, four aflatoxins primarily identified in foodstuffs are named as AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Among these four known aflatoxins, AFB1 and AFB2 are named as B-type aflatoxins because they are attached to a pentanone and can produce blue-color fluorescent under UV light, whereas AFG1 and AFG2 are termed as G-type aflatoxins because of their attachment to a 6-membered lactone and producing green fluorescent color feature. These aflatoxins are mainly produced by *A. flavus*, *Aspergillus parasiticus* (*A. parasiticus*), *Aspergillus nidulans* (*A. nidulans*), *Aspergillus pseudotamarii* (*A. pseudotamarii*), and *Aspergillus bombycis* (*A. bombycis*) [7, 8].

Toxigenic strains of *A. flavus* produce only B-type aflatoxins, but do not synthesize G-type aflatoxins due to the deletion of an unstable microsome enzyme and a-220 kDa cytosolic protein. The other aflatoxigenic species including *A. parasiticus*, *A. nidulans*, *A. pseudotamarii*, and *A. bombycis* can produce all four aflatoxins [8].

Numeral synthetical genes, such as aflatoxin regulatory protein gene (aflR), are required for aflatoxin biosynthesis and act as a huge neighbor gene cluster consisting of about 60–70 kb in original fungi (**Figure 1**) [8–10]. All corresponding gene-encoding enzymes and transcription factors produce aflatoxin production and regulate biosynthesis. Increasing evidence has proved that aflatoxin biosynthesis involves in at least 3 stages and 18 enzyme steps (**Figures 2–4**). The first stage, including the first (R01) to eighth reaction (R08) of biosynthesis, refers from acetyl CoA to hydroxyversicolorone. The primary product hydroxyversicolorone will be formed and regulated by transcription factors aflR and aflJ (**Figure 2**) [8, 10]. The second (biosynthesis

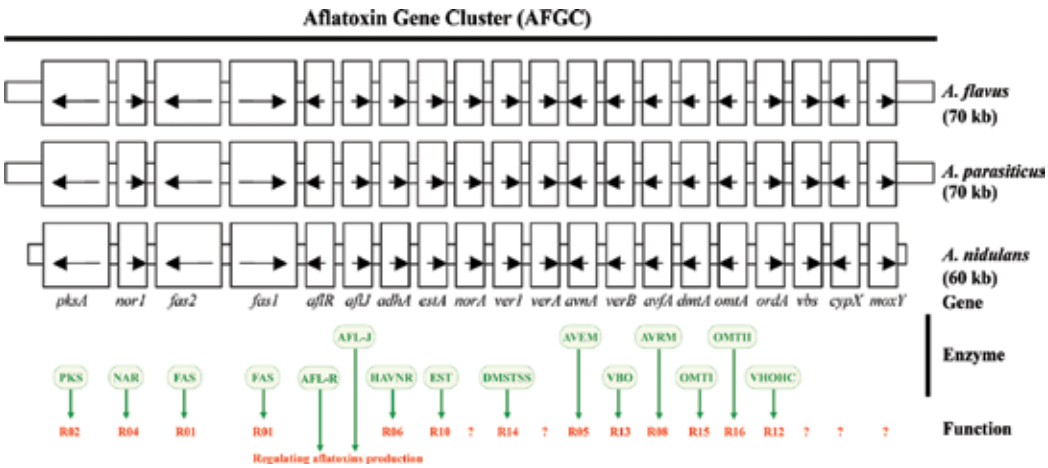

Figure 1. The aflatoxin gene cluster and their expression productions and functions. In the fungus-producing aflatoxins including *A. nidulans*, *A. parasiticus,* and *A. flavus*, genes encoding the enzymes and the transcription factors involving in aflatoxin biosynthesis commonly locate within a huge gene cluster of about 60–70 kb in the genomes. These genes, except for aflR and aflJ, involve in the 18 enzyme reaction steps (R01–R18) of aflatoxin biosynthesis, whereas aflR and aflJ expressing proteins are two important transcription factors and can regulate enzyme-related gene expression. "?" shows that the function of the corresponding gene is unknown (Note: adapted from Yabe and Nakajima [7]). *Abbreviations*. MCA, malonyl CoA; HAS, hexanoate synthase (also termed fatty acid synthase); PKS, polyketide synthase; NAS, Norsolorinic acid (NA) synthase; NAR, norsolorinic acid (NA) reductase; AVN, averantin; AVNM, averantin (AVN) monooxygenase; HAVN, 5′-hydroxyaverantin; HAVNR, 5′-hydroxyaverantin reductase; OVENC, 5′-oxoaverantin (OAVN) cyclase; AVRM, averufin (AVR) monooxygenase; VHAS, versiconal hemiacetal acetate (VHA) synthase; VHOHC, versiconal (VHOH) cyclase (also called versicolorin B synthase); VHAR, versiconal hemiacetal acetate (VHA) reductase; VBD, versicolorin B (VB) desaturase; DMSTSS, demethylsterigmatocystin (DMST) synthase system; OMTI, *O*-methyltransferase I; OMTII, *O*-methyltransferase II; OAE, OrdA enzyme.

reaction: R09–R12) (**Figure 3**) and third stages (biosynthesis reaction: R13–R18) (**Figure 4**) refer from hydroxyversicolorone to versicolorin B and from versicolorin B (VB) to the formation of ultimate products, respectively. These two stages involve in the formation of hydroxy- and non–hydroxy-versicolorone, and toxins. During the aflatoxin synthesis, more than 10 nicotinamide-adenine dinucleotide phosphate reduced form (NAPDH), one nicotinamide-adenine dinucleotide (NAD), and 2*S*-adenosylmethionine (SAM) are required. These cofactors may play a critical role in the control of aflatoxin biosynthesis [7–10].

2.2. The metabolism of aflatoxins in liver

Aflatoxins synthesized in the mycelia are finally excreted into such mediums as cereals (maize, wheat, sorghum, rice, and millet), nuts (peanuts, pistachios, walnuts, Brazil nut, and coconut), spices (chili, turmeric, paprika, black pepper, and ginger), and seeds. Epidemiological studies have exhibited that AFB1 is the most common in contaminated human foods [8, 10]. Once this aflatoxin in the mediums is taken into body, it is metabolized via two-stage reactions in the liver. The first-stage metabolisms include reduction reaction (ketoreduction to aflatoxicol), oxidative reaction (O-dealkylation to aflatoxin P1), and hydrolytic reactions (hydroxylation to aflatoxin M1, aflatoxin Q1, and aflatoxin B2). This stage reaction involves numerous enzymes such as cytochromes P450 (CYP450), monooxygenases, amino-oxidases,

alcohol dehydrogenases, epoxide-hydrolases, aldehyde-reductases, and ketone-reductases. The second-stage reaction mainly comprises covalent binding reaction (toxic produces) and conjugation reaction (excretion and detoxification). Through these metabolites, aflatoxins ultimately transform into nontoxic secretions and toxic products [10, 11].

2.3. The toxification of aflatoxins in liver

Toxification of aflatoxins in liver is mainly divided into acute and chronic toxic effects. Data from epidemiological, experimental, and clinical studies have shown that above 6000 mg exposure of aflatoxin through digestion will cause acute severe liver damage and subsequent

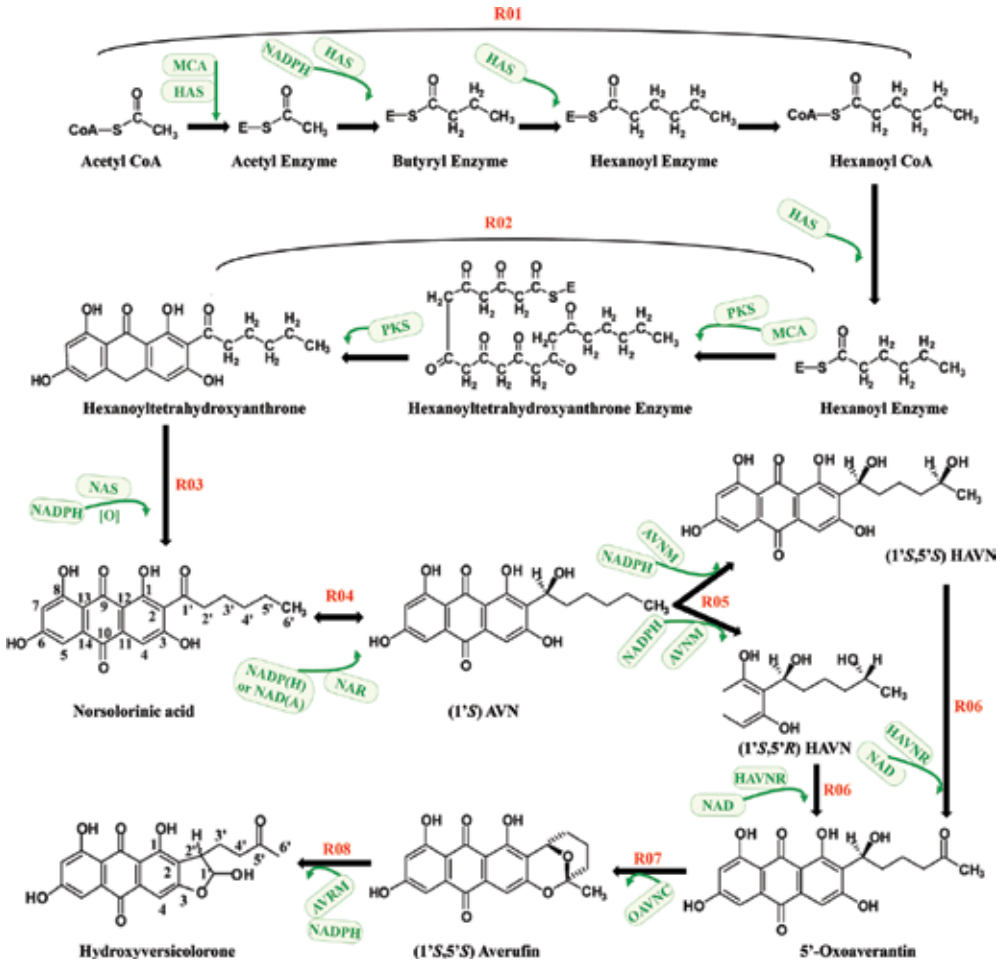

Figure 2. The first stage of aflatoxin biosynthesis. The first stage of aflatoxin biosynthesis, including the first (R01) to eighth reaction (R08) of biosynthesis, refers from acetyl CoA to hydroxyversicolorone. *Abbreviations*. MCA, malonyl CoA; HAS, hexanoate synthase (also termed fatty acid synthase); PKS, polyketide synthase; NAS, norsolorinic acid (NA) synthase; NAR, norsolorinic acid (NA) reductase; AVN, averantin; AVNM, averantin (AVN) monooxygenase; HAVN, 5′-hydroxyaverantin; HAVNR, 5′-hydroxyaverantin reductase; OVENC, 5′-oxoaverantin (OAVN) cyclase; AVRM, averufin (AVR) monooxygenase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form); CoA, coenzyme A. *Noted:* adapted from Yabe and Nakajima [7].

http://dx.doi.org/10.5772/intechopen.72883

illness or death. This kind of acute effect is mainly associated with malfunction of the liver induced by toxic metabolic products. For chronic toxic effects, chronic exposure of aflatoxins can induce DNA damage and produce genotoxicity and carcinogenicity. In the past decades, increasing evidence has proved that AFB1 as aflatoxins often induce genic mutations such as TP53 and are among the most carcinogenic substances known and the major cancerous hepatocarcinoma risk factor.

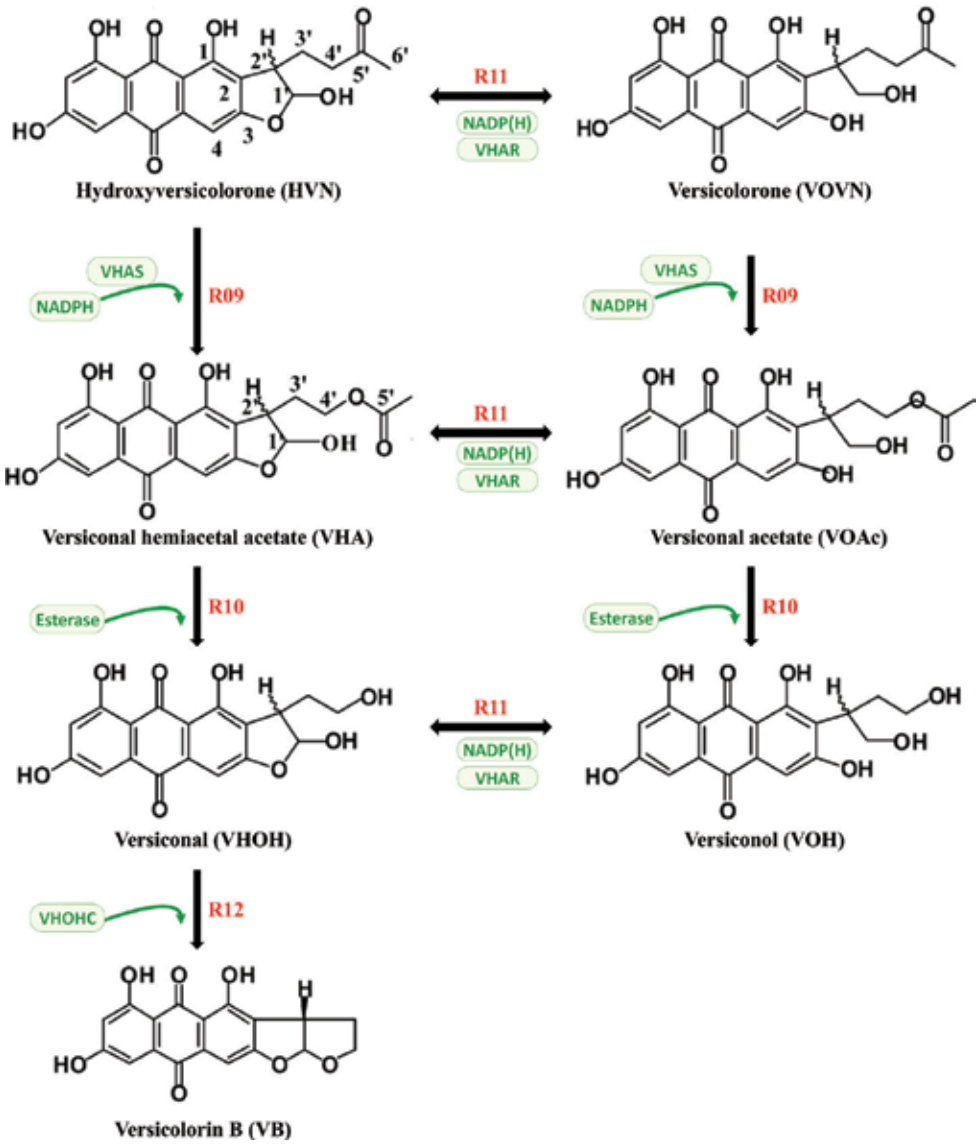

Figure 3. The second stage of aflatoxin biosynthesis. The second stage of aflatoxin biosynthesis, including the ninth (R09) to twelfth reaction (R12) of biosynthesis, refers from hydroxyversicolorone to versicolorin B (VB). *Abbreviations*. VHAS, versiconal hemiacetal acetate (VHA) synthase; VHOHC, versiconal (VHOH) cyclase (also called versicolorin B synthase); VHAR, versiconal hemiacetal acetate (VHA) reductase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form). *Noted:* adapted from Yabe and Nakajima [7].

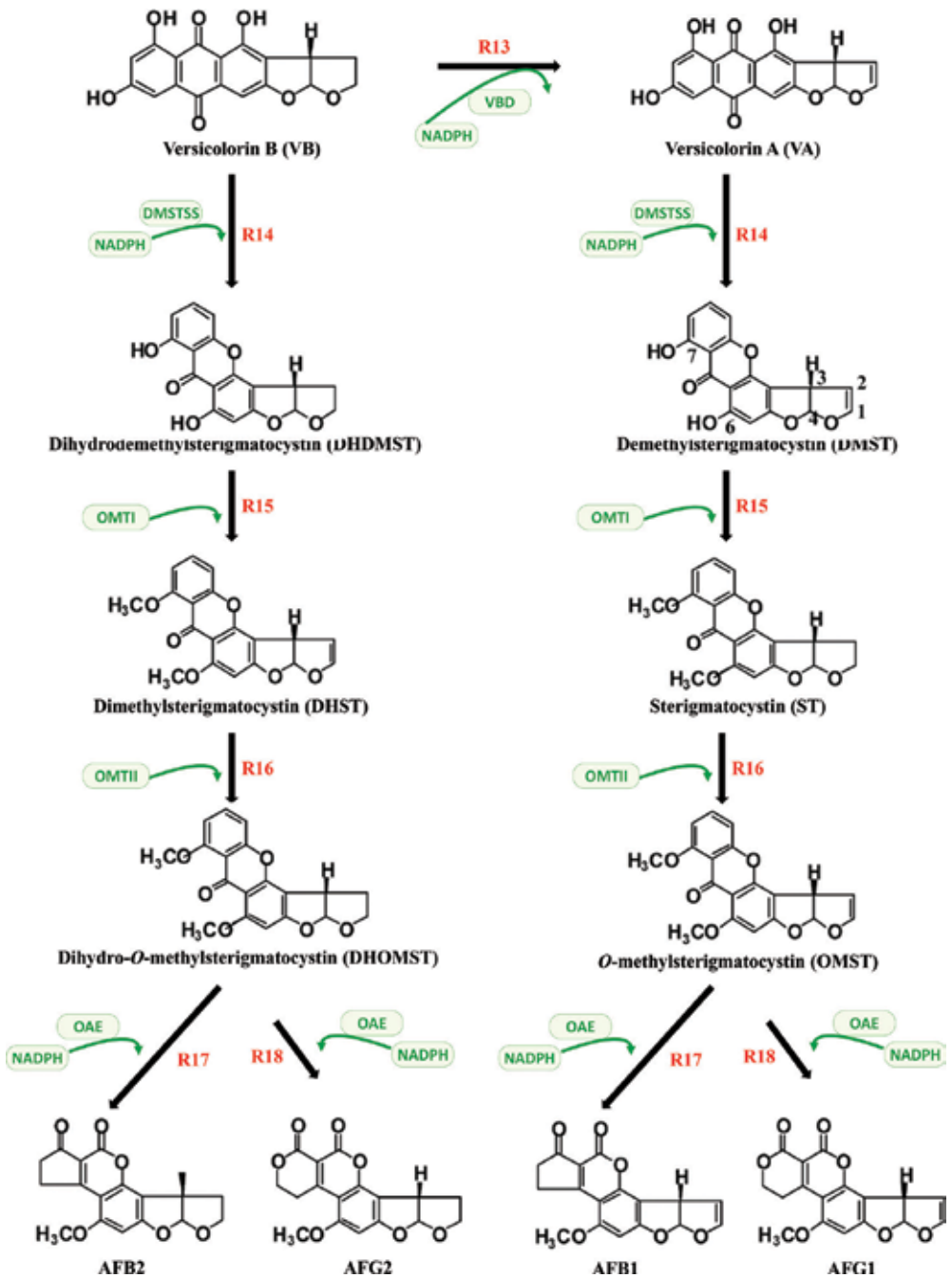

Figure 4. The third stage of aflatoxin biosynthesis. The third stage of aflatoxin biosynthesis, including the 13th (R13) to 18th reaction (R18) of biosynthesis, refers from versicolorin B (VB) to the formation of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). *Abbreviations*. VBD, versicolorin B (VB) desaturase; DMSTSS, demethylsterigmatocystin (DMST) synthase system; OMTI, *O*-methyltransferase I; OMTII, *O*-methyltransferase II; OAE, OrdA enzyme; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form). *Noted:* adapted from Yabe and Nakajima [7].

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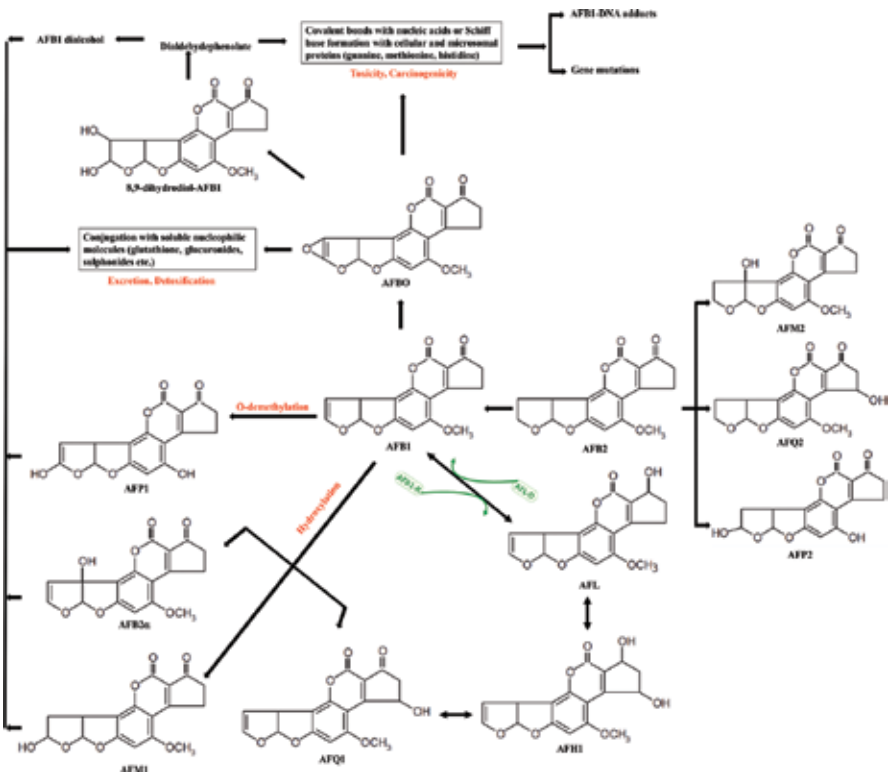

Figure 5. The metabolite of aflatoxins in the liver. Aflatoxins are metabolized via four metabolic pathways: O-dealkylation to aflatoxin P1 (AFP1), ketoreduction to aflatoxicol (AFL), epoxidation to AFB1-8,9-epoxide (AFBO, highly toxic, mutagenic, and carcinogenic), and hydroxylation to aflatoxin M1 (AFM1, highly toxic), AFP1, aflatoxin Q1 (AFQ1), or aflatoxin B2a (AFB2a). *Abbreviations*. AFM2, aflatoxin M2; AFP2, aflatoxin P2; AFQ2, aflatoxin Q2; AFL-D, aflatoxicol dehydrogenase; AFB1-R, aflatoxin B1 reductase. *Noted:* adapted from Wu and Jezkova [10].

3. The molecular mechanisms of aflatoxin-induced hepatocarcinoma

As described earlier, the main chronic toxification of aflatoxins is chronic liver damage and induced tumorigenesis of hepatocarcinoma. AFB1 has been proved as an I-type chemical carcinogen. Mechanisms of AFB1-induced hepatocarcinoma mainly involve in DNA damage and repair, the inactivation of tumor suppressor genes and the activation of oncogenes from genic mutations, abnormal immunoreaction, and inheritance alterations.

3.1. Aflatoxin-induced DNA damage

Increasing evidence has shown that the carcinogenicity of aflatoxins results from aflatoxin-induced DNA damage, including the formation of DNA adducts, DNA single strand breaks (SSBs) or double strand breaks (DSBs), chromosomal aberration damage (CAD), unscheduled DNA synthesis (USDS), abnormal chromatid exchange (ACE), the formation of micronuclei and macronuclei, and oxidation DNA damage. Of these DNA damages, AFB1-DNA adducts

are the most common damage types and consist of 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy–AFB1 adduct (AFB1-GA) and ring-opened formamidopyrimidine AFB1 adduct (AFB1-FAPYA). The formation of AFB1-GA begins from AFB1 covalent binding to DNA and its product 8,9-epoxide-AFB1 (AFBE) by CYP450 [12, 13]. This adduct can automatically not only give rise to AFB1-FAYPA, which is accumulated using a time-dependence and nonenzyme pathway, but also be transferred into AFP1, AFM1, AFQ1, and other products by metabolic enzymes.

Additionally, AFB1 also induces oxidation DNA damage such as 8-oxodeoxyguanosine (8-_{oxyG}). These damages induced by aflatoxins, if not timely repaired, can cause subsequent repair-resistant adducts and depurination or lead to error-prone DNA repair resulting in DSBs, SSBs, USDs, CAD, ACE, and frame shift mutations. Interestingly, the accumulation of DNA damages is positively associated with the time and the levels of aflatoxin exposure and modifies the risk of hepatocarcinoma through regulating the expression of some genes such as a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) [14], X-ray repair complementing 4 (XRCC4) [15], microRNA-4651 [16], and so on (**Table 1**). For example, Huang et al. [14] investigated the association between AFB1-DNA adducts via a hospital-based case control study and found increasing AFB1-DNA adducts negatively correlated with ADAMTS5 expression. It is known that ADAMTS5 may act as a tumor suppressor gene via decreasing vascular endothelial growth factor (VEGF) expression and inhibiting tumor angiogenesis and metastasis [17]. The downregulation of XRCC4 by increasing AFB1-DNA adducts decreases repair capacity for SSBs and DSBs and increases risk of tumor suppressor gene TP53 mutation and tumors [15, 18–22]. These genes progress the tumorigenesis and progression of hepatocarcinoma via regulating DNA repair capacity and angiogenesis. Although AFB1-DNA adducts are mainly produced in liver cells, they are also found in the immune cells and may regulate the immune function. Thus, DNA damage may be an important molecular event and may play a crucial role in the carcinogenesis of hepatocarcinoma caused by aflatoxins.

3.2. The mutagenesis of aflatoxins

Aflatoxin-induced DNA adducts can produce depurination, DSBs, the substitution of DNA bases, and frame shift mutations. In the past decades, the *in vivo* and *in vitro* studies have shown that the mutagenesis of aflatoxins can induce the mutation from GC to TA. As previously shown, mispairing of the aflatoxin-DNA adducts can cause both transition and transversion mutations [25–27]. In an *in vitro* non-sense analysis, Foster et al. found that the action form of AFB1 (namely AFBE) can induce more than 90% of GC to TA mutation [28]. This

Gene	Expression change	Role of change in the hepatocarcinoma carcinogenesis	Ref
ADAMTS5	Down	Angiogenesis, metastasis, prognosis	[14]
XRCC4	Down	Low DNA repair capacity, gene mutation	[15]
MicroRNA-4651	Up	Angiogenesis, metastasis, prognosis	[16]
MicroRNA-24	Up	Angiogenesis, metastasis, prognosis	[23]
MicroRNA-429	Up	Angiogenesis, metastasis, prognosis	[24]

Table 1. The change of gene expression related to DNA damage induced by aflatoxins.

mutation was further proved to locate in the GC-rich regions via the plasmid system identifying mutational target enzyme and named as hot-spot regions for aflatoxin-induced mutations [29–31]. Results from quantitative analyses based on the *in vitro* cell model, which was transfected by pS189 (a shuttle vector having mutative targets), also showed that more than 90% of mutative spectra caused by aflatoxins was GC to TA (about 50% of mutations) and GG to TC transversion (about 30% of mutations) [32]. It has been proved that the accumulation of these transversions will result in the mutations of some important genes such as TP53 and Ras and promote hepatocarcinogenesis [31, 33].

3.3. The abnormality of tumor suppressor genes induced by aflatoxins

Studies *in vivo* and *in vitro* have examined the abnormality of tumor suppressor genes by aflatoxin exposure (**Table 2**). Among these known genes, the abnormality of TP53 induced by aflatoxins has been proved to be an important molecule change [34, 35]. In high aflatoxin-exposure areas, the mutations of TP53 gene, especially hot-spot mutation at codon 249, are present among more than 40% of patients with AFB1-related hepatocarcinoma, whereas this kind of mutation is very rare among cases with null or low AFB1 exposure [14, 36, 37]. Therefore, the mutation at codon 249 of TP53 gene has been defined as a molecular symbol for hepatocarcinoma caused by AFB1 exposure. Results from clinical sample and experimental studies further display that consistent exposure of aflatoxins may result in the accumulation of TP53 mutant protein and abnormal DNA damage repair, apoptosis, and immunoreaction [38]. Other genes such as bcl2, p27, p16, and p21 are found to produce different expression or abnormal structural change under the conditions of aflatoxin expression (**Table 2**). Taken together, inactivation of tumor suppressor genes from mutation and increasing mutant expression may be a crucial step of malignant transformation for liver cells.

3.4. The abnormality of oncogenes induced by aflatoxins

In the past decades, the abnormality of oncogenes induced by aflatoxins has mainly been focused on c-myc and ras genes, involving in the activation, expression, and mutation of proto-oncogenes (**Table 3**). For example, Tashiro et al. investigated the effects of AFB1 exposure on oncogenes based on rat model with AFB1-induced hepatomas and found that the expression of both c-myc and c-Ha-ras was upregulated in all the tumors [65]. They also observed c-Ha-ras amplification and rearrangement [65]. In Fischer rat models with AFB1- and AFG1-induced liver tumors, Sinha et al. observed that aflatoxins can induce activation of N-ras and spot mutation of G to A at codon 12 of Ki-ras [66]. This type of activation and mutation will increase in the tissues with liver cancer than those with noncancers [66–69]. Results from *in vitro* studies have further proved that aflatoxins can induce gene mutations of oncogenes [70]. Together, these data suggest that aflatoxins may activate proto-oncogenes by inducing gene mutations and promote the carcinogenesis of hepatocarcinoma.

3.5. The interaction of aflatoxins and hepatitis B virus promoting hepatocarcinogenesis

The interaction of aflatoxins and hepatitis B virus (HBV) has been proved in the carcinogenesis of hepatocarcinoma by molecular epidemiological and clinicopathological studies and sys-

Gene	Study design	Change	Significance	Ref
TP53	Mice model with HNP	Expression ↑	DNA damage ↑	[39]
bcl2	Mice model with HNP	Expression ↓	DNA damage ↑	[39]
p27	Hepatocytes *in vitro*	Expression ↓	DNA damage ↑	[40]
p21	Hepatocytes *in vitro*	Expression ↓	DNA damage ↑	[40]
TP53	HCCs (n = 223)	Expression ↑, multiplot mutation	Carcinogenesis	[41]
TP53	HCCs (n = 124)	Mutation at codon 249: 60%	Carcinogenesis	[42]
H2AX	HCC cells *in vitro*	Phosphorylation	Carcinogenesis	[43]
BP1	HCC cells *in vitro*	Phosphorylation	Carcinogenesis	[43]
TP53	HCCs (n = 52)	Mutation at codon 249: 50%	Carcinogenesis	[44]
p16	HCCs (n = 40)	Methylation	Carcinogenesis	[45]
p53	HCCs (n = 40)	Multiplot mutation	Carcinogenesis	[45]
p53	AFB1-induced mutation *in vitro*	Multiplot mutation at CpG	Carcinogenesis	[46]
TP53	HCCs (n = 64) plus a meta-analysis	Mutation at codon 249: 36%, protein accumulation: 50%	Carcinogenesis	[47]
TP53	Mice model with HNP	Multiplot mutation	Carcinogenesis	[48]
TP53	HCC cells *in vitro*	AFB1-induced mutation at codon 249 promoting IGF-II expression	Carcinogenesis	[49]
TP53	Atcc-Ccl13 *in vitro*	Mutation at codon 249	Carcinogenesis	[50]
TP53	HCCs (n = 36)	Mutation at codon 249	Carcinogenesis	[51]
TP53	Mice model	Mutation at codon 249 and 346, mutant protein increasing	Carcinogenesis	[52–57]
TP53	HCCs (n = 60)	Mutation at codon 249: 69%	Carcinogenesis	[58, 59]
TP53	Hepatocytes *in vitro*	Multiplot mutation	Carcinogenesis	[60]
TP53	HCCs (n = 110)	Mutation at codon 249: 69%	DNA damage, carcinogenesis	[61]
TP53	HCCs (n = 15)	Mutation at codon 249 and 254	Carcinogenesis	[62]
TP53	HCC cells *in vitro*	AFB1-induced Mutation at codon 249	Carcinogenesis	[63]
TP53	HCCs (n = 18)	Mutation at codon 249: 53%	Carcinogenesis	[64]

Abbreviations. HNP, hepatic neoplasms; HCC, hepatocarcinoma.

Table 2. The change information of tumor suppressor genes induced by aflatoxins in hepatic cells and hepatocarcinoma cells.

Gene	Study design	Change	Significance	Ref
N-ras	HCCs (n = 36)	Mutation at codon 61	Carcinogenesis	[51]
c-myc	Mice model with HNP	Expression ↑, amplification, rearrangement	Carcinogenesis	[65]
c-Ha-ras	Mice model with HNP	Expression ↑, amplification, rearrangement	Carcinogenesis	[65]
Ki-ras	Mice model with HNP	Activation	Carcinogenesis	[69]
N-ras	Mice model with HNP	Activation	Carcinogenesis	[66]
Ki-ras	Mice model with HNP	Mutation at codon 12	Carcinogenesis	[66]
N-ras	Mice model with HCC	Activation	Carcinogenesis	[67]
Ki-ras	Mice model with HCC	Activation	Carcinogenesis	[67]
c-Ha-ras	Mice model with HNP	Mutation at codon 61: 40–60%	Carcinogenesis	[71, 72]

Abbreviations. HNP, hepatic neoplasms; HCC, hepatocarcinoma.

Table 3. The change information of oncogenes induced by aflatoxins.

tematically reviewed by several studies [73–75]. In brief, the first clinicopathological evidence of aflatoxins interacting with HBV was provided by Yeh et al. [76]. Through a case-control study design conducted in Guangxi Area, they found that these HBV-positive individuals with high AFB1 exposure consumption featured 10-times the mortality rate compared with those with low exposure consumption. Results from multivariable interactive analyses have further convinced that AFB1 multiplicatively interacted with HBV status for promoting hepatocarcinoma risk [77–80]. For example, Williams et al. reported that the risk of developing hepatocarcinoma was 6.37 for aflatoxin exposure, 11.3 for HBV infection, and 73.0 for the combination of aflatoxin and HBV [77]. The following several molecular epidemiological studies with large-size samples from areas with high aflatoxin exposure and high HBV infection in China showed remarkably multiplicative effect for hepatocarcinoma risk (multiplicative interaction: $63.2_{\text{(both positive)}} > 1.9_{\text{(AFB1 positive)}} \times 9.5_{\text{(HBV positive)}}$ [78–80].

This interaction of two hepatocarcinogenic causes has been proved in the transgenic mice models with overexpressing HBV large envelope polypeptide [81]. Results from this study exhibited that animals will produce more rapid and extensive hepatic dysplasia and hepatocarcinoma under the conditions with aflatoxin consumption [81]. Similar findings have also shown in the studies based on woodchuck and duck models [82–84].

The aflatoxins interacting with HBV infection promoting hepatocarcinoma development mechanically involve in the following aspects. First, HBV infection directly or indirectly increases the sensitivity of hepatocytes on the toxification of aflatoxins. Evidence from observation studies have displayed that HBV-positive carriers have more amount of aflatoxin adducts than those with negative HBV status, although they are from the same high aflatoxin exposure area [85, 86]. The active product of aflatoxin AFBE is found to significantly increase the risk of viral DNA integrating into damaged DNA strand [87]. This promotes malignant transformation of damaged hepatocytes by aflatoxins. Second, HBV

infection increases the mutation frequency at codon 249 of TP53 gene and coordinates with aflatoxins for abrogating the normal functions of TP53 (such as the control of cell cycle, DNA damage repair, and cell apoptosis), which contributes to multisteps of hepatic carcinogenesis [64, 88]. Third, the HBV X gene–expressing protein inhibits base excision repair potential and results in an increasing accumulation of aflatoxin-DNA adducts [89]. Finally, HBV infections can cause hepatocytic necrosis, inflammatory proliferation, and oxygen/nitrogen active products, which may increase the likelihood of aflatoxin-induced mutations and the cellular clonal expansion containing mutations [90–92].

3.6. The interaction of aflatoxins and inheritance alterations promoting hepatocarcinogenesis

Increasing evidence has exhibited that the genetic alterations in DNA repair genes increase the amount of AFB1-DNA adducts and the frequency of hot-spot mutation at codon 249 of TP53 gene and may promote hepatic toxification of aflatoxins [1, 19, 20, 22, 37, 93–98]. Joint analyses based on meta-analyses further showed this kind of toxic effects (**Table 4**) [1, 22]. The genetic variants in other genes, such as CYP450, glutathione *S*-transferase T1 (GSTT1), glutathione *S*-transferase M1 (GSTM1), and microsomal epoxide hydrolase (HEHY), also display similar modificative effects on aflatoxin-induced hepatocarcinoma [98–101]. Interestingly, the multiplicatively interactive effects between aflatoxins and genetic alterations in these genes have been identified in the risk elucidation of hepatocarcinoma related to aflatoxins [22]. Taken together, genetic deficiency in the DNA repair and detoxification capacity may play a vital role in the carcinogenetic process of aflatoxin-induced hepatocarcinoma.

3.7. The aflatoxin-caused immunosuppression promoting hepatocarcinogenesis

Increasing evidence from *in vitro* and *in vivo* studies has proved that the immunosuppression induced by aflatoxins plays an important role in the carcinogenesis of hepatocarcinoma. Several known mechanisms may involve in this progression step. First, aflatoxins can significantly suppress the functions of macrophages via affecting the expression and secretions of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-3, IL-6, and reactive intermediates (including nitric oxide, hydrogen peroxide, and superoxide anion) [102, 103]. The suppression of macrophages by aflatoxins may be also correlated with the arrest in the G1/G0 phase [104] and altered expression of CD14 (a cell surface protein functionally regulating immunoreaction) [105]. This suppression may result in the dysregulation of the immune response and homeostasis, which contributes to the accumulation of abnormal cells with DNA damage and altered genome induced by aflatoxins, and ultimately progresses tumorigenesis. Second, aflatoxin exposure can decrease the secretion of antibody such as IgA [106]. For example, Turner et al. investigated effects of aflatoxin exposure on antibody production based on a large molecular epidemiological study [106]. In their study, they tested the levels of saliva secretory IgA (sIgA) in Gambian children (n = 472) with different degree exposure of aflatoxins and found that these individuals with high aflatoxin exposure featured lower level of sIgA in their saliva compared to those without high exposure (50.4 vs. 70.2 μg/mg protein). Finally, aflatoxins may alter T-cell functions (including decreased T-cell populations and suppressed CD4+ T-cell function) and increase individuals' susceptibility to other carcinogens [77, 107].

Gene	RS#	Genotype	TP53M			DNA adducts	
			%	Risk	*P*	Mean	*P*
XRCC1	rs25487	CC	46.51	Reference		3.276	
		CT	45.25	2.419	3.371×10^{-11}	3.264	0.899
		TT	8.24	5.028	6.651×10^{-6}	3.640	0.026
XRCC3	rs861539	GG	32.17	Reference		2.990	
		GA	43.55	1.380	0.018	3.216	0.025
		AA	24.28	1.524	0.011	3.897	4.962×10^{-14}
XRCC7	rs7003908	AA	21.24	Reference		2.879	
		AC	46.06	1.883	1.372×10^{-5}	3.347	1.663×10^{-5}
		CC	32.71	2.089	4.368×10^{-6}	3.550	1.751×10^{-8}
XRCC4	rs28383151	GG	67.03	Reference		3.308	
		GA	21.68	1.688	0.001	3.405	0.069
		AA	11.29	3.829	7.387×10^{-6}	3.721	2.867×10^{-4}
XRCC4	rs3734091	GG	72.31	Reference		3.229	
		GT	17.56	2.799	9.191×10^{-7}	3.439	0.095
		TT	10.13	5.104	3.826×10^{-6}	3.654	0.005
XPD	rs13181	TT	34.41	Reference		2.926	
		TG	41.85	1.458	0.005	3.253	0.011
		GG	23.75	1.744	0.001	4.062	4.265×10^{-6}
XPC	rs2228001	TT	34.05	Reference		3.083	
		TG	48.30	1.500	0.002	3.332	0.001
		GG	17.65	1.818	0.001	3.666	3.404×10^{-22}

Noted: Adapted from Refs. [13] and [84]. *Abbreviations*. TP53M, hot-spot mutation at codon 249 of TP53 gene; RS#, the number of polymorphism.

Table 4. Polymorphisms in DNA repair genes and HCC risk.

Altogether, the data available to date make it clear that aflatoxins can exert an immunosuppressive effect via different pathways. However, more detailed mechanisms by which this effect is mediated remain unknown.

4. Limitation and further direction

In the past decades, the advance in pathological mechanisms of aflatoxin-related hepatocarcinoma held great promise. However, we are still far from a comprehensive view of this kind of potentials. First, the detailed metabolic step and corresponding enzymes, especially the first-stage

reaction and toxicity mechanisms, have not been elucidated. Second, although the activation of aflatoxins is found to act as a crucial step, it is unclear how the tumorigenesis of hepatocarcinoma is triggered by aflatoxins. Third, the vast literature for aflatoxin-induced hepatocarcinoma mainly focuses on the studies on AFB1, and some important information may have been lost. Fourth, in spite of some evidence of AFB1 inducing abnormal immunoreaction and interacting with hepatitis virus and genetic factors, they are at the primary stage and still far from elucidation. Therefore, the detailed toxicity mechanisms of aflatoxins and corresponding carcinogenesis mechanism will greatly benefit our understanding of aflatoxin-related hepatocarcinoma.

5. Summary

It has been shown that increasing exposure of aflatoxins may promote the carcinogenesis of hepatocarcinoma. Molecular mechanisms of aflatoxin-induced hepatocarcinoma involve in DNA damage, gene mutations, the inactivation of such tumor suppressor gene as TP53, the activation of proto-oncogenes, abnormal immunoreaction, and the interaction between aflatoxins and other carcinogens such as HBV. However, an understanding of aflatoxin-induced hepatocarcinoma is far from complete, and further research in this field is looked forward to elucidating more detailed mechanisms responsible for hepatocarcinoma related to aflatoxins in the future.

Conflicts of interest and source of funding

The authors declare no competing financial interests. This study was supported in part by the National Natural Science Foundation of China (Nos. 81760502, 81572353, 81372639, 81472243, 81660495, and 81460423), the Innovation Program of Guangxi Municipal Education Department (Nos. 201204LX674 and 201204LX324), Innovation Program of Guangxi Health Department (No. Z2013781), the Natural Science Foundation of Guangxi (Nos. 2017GXNSFGA198002, 2017JJF10001, 2017GXNSFAA198002, 2016GXNSFDA380003, 2015GXNSFAA139223, 2013GXNSFAA019251, 2014GXNSFDA118021, and 2014GXNSFAA118144), Research Program of Guangxi "Zhouyue Scholar" (No. 2017-38), Research Program of Guangxi Specially-invited Expert (No. 2017-6th), Research Program of Guangxi Clinic Research Center of Hepatobiliary Diseases (No. AD17129025), and Open Research Program from Molecular Immunity Study Room Involving in Acute & Severe Diseases in Guangxi Colleges and Universities (Nos. kfkt20160062 and kfkt20160063).

Abbreviations

AFB1	aflatoxin B1
AFB2	aflatoxin B2
AFG1	aflatoxin G1

AFG2	aflatoxin G2
AFP	α-fetoprotein
A. flavus	*Aspergillus flavus*
A. parasiticus	*Aspergillus parasiticus*
A. nidulans	*Aspergillus nidulans*
A. pseudotamarii	*Aspergillus pseudotamarii*
A. bombycis	*Aspergillus bombycis*
HBV	hepatitis virus B
HCV	hepatitis virus C
Hepatocarcinoma	hepatocellular carcinoma
NAPDH	nicotinamide-adenine dinucleotide phosphate reduced form
NAD	one nicotinamide-adenine dinucleotide
SAM	*S*-adenosylmethionine
CYP450	cytochromes P450

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Chapter 7

Evaluation and Surgical Management of Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75164

Abstract

Hepatocellular carcinoma (HCC) is the most frequent primary malignant tumor of the liver, being the sixth most common cancer in the world and the third cause of cancer mortality. Most of the patients with HCC have an established background of cirrhosis and chronic liver disease. Magnetic resonance imaging (MRI) is the best technique for evaluation of the liver nodules in patients with cirrhosis, especially when a HCC is suspected. HCC staging is mandatory to select the appropriate primary and adjuvant therapy and to evaluate the prognosis. Hepatic resection is the treatment of choice in non-cirrhotic patients who have been diagnosed with HCC. In this chapter we underline the main diagnostic methods used for HCC staging, together with the treatment possibilities, highlighting the importance of surgical management, conventional or minimally invasive.

Keywords: hepatocellular carcinoma, hepatic resections, laparoscopy, ablative therapy

1. Introduction: generalities

Hepatocellular carcinoma (HCC) is the most frequent primary malignant tumor of the liver, which is arising from hepatocytes, the liver's parenchymal cells. Most of the patients with HCC have an established background of cirrhosis and chronic liver disease due to hepatitis B virus or hepatitis C virus. HCC is the sixth most common cancer in the world and the third cause of cancer mortality [1].

2. Diagnosis

Clinical features of HCC may include pain in the upper right quadrant and weight loss. Most of the patients diagnosed with HCC are patients known with liver cirrhosis. Despite this fact, there is a rare complication such as rupture of a liver tumor with intra-abdominal bleeding, which will need immediate surgical care [2]. These patients will present with acute abdominal pain, peritoneal irritation and hypotension. Other patients present with nonspecific signs such as fever, jaundice, ascites, anorexia or encephalopathy [3].

Clinical examination can reveal an abdominal mass in the upper right quadrant or hepatomegaly. Obstructive jaundice can indicate tumor extension into the extrahepatic biliary structures [4].

HCC can metastasize to any organ, the most frequent being metastasis to bone, lung or other abdominal viscera; so, patients can present with various clinical signs and symptoms related to the affected organs. Watery diarrhea is more common in patients with cirrhosis and HCC because of the increased production of intestinal secretory substances such as gastrin and vasoactive intestinal peptide (VIP) [5, 6].

Alpha-1 fetoprotein is the most commonly used marker for HCC. Patients with AFP > 400 ng/ml tend to have a greater size, bilobar involvement, portal vein thrombosis and decreased survival [7]. If the tumor producing AFP is left untreated, the AFP value will increase over the time, so this marker can be used for detecting tumor progression. APF may be increased in a variety of other malignancies and in patients with chronic liver disease without HCC, particularly in hepatitis C [8]. The sensitivity, specificity and positive predictive value of AFP range from 39 to 64%, 76 to 91% and 9 to 32% [8]. Patients with values of AFP greater than 1000 ng/ml have a higher incidence of vascular invasion (61%) compared with patients with values of AFP <1000 ng/ml [9].

Other clinical biomarkers used for the diagnosis of HCC are: microRNAs [10], des-gamma-carboxyprothrombin (DCP) [11], glypican-3 (GPC3) [12], proteomic profiling [13], and alpha-L-fucosidase [14].

Imaging has an important role in the diagnosis of HCC. Even if over the past decades the imaging technology has improved and the hepatic lesions are better characterized, detection of the small tumors continues to be difficult especially in patients with liver cirrhosis whose parenchymal architecture is abnormal. The most common imaging techniques used for evaluation of the liver parenchyma are as follows: ultrasound scanning (US), CT scan, MRI, and angiography.

Ultrasound scanning is the most used technique, and it is performed as a routine test for screening focal hepatic lesions. Ultrasound imaging has now been replaced in diagnosis by CT scan and MRI. Contrast-enhanced ultrasound (CEUS) uses contrast agents such as intra-arterial dioxide carbon and helium. Also, application of color Doppler sonography can be useful in the assessment of intrahepatic vascular flow and the Doppler of the portal vein can differentiate bland thrombus from tumor invasion.

Contrast-enhanced ultrasonography (*CEUS*) can offer information about the nature of the liver tumor which cannot be obtained with conventional ultrasonography. CEUS is safe, and it is

usually performed after detection of a focal lesion on standard US. The characterization of the hepatic lesion depends on all phases of contrast enhancement.

Most of the HCC are characterized by arterial phase enhancement and wash-out of the contrast during the late phase. According to some studies, more the differentiated a lesion is, the more gradually it is to washout [15, 16].

CEUS is an alternative for CT and MRI especially when there are contraindications for these investigations and it offers equivalent accuracy to CT and MRI if there is an experienced and skilled operator [17, 18].

CT scan is an important investigation for the characterization of the HCC. It includes 4 phases: pre-contrast, hepatic arterial phase, portal venous and delayed phases.

HCC must be differentiated from regeneration nodules, hemangioma, focal fat, dysplastic nodules and peliosis [19].

Factors such as injection of the contrast, tumor size and vascularity can affect the diagnostic accuracy of the HCC. In small tumors (less than 2 cm), the efficacy of CT is diminished due to the hypo-vascularization of small-sized tumors. The sensitivity of four phase CT in detecting HCC was up to 100% for tumors larger than 2 cm, 93% for tumors size between 1 and 2 cm and 60% for tumors less than 1 cm [20–22].

Multidetector helical CT (*MDCT*) is a new technique. which allows collection of early (18–28 s after administration of the contrast agent) and late or early parenchymal (35–45 s) arterial phase images. This new technique has improved the sensitivity and positive predictive values [23, 24]. Vascular tumors appear hypodense compared with liver parenchyma during the equilibrium phase (3–5 min after the administration of the contrast agent) and this technique is compared with MRI for early detection of small HCC (<1 cm) [24, 25].

Magnetic resonance imaging (*MRI*) is the best technique for evaluation of the liver nodules in patients with cirrhosis. HCC aspect varies on MRI because of the following factors: hemorrhage, degree of fibrosis and necrosis and histologic pattern. MRI is more accurate than CT or ultrasonography in detecting and characterization of HCC even for patients with liver cirrhosis. HCC appears hyper-intense on T2-weighted images while in T1-weighted images it may appear hypointense, isointense or hyperintense.

The sensitivity of MRI depends on tumor size, and it is about 95% in tumors larger than 2 cm and reduced to 30% for tumors, which are less than 2 cm in size [26].

Even if the MRI is the best investigation to characterize a liver nodule and to put the diagnosis of HCC, often the nodules might not be distinguished so a histological examination or advance imaging modalities will be necessary.

Angiography can be used to define hepatic anatomy before surgical resection.

Liver biopsy is performed with fine needle aspiration biopsy (FNAB) under ultrasonography or CT guidance and is considered the best method for a sure diagnosis of HCC. The sensitivity and specificity are about 96 and 95%, respectively, superior to any other test [27]. Sometimes, because the HCC lesions cannot be accurately located by radiographic methods, it is necessary

to perform open surgical biopsy. The most important complications are the risk of tumor spreading along the needle tract, estimated at up 3%, important bleeding or infectious complications [7] [28–30]. Contraindications for liver biopsy are platelet count <50,000 per mm^3 or the international normalizing ratio (INR) > 2 [7].

3. Diagnostic guidelines

According to European Association for the Study of the Liver (EASL):

- HCC lesions of greater than 2 cm in diameter can be diagnosed non-invasively in patients with cirrhosis based on radiographic criteria;
- Nodules with arterial hypervascularization in two imaging modalities or in only single imaging modality associated with values of AFP > 400 ng/ml in the cirrhotic liver is considered HCC [31];
- Evaluation of the liver nodules should be performed by US, CT and MRI; liver biopsy is not mandatory [32];
- EASL recommend repeated US every 3 months for lesions which are smaller than 1 cm, until it grows [31];
- Nodules between 1 and 2 cm in size are more likely to be HCC and confirmation by liver biopsy is recommended [33].

According to American Association for the Study of Liver Disease (AASLD):

- AFP > 200 ng/ml should lead to diagnostic suspicion of HCC and requires more investigation;
- Nodules <1 cm should be repeatedly imaged for up to 2 years;
- Nodules between 1 and 2 cm should be investigated with two techniques: CEUS, CT scan, MRI. If there is a hypervascularity with washout in the portal venous phase the lesion can be diagnosed as HCC [34];
- Nodules larger than 2 cm can be diagnosed as HCC with a use of only one imaging modality (arterial hypervascularity with wash-out in the early or delayed venous phase) [35];
- Liver biopsy is recommended if the vascular pattern is not characteristic for HCC on imaging modalities [34].

4. Stadialization

HCC staging is mandatory to select the appropriate primary and adjuvant therapy and to evaluate the prognosis. There are eight different staging systems available for the management of HCC but none of them are universally accepted. The currently available staging systems for

HCC include: pathologic tumor-node-metastasis (pTNM) [36], Okuda [37], Cancer of the Liver Italian Program (CLIP) [38] and Barcelona Clinic Liver Cancer (BCLC) [39].

BCLC staging system seems to be the best for selection of early-stage HCC that should benefit from orthotopic liver transplantation, hepatic resection or local ablation while the CLIP score may be more useful at stratifying patients who are not candidates for resection or transplantation.

5. Treatment

Nowadays, many of the patients with HCC are diagnosed at an early stage when there are no signs of an advanced cancer. In the past, most of the patients were diagnosed only when they became symptomatic and no treatment had a chance of being effective or to improve the survival rates. There are a number of treatments available which seems to improve the survival rates, but to achieve the best results a careful selection of the patients is needed. Liver transplantation is the best option treatment for the patients with solitary HCC in the setting of decompensated cirrhosis and for those with early multifocal disease (up to 3 lesions, none larger than 3 cm) [40, 41], while for the patients with solitary tumors in well-compensated cirrhosis the best treatment strategy is under debate [42]. Treatments which offer the best survival rate are surgical resection, liver transplantation, percutaneous ablation and transarterial chemoembolization [40, 43]. Systemic chemotherapy has been demonstrated that has no benefits on survival rates [44, 45], while agents like tamoxifen [43], anti-androgens [46] or octreotide [47] are completely ineffective.

Hepatic resection is the treatment of choice in non-cirrhotic patients who have been diagnosed with HCC (**Figure 1a** and **b**). Patients with cirrhosis have to be very well selected for surgical resection due to the high risk of postoperative liver failure which can lead to death after the surgery. Cirrhotic patients have a higher rate of decompensation if they are operated with right hepatectomy than if a left hepatectomy is performed; however, the 5-year survival rate after resection can exceed 50% [42, 48, 49]. Before the surgery there are some specific factors which need to be considered:

- Stage of the tumor;
- Size of the tumor;
- Presence/absence of a chronic liver disease and portal hypertension assessed clinically or by hepatic vein catheterization. If the upper endoscopy shows varices or diuretic treatment is necessary, the portal hypertension is severe and there is no need for catheterization of the hepatic veins;
- Quality and volume of the future functional liver remnant.

The most important causes of death after liver resections are postoperative hemorrhages, liver failure and sepsis, but all these complications have a lower incidence due to the improvements of the surgical techniques (Pringles maneuver), the development of ultrasonic dissectors and vascular staplers.

To perform a *right hepatic resection*, you have to mobilize completely the right lobe of the liver to have control on the right hepatic vein before the parenchymal transection. Sometimes the

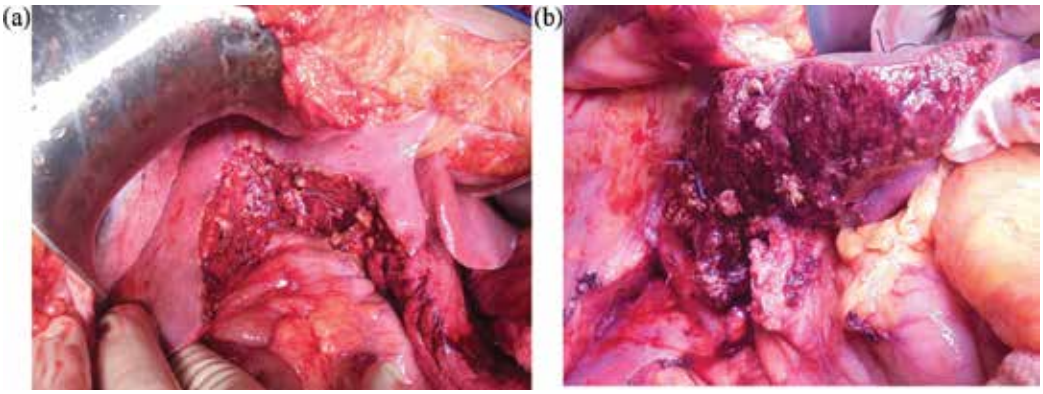

Figure 1. (a) Segment V resection. Intraoperative aspect after removal of the specimen (from the personal archive of the authors). (b) Right hepatectomy. Intraoperative aspect after removal of the specimen (from the personal archive of the authors).

size of the tumors does not permit to mobilize the right lobe of the liver and to expose the anterior surface of the inferior vena cava, so the surgeon has to perform an anterior approach. The anterior approach implies initial completion of parenchymal transection before mobilizing the right lobe of the liver and after hilar dissection is performed to control the right hepatic artery and portal vein. Intraoperative ultrasound is useful to mark on the Glisson capsule the plane of parenchymal transection. Transection is performed from the anterior surface of the liver down to right side of the liver hilum and down to the anterior surface of the inferior vena cava; then the right hepatic vein is isolated, clamped, divided and sutured. Only after the specimen is removed from the inferior vena cava, the right hepatic lobe is mobilized from the abdominal cavity by dividing the triangular ligament and other posterior attachments [50, 51].

Even if the anterior approach can be potentially dangerous because of the massive bleeding which can occur when deeper plane of the parenchyma is transected, it is an effective alternative when difficulty is encountered during liver mobilization using the conventional technique [52].

One of the most important factors which can lead to recurrence are microportal invasion and intrahepatic metastasis, these being associated with a poor prognosis. Anatomic resection implies the systematic removal of a hepatic segment or segments bearing the tumors (**Figure 2**). This technique has been shown to be effective in eradicating intrahepatic metastasis of HCC and it is associated with a prolonged survival. From the oncological perspective, anatomical resections which include satellite lesions are more efficient than limited resections without a surrounding margin [53].

Laparoscopic liver resection was initially used for non-anatomic liver resection for peripheral benign tumors (**Figure 3**), but nowadays, with the development of instrumentation and techniques, it has become a safe and feasible option for both benign and malignant liver lesions [54]. Regarding the advantages of laparoscopic liver resection, there are some advantages comparing with conventional liver resection such as reduced postoperative pain, less blood loss, less operative morbidity and a shorter length of hospitalization, while the long-term outcomes are similar especially for cirrhotic patients [55, 56].

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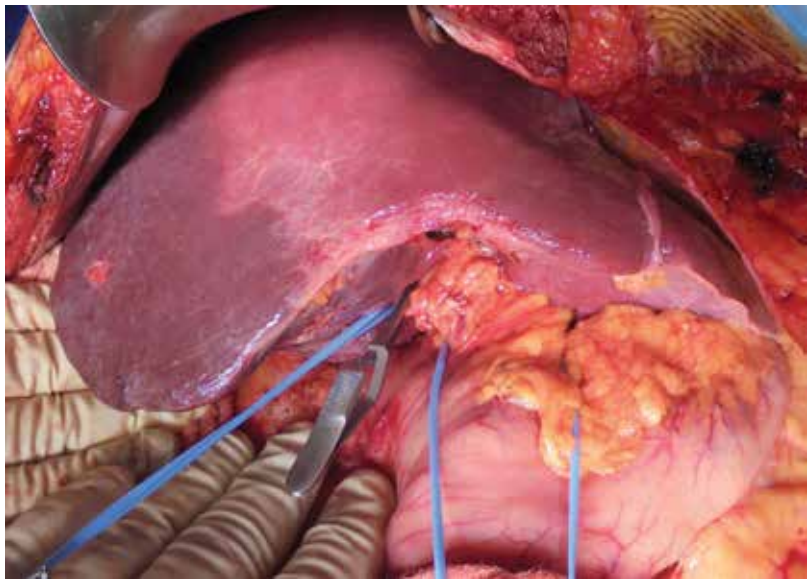

Figure 2. Anatomical resection of the VI–VII segments. Delimitation of the transection line after clamping the VI–VII pedicle (from the personal archive of the authors).

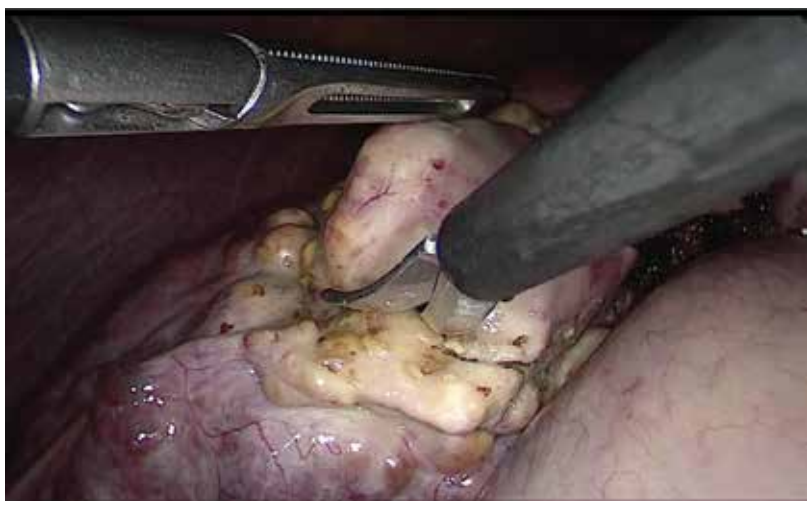

Figure 3. Laparoscopic liver resection of a HCC nodule (sg V) (from the personal archive of the authors).

Most of the patients with HCC are not suitable for the surgery due to the extent of the disease and because there is a high risk of liver failure. However, the patients with HCC who undergo surgery have a high risk of recurrence. The 5-year recurrence rate is about 77–100% and the median survival after the recurrence is between 7 and 28 months [57].

Predictors factors for the poor outcomes in HCC are the same for all therapeutic methods and they are: more than three tumors, tumor larger than 5 cm, portal vein invasion, intrahepatic metastases, absence of a tumor capsule, advanced TNM stage (III or IV), hepatitis C viral infection, and Child-Pugh class C [58, 59].

Liver resection may be used before the liver transplantation in three situations:

- Resection is used as primary treatment and liver transplantation will be an option for patients who develop liver failure or recurrence of the tumor;
- Resection is used as an initial treatment for patients who may undergo for liver transplantation according to detailed examination of the history pathological examination;
- Resection is used as pre-treatment for the patients which are already enlisted for liver transplantation.

Liver transplantation is the best treatment option for patients diagnosed with HCC and cirrhosis Child-Pugh B and C. The Milan criteria [60] are a generally accepted set of criteria used to assess suitability in patients for liver transplantation with cirrhosis and HCC. These criteria are:

- single tumor with diameter ≤ 5 cm, or up to 3 tumors each with diameter ≤ 3 cm;
- no extra-hepatic involvement;
- no major vessel involvement.

Living-donor liver transplantation is a liver transplantation option which has developed over the last years due to the limited availability of deceased-donor organs and can be offered for patients with HCC if the waiting time is long enough to allow tumor progression leading to exclusion from the waiting list [61]. This technique uses the right or left hemiliver from a healthy donor and should be performed by expert surgeons to ensure the lowest morbidity and best outcome. Complications may appear in 20–40% of the donors, while the mortality risk for the donor is still 0.3–0.5% [62].

One of the main problems after the liver transplantation for HCC is the risk for recurrence of the tumor which occurs in 8–20% of the patients. Usually, the recurrence appears in the first 2 years after liver transplantation and is associated with a median survival less 1 year [63].

For better results of the liver transplantation, there are some treatment options which can be performed before liver transplantation, such as liver resections or alcohol injection, radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and transarterial radioembolization/selective internal radiotherapy (TARE/SIRT). The main purpose of this treatment strategy is to reduce the size and number of the tumors in patients who do not have the accepted criteria for liver transplantation [64]. Most of the above techniques have been used as locoregional therapy for HCC recurrence in patients with limited disease.

Ablative techniques are useful for patients, which are not suitable for resection or liver transplantation. Ablation can be done percutaneous, in open surgery or by laparoscopic approach and its purpose is to destruct the tumor cells by modifying the local temperature. The efficacy

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of the percutaneous ablation is evaluated after 1 month with a CT scan (absence of the contrast uptake within the tumor reflecting tumor necrosis, while the persistence of contrast uptake indicates treatment failure) [31]. Recurrence rate is higher after ablation and the recurrence will occur nearby of the treated nodule due to the presence of microscopic satellites. Ablation must be performed under ultrasound guidance (**Figure 4**). Ablation techniques use chemical substances (ethanol, acetic acid, boiling saline) or surgical devices which modify the temperature of the tissue (radiofrequency, microwave, laser and cryotherapy).

Ethanol injection is highly effective for small HCC and has a low rate of complications, while the necrosis rate is about 90–100% of the HCC smaller than 2 cm. If the tumor size is between 2 and 3 cm, the necrosis rate is reduced to 70 and 50%, respectively, for the tumor size between 3 and 5 cm [65, 66]. Patients with Child-Pugh A class and HCC with successful tumor necrosis can achieve a 50% survival at 5 years [67, 68].

Radiofrequency ablation (RFA) is an option of treatment which has better result than ethanol injection and requires fewer treatment sessions [69, 70]. This type of treatment requires an insertion of single or multiple cooled tip electrodes or single electrodes with j-hooked needles that deliver heat around a wide region inducing necrosis of the tumor. This treatment is more efficient than ethanol injection but has a higher cost and a higher rate of complications such as

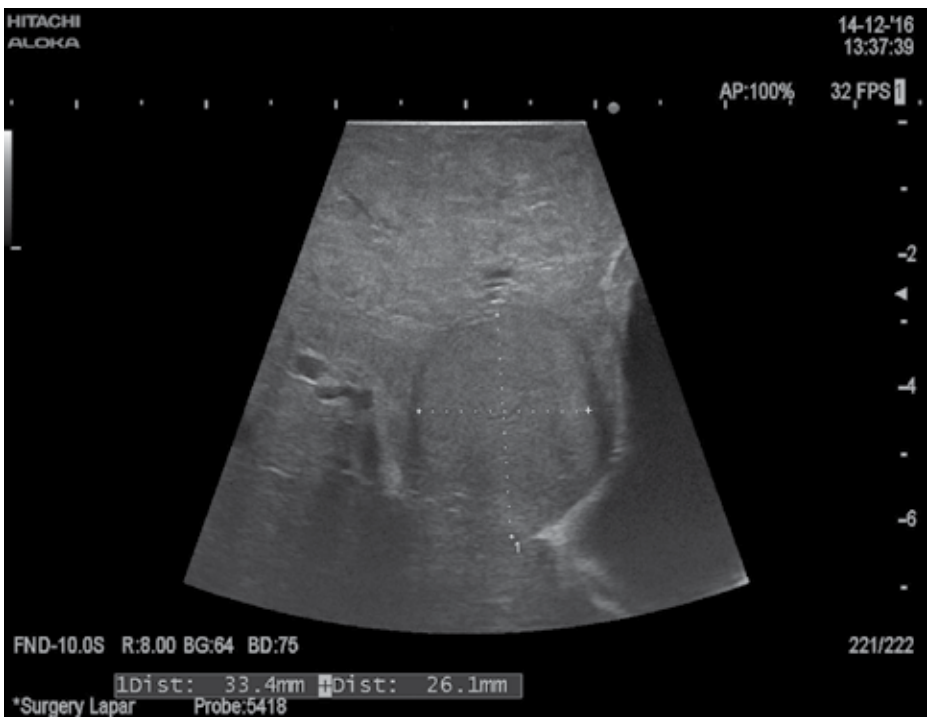


Figure 4. Intraoperative laparoscopic ultrasound of the liver showing a HCC nodule in segment V, next to the gallbladder (from the personal archive of the authors).

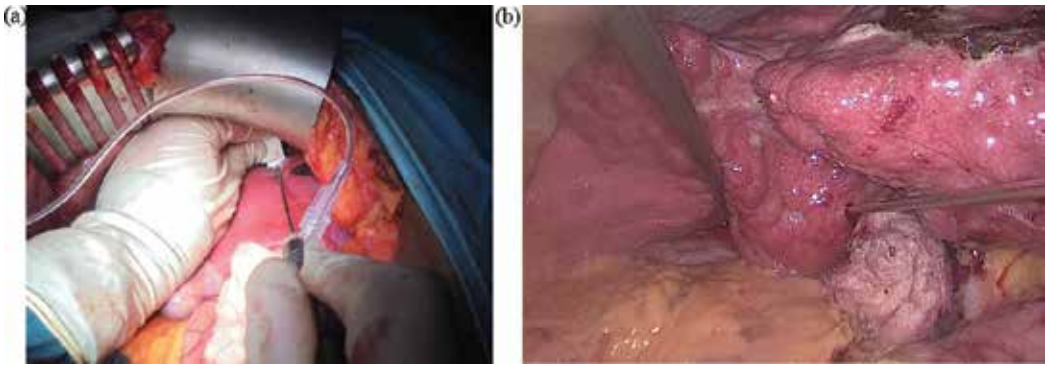


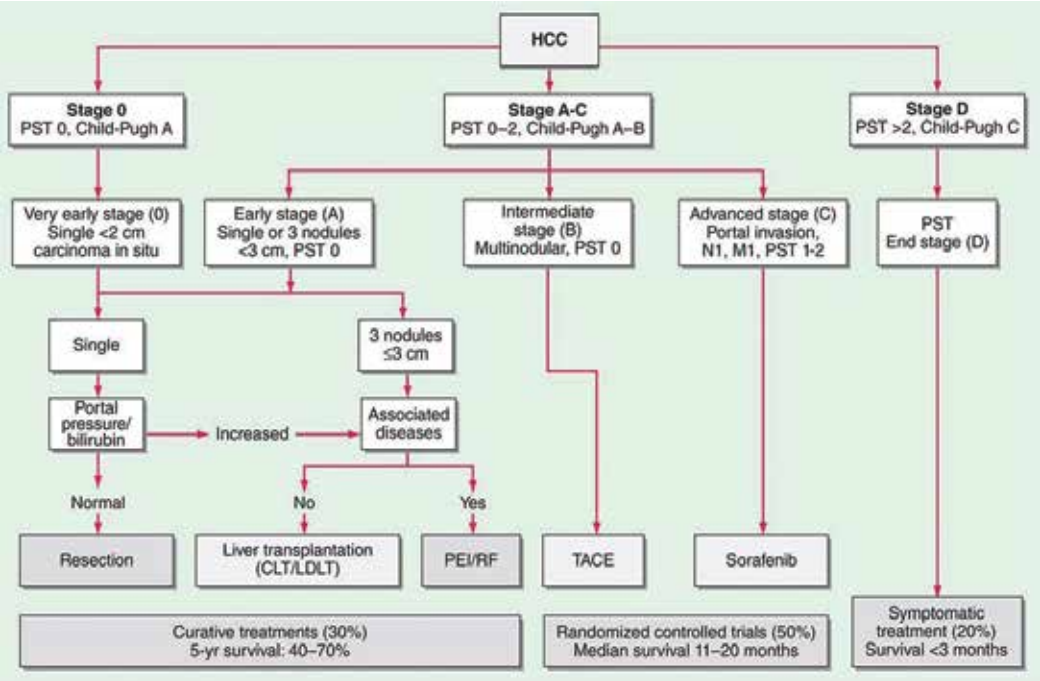
Figure 5. (a) US-guided intraoperative RFA of a liver tumor (from the personal archive of the authors). (b) Laparoscopic RFA of a HCC nodule. Intraoperative aspect (from the personal archive of the authors).

peritoneal bleeding or pleural effusion [69–72]. RFA can be performed by percutaneous under ultrasound guidance, open surgical approach or laparoscopic approach (**Figure 5a** and **b**). Some of the HCC cannot undergo for RFA due to their localization.

Transarterial embolization and chemoembolization are types of treatment which have developed over the last years because HCC exhibits intense neo-angiogenic activity and should be considered for patients who are not suitable for surgical resection or percutaneous ablation [61]. In patients with early-stage HCC, the blood supply comes from the portal vein and only when the tumor is larger it has an arterial blood supply from hepatic artery. This treatment purpose is to obstruct the hepatic artery to induce ischemia to the tumor. Hepatic artery obstruction is performed during an angiographic procedure and is known as transarterial embolization (TAE). If the transarterial embolization (TAE) is associated with the injection of chemotherapeutic agents in the hepatic artery, the procedure is known as transarterial chemoembolization (TACE). The procedure needs advanced catheterization of the hepatic artery and the specific lobar and segmental branches to be as selective as possible and to reduce the damages of the nontumoral liver parenchyma. Chemotherapic agents such as adriamycin or cisplatin must be injected prior to arterial obstruction [73]. Contraindication for TAE/TACE is the lack of portal blood flow due to portal vein thrombosis, portosystemic anastomoses or hepatofugal flow [61]. Also, patients which advanced staged disease (Child-Pugh B and C) should not be considered for this treatment due to the high risk of hepatic failure. Side effects of intraarterial injection of the chemotherapeutic agents are nausea, vomiting, alopecia and sometimes, renal failure. After the transarterial embolization, the so-called post-embolization syndrome can appear, which consists of fever, abdominal pain and ileus. Post-embolization syndrome is usually self-limited in less than 48 hours, but sometimes patients can develop hepatic abscess or cholecystitis. Regarding the response to this treatment there are no significant differences between TAE and TACE, the reported rate of objective response ranging from 16 to 60%, with a significant improvement in survival [43, 73].

Treatment algorithm is described in the next figure based on the Barcelona Clinic Liver Cancer (BCLC) staging classification [74]:

http://dx.doi.org/10.5772/intechopen.75164



6. Perspectives

There are several areas where active research is needed, starting from molecular pathogenesis, to detection, diagnosis and treatment. Despite recent progress in the management of HCC, treatment of patients with portal vein thrombosis remains still a challenging area. Current clinical guidelines recommend Sorafenib only. However, besides Sorafenib, various therapies including surgery, TACE, external radiation therapy, hepatic artery infusion chemotherapy (HAIC) and radio-embolization may be considered in selected patients; the usefulness of combined treatment needs to be verified. Newer therapeutic options such as immunotherapeutic agent and oncolytic virus are under investigation [75].

7. Conclusions

Management of HCC continues to be improved due to development of newer therapies which are combined with liver resection and liver transplantation. These therapies become better tolerated and more precise even in patients with advanced liver disease. Better surveillance of cirrhotic patients allowed an early detection of HCC and permitted treatments to have a higher rate of cure. For the patients who present with HCC and moderate to severe liver insufficiency, liver transplant remains a critical method to eliminate the cancer and cure the underlying liver disease with a lower risk of recurrence than resection or ablation. The best results for liver resection are obtained in patients with small solitary tumors, but there is a high rate of disease

recurrence due to cell dissemination prior to treatment. Improved survival for patients treated with Sorafenib for advanced disease increases enthusiasm for additional therapies for HCC.

Nowadays, the improvement of the surveillance will allow detection of the early stage of HCC when the loco-regional treatment is effective and transplantation is reserved only for selected cases. Alfa-fetoprotein and ultrasound scan should be used every 6 months for surveillance in high-risk individuals.

Acknowledgements

Adrian Bartoș is the coordinator of this chapter.

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Liver Transplantation

Chapter 8

Liver Transplantation in Acute Liver Failure: Indications and Outcome

Rocío González Grande and Miguel Jiménez Pérez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72664

Abstract

The term acute liver failure (ALF) refers to the acute (<26 weeks) and severe worsening in liver function associated with encephalopathy in a person with no underlying chronic liver disease. ALF constitutes a critical clinical syndrome that is potentially reversible but has a very variable prognosis. No specific treatment is available, and liver transplantation (LT) is the treatment of choice in many cases. However, the challenge remains of identifying those patients with a poor likelihood of spontaneous recovery of liver function and for whom the indication and time of LT in order to guarantee survival (based on identification of prognostic factors) need to be established. In Europe, 8% of LT are due to ALF. Although the results of LT due to ALF have improved over recent years, they are still far from those seen after elective LT.

Keywords: liver transplant, acute liver failure, prognostic score, outcome

1. Introduction

Acute liver failure (ALF) is defined as the presence of acute liver injury, that is, a rise in transaminases at least three times the upper limit of normal, jaundice, and coagulopathy, together with the onset of encephalopathy in a person with no previous liver disease [1]. Exceptions to this definition include the acute onset of Wilson disease, autoimmune hepatitis, and the Budd-Chiari syndrome, as well as reactivation of the hepatitis B virus (HBV) [2].

Though no consensus exists on the severity of the coagulopathy or the encephalopathy marking the transition from acute liver injury to ALF, an INR ≥1.5 and any degree of encephalopathy are generally accepted [3]. Clinically, ALF is classified according to the interval between the onset of jaundice, considered as the initial symptom, and the encephalopathy. The ALF is considered to be hyperacute if the encephalopathy appears within

7 days of the jaundice, acute if it appears between 8 and 28 days, and subacute when it appears beyond 28 days [4]. The disease is considered chronic if it has a history of more than 26 weeks.

Table 1 summarizes the causes of ALF. Worldwide, infection is the most common cause, though in developed countries drug-induced hepatic injury is responsible for up to 50% of cases. As many as 30% of cases are of unknown etiology [5].

The main causes of death due to ALF are infection and decerebration from cerebral edema. The medical management of patients is based on support measures, early identification, and treatment of complications until spontaneous recovery of liver function or liver transplantation (LT) [6].

Establishing the indication for and time of LT in a patient with ALF should be as precise as possible in order to avoid, on one hand, unnecessary risks for a recoverable patient and on the other an increased likelihood of death associated with a delay in transplantation.

Viral
HAV, HBV, HCV, HEV
CMV, EBV, HSV, VZV, dengue
Pharmacologic/toxic
Paracetamol (acetaminophen)
Idiosyncratic drug reaction
Amanita phalloides
Vascular
Budd-Chiari
Ischemic hepatitis
Pregnancy
Preeclampsia
HELLP
Fatty liver of pregnancy
Others
Wilson disease
Autoimmune hepatitis
Lymphomas and other neoplastic diseases
Hemophagocytic lymphohistiocytosis
Cryptogenic

Table 1. Etiology of the acute liver failure.

http://dx.doi.org/10.5772/intechopen.72664

2. Indication for liver transplantation in acute liver failure

Liver transplantation in ALF has represented an inflection point in the survival of affected patients, who previously suffered a mortality rate of almost 85% in the pretransplant era [7].

Indicating LT too soon involves the possibility of performing the transplant in patients who may still experience spontaneous recovery with complete liver function, thereby adding the risks associated with an urgent transplant and lifelong immunosuppression, in addition to the waste of a valuable organ. However, delaying the decision to transplant in patients with ALF can increase the risk of infection, irreversible brain damage, multiorgan failure, or even death. Accordingly, selection of ALF patients who need LT should be based on the early identification of factors predicting a poor clinical outcome, as well as application of prognostic models combining different parameters. Unfortunately, the prognostic models available have certain limitations, low sensitivity and specificity, and worse predictive value than desired [8].

2.1. Predictive factors

The etiology is considered a predictive factor. Around 60% of patients with ALF due to paracetamol intoxication, hepatitis A, ischemic hepatitis, or pregnancy may survive with no need for transplantation, whereas only 30% of cases with drug-induced liver injury, autoimmune hepatitis, and various cases of unknown etiology achieve spontaneous recovery [9].

The duration of symptoms has traditionally had a prognostic value. The subacute presentation of ALF is associated with a worse prognosis than acute and hyperacute ALF, though these differences are probably conditioned by the etiology of the subacute failure [10].

Encephalopathy, although it forms part of the definition of ALF, should also be classified. Patients with grades 1–2 encephalopathy have an excellent prognosis, whereas grades 3–4 encephalopathy is associated with a low likelihood of spontaneous resolution [11] and is thus criteria for admission to the intensive care unit, with a recommendation to measure the intracranial pressure as a marker of the preservation of brain perfusion [12]. Coagulopathy, as a direct indicator of liver function, is considered to predict the severity. Generally measured using the prothrombin time (PT) or the International Normalized Ratio (INR), a PT over 90 s or an INR >4 is associated with a mortality rate above 90% [13]. Factor V levels <20% in patients younger than 30 years and levels <30% in those older than 30 years indicate a worse prognosis.

The histologic findings have also been proposed as predictors of the outcome and the likelihood of spontaneous recovery. Though some series have related the risk of death with the presence of >50% hepatocyte necrosis, the little representativity of the samples together with the risk involved in performing a liver biopsy in patients with coagulopathy generally advise against routine histologic study in patients with ALF, and nor should the decision to transplant be based on biopsy findings. A liver biopsy could be indicated in cases of diagnostic doubt, especially to rule out neoplastic causes, which contraindicate LT [14]. Other factors, such as age, body mass index, serum bilirubin, creatinine, hypoglycemia, lactate levels, and pH changes, can also be considered determinant.

All these factors help to identify patients with ALF who have a worse prognosis. Recently, the EASL established recommendations for the early transfer to transplant centers of patients with ALF if they fulfill the following criteria [15]:

- ALF due to paracetamol or hyperacute causes:
  - Arterial pH <7.3 or HCO_3 <18
  - INR >3.0 day 2 or >4 thereafter
  - Oliguria and/or elevated creatinine
  - Altered level of consciousness
  - Hypoglycemia
  - Elevated lactate unresponsive to fluid resuscitation
- Non-paracetamol:
  - Arterial pH <7.3 or HCO3 <18
  - INR >1.8
  - Oliguria/renal failure or Na <130 mmol/L
  - Encephalopathy, hypoglycemia, or metabolic acidosis
  - Bilirubin >300 μmol/L (17.6 mg/dL)
  - Shrinking liver size

2.2. Prognostic models

The currently available prognostic models should be applied continuously during the follow-up and clinical management of patients with ALF, even though they are not universally accepted or their recommendations established.

In 1989, the King's College Hospital criteria (KCC) were established (**Table 2**) [16]. These are based on cohort studies and are widely used at the present time. They are based mainly on the etiology, differentiating between ALF secondary to paracetamol and ALF of other causes. They are highly specific, that is, patients who fulfill these criteria have a high likelihood of death if they do not undergo LT. However, their sensitivity is low, as seen from the death of patients who do not meet the criteria, especially in patients with causes other than paracetamol [17]. A meta-analysis found a specificity of 82% for etiologies other than paracetamol and 92–95% for causes related with paracetamol. The sensitivity was about 68%. Both sensitivity and specificity increase if the criteria are applied dynamically [18]. In an attempt to improve the predictive value of the KCC, the measurement of lactate as an indicator of tissue dysfunction and failure of hepatic clearance has been added to the criteria in the UK. This is particularly useful in cases of paracetamol toxicity. An admission arterial lactate >3.5 or >3 mmol/L after fluid resuscitation is a marker of poor prognosis [19, 20].

AFL due to paracetamol
• Arterial pH <7.3 after resuscitation and 24 h since ingestion
• Three following criteria:
• Hepatic encephalopathy grades 3–4
• Serum creatinine >300 mol/L (3.4 mg(dl)
• INR >6.5
ALF not due to paracetamol
• INR >6.5
• Three out of five following criteria:
• Etiology: indeterminate etiology hepatitis, drug-induced hepatitis
• Age <10 years or >40 years
• Interval jaundice encephalopathy >7 days
• Bilirubin >300 mol/L (17 mg/dl)
• INR >3.5

Table 2. King's College criteria.

The Clichy criteria, established in 1986, also derive from cohort studies in patients with fulminant hepatitis B (**Table 3**) [21]. Validation studies found less accuracy than for the KCC, with a positive predictive value of 89%, but a negative predictive value of 36%. They are, therefore, very deficient for identifying potential survivors without a LT [22].

The MELD score (**Table 4**), adopted by the United Network for Organ Sharing (UNOS) and The Organ Procurement and Transplantation Network (OPTN), has been validated as a predictor of short-term mortality in patients with hepatic cirrhosis. Retrospective studies have shown that the MELD score has a similar predictive value to the KCC for AFL-associated mortality [23]. In the USA, the prospective data from the Acute Liver Failure Study Group (ALFSG) showed that a MELD >30 in patients with ALF due to paracetamol has a negative predictive value of 82%, such that patients with a MELD <30 have a high likelihood of survival without a LT and with a slightly lower score in cases not related with paracetamol [24].

• Confusion or coma (hepatic encephalopathy 3–4)
• Factor V <20% of normal if age <30 years or factor V <30% if age >30 years

Table 3. Clichy criteria.

$9.57 \times \log^{e(\text{creatinine})} + 3.78 \times \log^{e(\text{bilirubin})} + 11.2 \times \log^{e(\text{INR})} + 6.43$

Table 4. MELD (MELD calculator).

In an attempt to improve the prognostic accuracy in ALF patients, other indicators of liver dysfunction have been suggested, such as measures of hepatic metabolism with markers labeled with indocyanine green [25], as well as predictive models of mortality used in other clinical situations. The APACHE II system, designed to predict mortality in intensive care patients, has also been applied in ALF patients, but no cut point has been set demonstrating that it is superior to the KCC and nor can it be applied early on [26].

A prognostic index designed by the ALFSG included variables at the time of presentation of the condition, such as bilirubin, encephalopathy grade, INR, phosphorus, and serum levels of M30 (a direct marker of hepatocyte apoptosis). Although the prognostic value of this index was greater than the KCC and MELD score, measurement of M30 is not generally available [27].

Comparison between these different models, which share some parameters, has found no superiority of one over the others, and no universal recommendations have been established. It is, however, accepted that ALF should be strictly assessed at the reception center and, if the patient meets the criteria for a poor prognosis, they should be referred as soon as possible to a transplant center where the available predictive models can be applied dynamically, mainly the KCC and Clichy criteria, to determine the indication for LT. American and European series show that 50% of patients admitted with ALF receive a LT [28]. Once the indication for a transplant has been made, the patient is included on the active list, in most countries with a higher priority than patients with other indications, thus ensuring an early transplant, usually within days of being placed on the list.

If a donor organ becomes available, the situation of the patient should be reassessed by the transplant team, in order to identify a likely clear improvement after transplant or else an absolute contraindication for transplant, mainly the presence of irreversible brain damage.

3. Outcome of liver transplant in acute liver failure

In Europe LT due to acute or subacute liver failure accounts for some 8% of LT. Patient survival after LT for this reason is 79, 71, 69 and 61%, at 1, 3, 5 and 10 years, respectively [29]. This survival rate is slightly lower during the initial years (first and third) than LT for other reasons but then becomes similar. Most deaths occur between the first and third years posttransplant due, mainly, to neurologic complications and sepsis [30]. Some centers have reported survival rates of up to 86% [31]. Overall survival is probably greatly influenced by patient age, with data from the European Transplant Registry showing 1- and 5-year survival of 51 and 42% in patients older than 60 years [29].

3.1. Factors influencing the results

Multiple factors have been associated with the outcome of patients who receive a LT due to ALF. Three studies [4, 32, 33] have identified a recipient age above 45–50 years as a poor prognostic factor, attributing this to the reduction in physiologic reserve with effect from these ages [4]. A body mass index (BMI) >29 was identified in one study [32]. On the other hand, no specific factor associated with the severity of the ALF, such as coagulopathy, has been found to be associated with a poor prognosis, although the degree of kidney failure, mechanical ventilation, and the use of inotropic drugs were found to be predictive factors in these studies.

Such donor characteristics as age >60 years, ABO incompatibility [34, 35], and the use of a split or small liver have also been related with worse results [18, 36].

Survival has improved greatly over the last decade. This is the result of better management of ALF patients, leading to a lower incidence of pretransplant complications (e.g., renal failure, respiratory problems, sepsis), a lower grade of encephalopathy, and the use of more isogroup grafts. Identification of prognostic factors as well as the creation of transplant indication criteria like the Clichy [37] or King's College [16] criteria has also contributed to this improvement. The earlier indication for transplant, which in turn contributes to the use of more compatible organs and the patient receiving the transplant in better conditions, has been the foundation for the improvement in results over recent years.

The rapid localization of organs for transplant in ALF patients is an important factor that has also contributed greatly to the better results. In countries like Spain, with a high donation rate, it proves relatively easy to find a compatible organ fairly quickly, with 50% of these patients receiving a transplant within 24 h of becoming active on the waiting list, while the mean time to transplant is 40 h. In Spain this has resulted in only around 7% of ALF patients dying while still on the waiting list compared with 30% in the USA [38].

Thus, the optimal selection of candidates for transplant plus the identification of poor prognostic factors and the exclusion of those patients who will not benefit from LT due to their situation have contributed to the improved results. The development of extracorporeal bioartificial systems, improved organ procurement, and the use of organs from living donors can all contribute to future improvements.

4. Conclusion

Acute liver failure is a potentially severe clinical condition that is associated with a high rate of mortality. Selection of those patients who will benefit from a liver transplant should be based on the early identification of prognostic factors. Survival of patients who receive a transplant due to ALF has improved over recent years, though it is still somewhat lower than that of patients who receive a LT for other reasons.

Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 9

Trends and the Current Status of Living Donor Liver Transplant

Ramesh K. Batra and David C. Mulligan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74818

Abstract

The need for liver transplant and its timely nature are both equally vital for a patient with end stage liver disease. But the ever-growing need for liver transplant across the entire world threatens the two reasons that justify its very existence. The popularity of living donor liver transplant has met great enthusiasm amongst the transplant physicians and surgeons, as it is timely, and also yields superior survival benefit as compared to a deceased donor liver transplant. Living donor liver transplant has been constantly adapting to meet the needs of patients and the expanding wait list. The need for a living donor liver transplant is not the same amongst the various parts of the world, because the population and the disease burden is different. We looked at the trend of living donor liver transplant across the world and also the change in practices over time including a glimpse of what lies ahead for the next decades.

Keywords: living donor liver transplant, deceased donor liver transplant, graft recipient weight ratio, small for size syndrome, United Network for Organ Sharing

1. Introduction

Liver transplant is the gold standard treatment for end stage liver disease (ESLD). This also, being the most viable option for ESLD patients, as there is no bridging dialysis unlike end stage renal disease (ESRD). ESLD patients therefore succumb to their disease in absence of a timely liver transplant. In addition to the disease burden, the expanding list of waitlisted patients and the relative shortage of deceased donor livers have subdued the attempts to bridge the gap between the need v/s availability of deceased donor livers for transplant. Therefore, to alleviate death on liver transplant waiting list, the donor organ pool was expanded by living liver donation in the year 1989. In the decades to follow, the success of

living donor liver transplantation (LDLT) and the growing burden of the waiting list, boosted a revolutionary momentum amongst transplant physicians around the world, offering living donor liver transplants for their waiting patients. But, the momentum of living donor liver transplant was variable in different regions across the world. There were many factors that contributed to this variability, the main ones being, (1) availability or access to deceased donor organs, (2) etiological factors of liver failure and (3) individual surgical practices. The latter pertains to the evolutionary changes of experience within the LDLT centers. The learning curve of LDLT for the donor operation was relatively short given the previous experience of hepatectomies for liver cancer, yielding an expeditious expansion of the living donor liver pool.

2. History

The revolutionary success of liver transplant at the University of Colorado in 1967, under Dr. Thomas Starzl and colleagues [1] came after seven unsuccessful attempts of the team. Although, this was a considerable technical milestone for transplantation, but the survival after liver transplant at 1 year was dismal, 28.8–50% [1]. But after the discovery of cyclosporine by Sir Roy Calne, its use in liver transplant paved the path for a modern era of liver transplants with survival rates at 1 year rising to 78.6% [1]. The success of whole organ deceased donor liver transplant further led to another technical milestone of split liver transplant from a deceased donor in Germany [2]. Soon to follow was the success of living donor liver transplant at the University of Chicago in 1989, by Christopher Broelsch and colleagues. Around the same time, LDLTs in Australia, Brazil and Japan were successfully performed [2], but as an emergency procedure. But, all these pioneered LDLTs had one thing in common, that they were in-fact pediatric liver transplants utilizing the left lateral segment grafts from the donor liver, and as the LDLT experience grew, various anatomical split liver transplants for LDLT were performed with promising results for both adults and children. As the individual experience of transplant centers grew over the learning curve of at least 15 LDLTs, this allowed to broaden the acceptance of donor candidates for a safer donor surgery, whilst also expanding the acceptance of LDLT to benefit more recipients. Over the years, this has greatly helped reduce death on liver waiting list, whilst also allowing friends and family members to actively participate in the well-being of their loved ones by sharing a section of their healthy liver.

3. Liver transplant wait list mortality and MELD score

Wait-list mortality for liver transplant patients is variable across the world. This is mainly due to the variability in access to organs and etiological factors of liver disease. Wait-list mortality, therefore can be as high as 50% in Asia [3] and about 10% in Australia [4]. In United States, over the last two decades, it has averaged at 10% [5].

In US, with the introduction of model for end-stage liver disease (MELD) by United Network for Organ Sharing (UNOS) in 2002, livers are allocated based on MELD score using serum

bilirubin, creatinine, sodium, and INR. According to the policies set forth by UNOS, certain subsets of the wait-listed patients qualify for exception points to this calculated physiologic MELD score. This allows for a preferential access to the allocated liver allografts. This helps minimize death on waiting list for patients who are much sicker than it is captured with their calculated physiologic MELD score or have disease processes that will progress but not cause immediate mortality, which includes patients with hepatocellular carcinoma.

Hepatocellular Carcinoma (HCC) is the leading cause of cancer mortality in the world [6], with overall 1 year survival rates below 50% and five-year survival as low as 10% for advanced disease [7]. Surgical resection definitely improves survival in certain HCC cases. However, many patients have unresectable disease or are cirrhotic, which dampens the survival benefit of resection in such cases [8]. For these subset of patients, liver transplantation has delivered excellent survival benefit, compared to resection [9–11]. Therefore, the survival advantage of liver transplant over other treatment options for HCC patients supported their inclusion to the MELD exception policies of UNOS. The MELD exception points therefore allowed for earlier liver transplantation of the qualified patients, thereby reducing the wait-list mortality to 4.49% for patients listed with MELD exception points. However, the preferential allocation and subsequent liver transplant came at the cost of a significantly higher waiting list mortality of 24.6% for patients without MELD exceptions [12]. This raises an ethical controversy of unequally sharing the public resource of deceased donor organ pool. Although the aim of MELD based allocation, was to objectively quantify the sickness of a cirrhotic patient allowing a better way of resource allocation. However, the disadvantage of certain factors causing immediate mortality that were not measured by the MELD score, like bleeding complications and encephalopathy, unknowingly advantaged the MELD exception group. This led to higher wait-list mortality (24.6 VS 4.49%), higher mean waiting time (180% higher) and a lower transplant rate (40% vs. 79%) for patients without MELD exceptions [12].

4. The need for LDLT

For the western world, LDLT is a viable alternative to bridge the gap between the need for liver transplant and the limited deceased donor liver allografts. It therefore allows for better resource allocation by increasing the total donor organ pool. However, for the eastern world, LDLT serves as the lifeline, as it provides for the majority of liver transplants (**Figure 1**) [13]. This is due to the disproportionately low number of deceased donors in the East vs. the West. In the year 2000, there were 0.07–6.5 deceased donors per million populations in Asia, whereas 35.1 and 25.2 deceased donors per million population in Spain and the US respectively (**Figure 2**) [13]. The lack of deceased donation rates in the eastern world has been largely crippled by the cultural and religious barriers of the public, limiting acceptance of the concept, and therefore practice of organ donation for transplant [14, 15]. Due to these factors, Asia follows a much stricter organ allocation based entirely on MELD score with no exception points. Hence tumor progression due to the longer waiting time rendering a patient non-transplantable has been a grave concern. For these subset of patients, LDLT allows for a timely transplant to limit further spread of HCC and also providing another valuable resource and choice to patients waiting for a liver transplant.

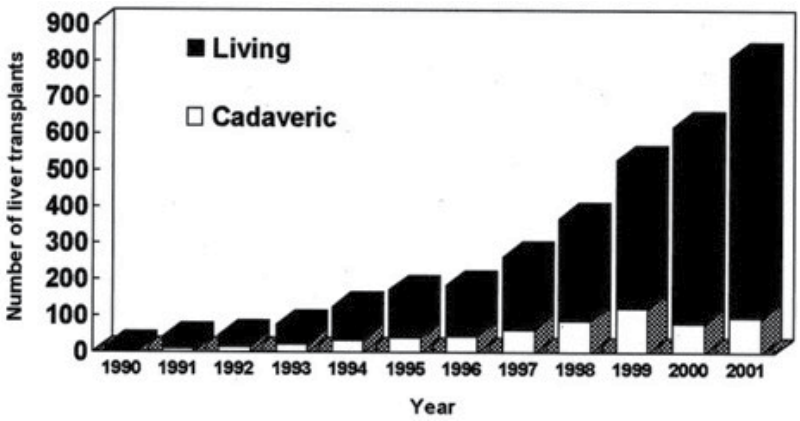

Figure 1. Comparison of annual deceased (cadaveric) and living-donor liver transplantation rates in Hong Kong, Japan, Korea, and Taiwan (pooled data) [13].

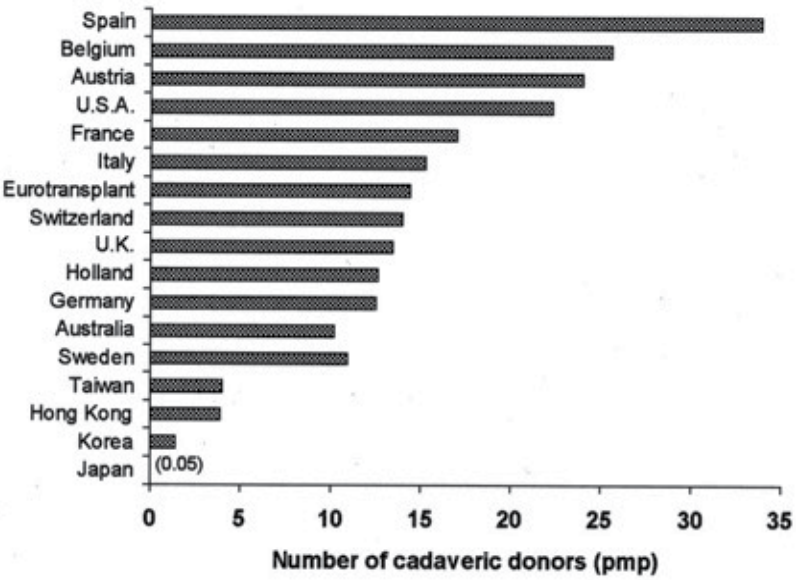

Figure 2. Number of deceased (cadaveric) donors per 1 million population (pmp) in different countries in the year 2000 [13].

5. Living donor liver transplant: statistics

In United States, until 1991, LDLT, an alternative to deceased donor liver transplant (DDLT) was offered at only one center. But by 2001, 67 transplant centers were offering LDLT for their patients. Its contribution to the US donor organ pool for livers started slow, but expanded

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exponentially within a decade. In 1991, the 22 LDLTs (0.75% of all liver transplants) at only one center in United States, rose to 524 total LDLTs (10% of all liver transplants) by the year 2001 [16]. This opportunity to offer LDLT for patients was met with much greater enthusiasm in Asian transplant centers, who had been suffering with low deceased donation rates due to the earlier discussed barriers. Asian transplant centers (comprising of Japan, Hong Kong, Korea and Taiwan) started with about 50 LDLT cases in 1999, but in less than a decade, rose to 1387 (>90% of all liver transplants) by 2005 [17], a much greater rise when compared to United States.

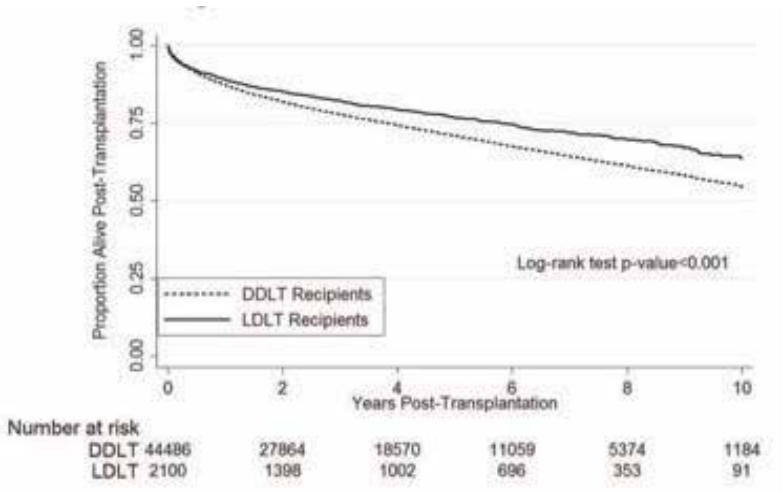

Figure 3. Post-transplant patient survival [18].

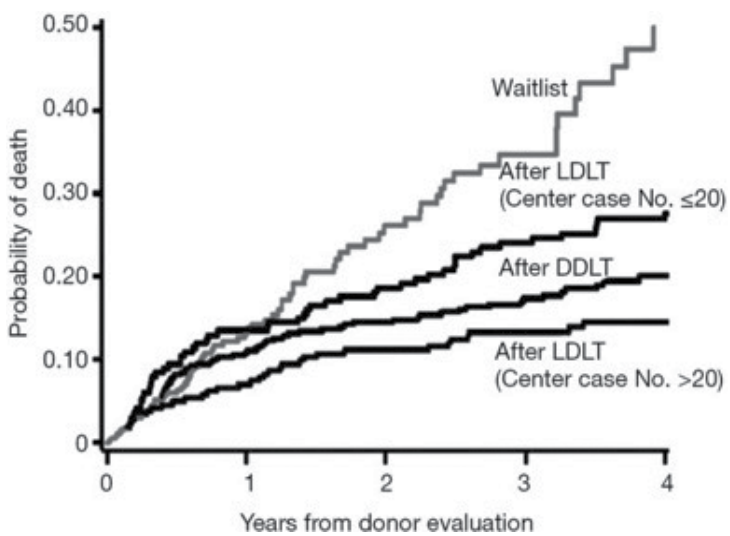

Figure 4. Cumulative risk of death whilst waiting for a liver transplant, compared to LDLT based on center experience and DDLT [19].

The encouraging growth in LDLT volume and its timed elective nature, yielded superior graft and patient survival when compared to DDLT (**Figure 3**) [18]. But it is noteworthy, the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) group in United States showed that a low volume LDLT transplant center (center case no. <20) had far poorer outcomes when compared to DDLT (**Figure 4**) [19]. This echoes the learning curve that follows a novel surgical technique but also the updation of the infrastructure that a transplant center needs during the initial experience.

6. Trend of living liver donors

As the experience grew with LDLT amongst the transplant surgeons, there was constant fine tuning of the technique for both the donor and recipient operations. The refinement of technique allowed for the donors who were once considered anatomically unfeasible to be much more openly accepted, further increasing the living liver donor pool available for transplant.

6.1. Donor age

The majority of living liver donors in United States are <50 year old patients. But in the three decades since the first successful LDLT in Chicago, the living liver donor pool for donors >50 years and also for >65 year old, has doubled (**Figure 5**), whilst reducing the percentage share of the living donor pool from <50 year old donors. This is largely due to the reduced no. of <50 year old live liver donors available, further complicating the increased burden on the liver transplant waiting list in United States. Therefore, this increased demand has somewhat driven the acceptance of extended age spectrum for living liver donors.

Increased donor age, i.e., above 50 years has profound negative impact on long term patient survival after a Deceased Donor Liver Transplant (DDLT) [21]. This is thought to be largely due to the poor tolerance of such liver allografts to longer cold ischemic times and the ischemia-reperfusion injury that follows. In LDLT too, donor age > 50 years negatively impacts recipient survival [22, 23], but not at a significant rate when compared to the DDLT experience with older donors. But the reduced patient survival is hard to explain, especially when LDLT has much shorter cold ischemic times which are significantly higher in a DDLT.

Liver allograft regeneration in LDLT has been shown to be impaired in older liver donor allografts [22], however this effect seems to disappear in a prospective study [24]. At a molecular level, activation of phosphorylated-Signal Transducer and Activator of Transcription 3 (p-STAT3) gene through signal transduction of cytokines protects hepatocytes from apoptosis and oxidative stress [25] and p-STAT 3 is under-expressed in LDLT from donors>50 years [25]. This imbalance of anti-apoptotic and anti-oxidative functions at the cellular level could explain the reduced graft and patient survival in living donor liver transplant from older donors.

Therefore, although LDLT is preferable from a younger donor, but LDLT from older donors, still confers a far superior survival advantage in comparison to waiting on the liver transplant wait-list.

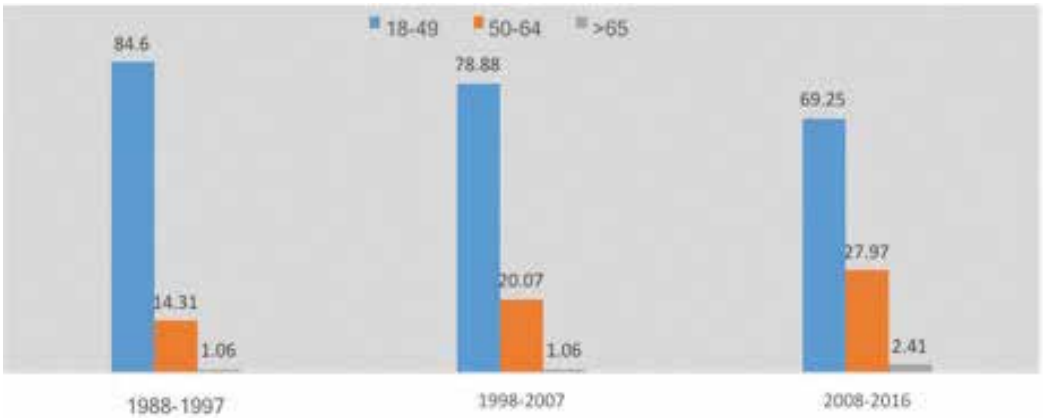

Figure 5. Age distribution of living liver donors in the last three decades in the US [20].

6.2. BMI and macrosteatosis

Hepatic steatosis marginalizes the quality of the allograft by compromising graft and patient survival in short and also long term [26, 27]. It is also associated with higher incidences of Primary Non-Function (PNF) [27] in DDLT. The positive predictive value of PNF after a DDLT based on a deceased donor liver biopsy can be as high as 90% [28], therefore transplant centers when accepting donor livers>30% macrosteatosis on liver biopsy are cautious, and control other variables that can jeopardize the outcomes, like cold ischemic time and warm ischemic time [29]. But, such detailed causal relationship of hepatic steatosis with PNF has not been well studied in LDLT. Furthermore, to establish the true level of hepatic steatosis, a liver biopsy is needed which carries although low but un-needed risks for the living donor, i.e. 5% risk of serious complication and 1% risk of significant bleeding and 1 in 10,000 risk of a fatality [30, 31]. Therefore, transplant centers tend to avoid routine liver biopsies in living liver donors during evaluation, but some, instead rely on the BMI as an indicator of hepatic steatosis [32]. But obesity has been rising in the last two decades across the world. In 2012, in United States, 69% people were overweight (BMI > 25) and 35% obese (BMI > 30) [33]. Therefore, in presence of the obesity epidemic, and the rising liver transplant waiting list, should transplant centers be selective in choosing living liver donors with BMI > 30, especially when it has been demonstrated that BMI > 30 is not a contraindication for live liver donation [34]. The yield of such a donation also had comparable results for donor safety and donor complications in both short and long term when compared with live liver donors with BMI < 30. Furthermore, the recipients also enjoy similar graft/patient survival both in short and long term (**Figure 6**) [34]. Therefore, when considering obese live liver donors, accepting Graft Recipient Weight Ratio (GRWR) of a higher value (1.42 vs. 1.17, $p = 0.0001$) can yield the desired donor safety and comparable recipient outcomes [34]. However, there is no ideal GRWR, that is considered optimal in an obese live liver donor, because values as low as 0.74 have successfully achieved comparable and good recipient outcomes [35]. It is also thought that various techniques for Graft Inflow Modulation (GIM) allows for accepting and safely transplanting a lower GRWR liver allograft in LDLT to minimize Small For Size Syndrome (SFSS) [36], whereas others are of

the belief that higher portal pressures on the contrary help in liver regeneration [35, 37] thereby avoiding the need for GIM.

6.3. The debate of right vs. left lobe donor hepatectomy

The very first few LDLTs performed were in pediatric patients, utilizing Left Lateral Segment (LLS) liver grafts. This was technically easier for the recipients, whilst offering higher level of donor safety. Both these factors were paramount in gaining the needed success and popularity of LDLT across the world. Meanwhile in adult recipients, right lobe versus left lobe LDLT was debated heavily for two decades. The debate aimed at balancing donor safety and recipient outcomes. Although, logic and ethics favored donor safety, but the recipient risks and outcome were equally important, thus feeding the debate. Normally, the right lobe of the liver is larger and denser than the left lobe, which is much smaller and flatter. Therefore, a left lobe hepatectomy generates a smaller allograft providing higher safety for the donor but limiting the choice of recipient. This blunted the popularity of LDLT in adult patients. But in 1997, the feasibility of using a right lobe liver graft safely in adults by overcoming the graft size matching, opened the gateway for adult LDLTs [38, 39].

In United States, right lobe living donor hepatectomy remained the choice for 95% adult LDLTs, between 1998 and 2009 [40]. Although the number of left lobe living liver donors were smaller for statistical inference, nonetheless, A2ALL consortium concluded, a higher rate of donor complications with left lobe donation. The Turkish group also noted similar findings, whereby performing 91% right lobe LDLTs between 2007 and 2011, they experienced higher donor complications for left lobe liver donation [41]. Arguably both in the US and Turkey, the number of left lobe liver donation was far smaller for a meaningful covariate analysis, instead, it hinted towards the importance of individual surgeon experience in right or left lobe donor hepatectomy.

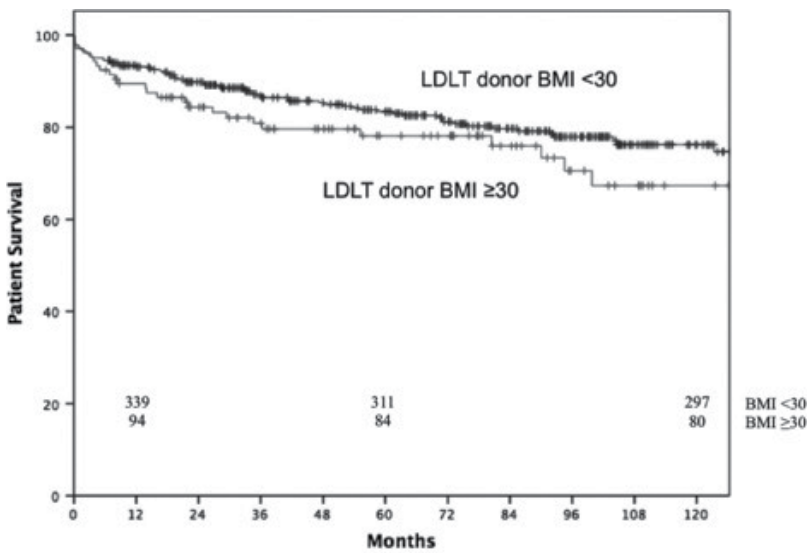

Figure 6. LDLT patient survival based on BMI [34].

The experience in the east was entirely different at the five-large volume Asian transplant centers (Seoul, Hong Kong, Taiwan, Kyoto and Tokyo), wherein 38% were left hepatic lobe donation versus 62% right lobe between 1990 and 2001, with lower complication rates for left lobe donation 7.5 vs. 28% [42]. Furthermore, when the transplant centers performed more left lobe hepatectomy as compared to the right (762 vs. 500), the lower donor complication rates for the left lobe liver allografts even reached statistical significance (18.8 vs. 44.2%, $p < 0.05$) [43].

In summation, the combined experience of living donor hepatectomy across the eastern and western world resonates, that the success of surgery with lower donor complication rates is heavily dependent on the experience of surgeons and their individual practice, than just the laterality of the hepatic lobectomy. Inherently, a living donor transplant carries high stakes, as a donor death or higher complication rate can significantly impact the LDLT practice of an entire nation. In 2001, United States performed a record no. of LDLTs, i.e., 524. But the infamous living liver donor death later that year, crippled the LDLT practice of US to this date, annually averaging to only about 300 LDLTs.

7. Advances in LDLT

7.1. Dual lobe liver transplant

Donor Safety is paramount to the success of LDLT and also for the transplant program. Therefore, inadequate graft size is a major obstacle, considering the low number of donors willing and suitable to donate. Inadequate graft size along with portal hyper-perfusion can lead to Small For Size Syndrome (SFSS), a clinical syndrome characterized by postoperative coagulopathy and liver dysfunction. Various modes of GIM have been proposed to minimize the portal hyper-perfusion and also minimize congestion, but little focus has been on how to encompass the low GRWR. Therefore, dual lobe liver transplant, a technical advancement to the standard LDLT has been described and safely practiced [44] by certain centers. Herein, dual allografts from two separate donors are transplanted heterotopically and orthotopically in one recipient; yielding higher liver volume for better recipient outcome and at the same time, be safer for the living donor(s) (**Figure 7**). Besides being a technically complex operation, it comes with immunological challenges of acute rejection between the two grafts and the recipient, and also between the grafts itself, including the risk of Graft Versus Host Disease (GVHD).

7.2. ABO-i liver transplant and paired exchange

ABO-incompatible (ABO-i) living donor liver transplants have been in discussion and sparse use for almost three decades, and is reserved for urgent cases only. This is because the five-year patient survival rates in adults are abysmally low at 22% [45]. Therefore, ABO-i LDLT has unpopular amongst transplant surgeons. But in 2003, Rituximab, an anti-CD20 monoclonal antibody was introduced in liver transplantation with excellent graft and patient survival rates for ABO-i LDLT [46]. Since then, there have been various immune-modifications by adding plasmapheresis, splenectomy or immunoadsorption columns to Rituximab therapy, in order to successfully cross the blood group incompatibility barrier.

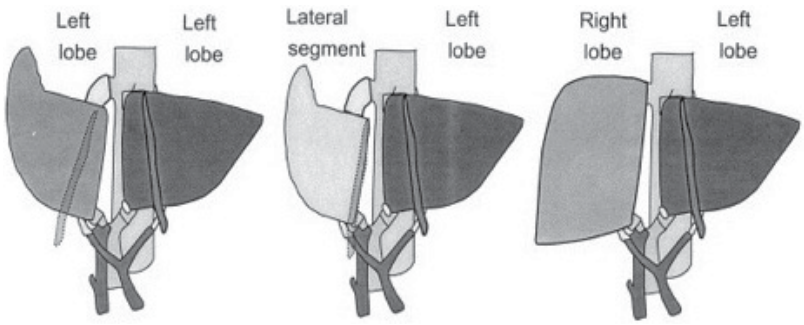

Figure 7. Adult to adult living donor liver transplant, using dual grafts [44].

The other alternative to blood group incompatible liver transplant is liver paired exchange. This requires a very high level of coordination between multiple transplant teams as it can be a logistical puzzle. A paired exchange liver transplant in essence, offers superior survival advantage over an ABO-i liver transplant, but the level of communication can strain the system to fail. There are also socio-cultural issues amongst patients in accepting someone else's organ, therefore the success rate of liver exchange matching can be as low as <10% [47]. But its conceptual possibility has been well established with excellent outcomes.

ABO-i liver transplants have a valuable role in patients where socio-cultural barriers deter them from participating in the exchange program. At the same time, certain patients cannot tolerate higher level of immunosuppression and therefore will not be suitable for an ABO-i liver transplant, requiring higher levels of immunosuppression and immune-modulation. But, the availability of multiple options offer valuable and real choices for patients, that meet their individual needs and agrees with their beliefs.

7.3. Tolerance in liver transplant

The liver has been considered very tolerant in solid organ transplantation, but its mechanism is still unclear. Application of regulatory T (T-reg) cells are in experimental stages, but have offered promising early results in solid organ transplantation including LDLT. However, most studies have focused on the applicability and short-term success as of now, but there is a real concern of chronic rejection with auto-antigens in T-reg therapies.

8. Summary

After the first decade following the inception of LDLT, there were refinements to the surgical technique and in the process of evaluation to select a suitable donor. There were also lessons learnt on how best to select a suitable recipient for LDLT. After being surgically refined, the second decade offered advancements to the learnt lessons on how to extend the donor acceptance boundaries, and at the same time, how best to match the extended spectrum donor to the

most appropriate recipient. This benefitted the growing need for liver transplant versus the sparse availability and poor access to deceased donor livers.

The current decade has been focused on entertaining further advancements not just in technique, but in immunosuppression and disease control i.e. hepatitis C treatment.

LDLT in summary has come a long way since 1989, and the future progress is yet to be seen in the direction of 3D-bioprinting, cell therapy and crossing the barrier into successful xenotransplantation.

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Section 6

Liver Gene Therapy

Chapter 10

Liver Gene Therapy: Employing Surgery and Radiology for Translational Research

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72665

Abstract

Gene therapy is a therapeutic strategy that aims to employ nucleic acids as drugs for the transient or permanent treatment of inherited or acquired pathologies. Based on the type of vector employed for the gene transfer, gene therapy can be classified as viral gene therapy and nonviral gene therapy. Nonviral gene therapy is less efficient but safer than viral gene therapy. Hydrodynamic naked DNA transfer has shown great translational potential, achieving therapeutic levels of a human protein in the murine model. The translational process of the procedure has already been performed. Different radiologic and surgical approaches permitted pressurizing the liver in vivo by excluding its vascularization partially or totally. These approaches mediated a tissue rate of human alpha-1-antitrypsin protein translation (100–1000 copies per cell) close to those obtained with the mouse gold standard model in a safe mode that could be translated to human settings.

Keywords: gene therapy, liver, hydrodynamic, radiologic, catheterization, transplantation, surgery

1. Introduction

Gene therapy is a therapeutic strategy that uses nucleic acids, in any of their forms, as a drug for the transient or permanent treatment of inherited or acquired pathologies [1]. From a regulatory point of view, these drugs are considered in the European Regulation 1394/2007 and the Directive 2001/83/CE of the European Parliament. These, basically, establish the following:

"A gene therapy drug is a biologic drug with the following features:

a. it includes an active principle that contains a recombinant nucleic acid, or it is constituted by this, and it is employed or administered in human beings, aiming to regulate, repair, substitute, add or remove a gene sequence;

b. its therapeutic, prophylactic or diagnostic effect depends directly on the nucleic acid sequence or its expression product."

Gene therapy consists of the transfer of a gene with clinical interest to the target tissue or organ of a patient. This transfer can be mediated by a vector or vehicle or employing naked DNA. Gene therapy aims that the gene reaches the target cells with sufficient molecular bioavailability for the host cell's decoding machinery to decode the gene sequence and produce the protein encoded by it [2].

The correct production of the protein encoded by the transferred gene would permit:

a. adding a new function to the target cell

b. recovering a lost or diminished function

c. inhibiting or modulating an exacerbated function

d. editing the genome to correct the production of a defective protein

1.1. Gene therapy strategies

There are different alternatives to perform the gene transfer. Depending on the resource employed for the delivery procedure, gene therapy can be classified as viral and nonviral.

1.1.1. Viral gene therapy

Viral gene therapy employs a viral vector to carry the DNA of interest to the nucleus of the target cell [3]. This viral vector consists of the sequence of a virus, integrative or not, without the pathogenic sequences (related with its replicative ability), which are substituted by the therapeutic gene of interest. This strategy takes advantage of the viral ability to access the cells and employ their decoding machinery to translate its own genome. Viral gene therapy offers the following advantages:

a. It mediates a more efficient transfer of the gene;

b. Its administration could be systemic since the virus structure protects the gene from the circulating nucleases;

c. It permits developing permanent (employing viral vectors with the ability to integrate within the host cell genome) or transient (employing non-integrative viruses such as adenoviruses) therapies;

d. It is possible to select the target cell since some viruses present tropism.

However, they also present disadvantages to be considered:

a. Some viruses can induce intense immune responses, limiting the repeated doses (especially relevant in adenoviral vectors);

b. The exact place of the host genome where viruses integrate their genome is still unknown, hence being possible to alter the normal cell functions (insertional mutagenesis, tumor transformation);

c. Although not probable, it is possible that the viral particle without pathogenic features could recover them by genetic recombination, resulting in a potential risk for its clinical use.

1.1.2. Nonviral gene therapy

Nonviral gene therapy consists of the delivery of DNA mediated by the use of a nonviral vector [4–6] or the delivery of naked DNA [7], by physical procedures. With the aim of protecting the delivered DNA from its degradation exerted by circulating nucleases, different types of nonviral vectors have been designed. These vectors can facilitate the DNA (negative net charge) access into the cell through its plasmatic membrane, also negatively charged. Among the different models of nonviral vectors, we can find:

a. Liposomes—formed by the inclusion of DNA molecules within the lipid's concentric layers. They have the ability to protect the gene and facilitate its cell internalization by endocytosis and/or fusion with the cell membrane in order to release the DNA inside the cell [6, 8].

b. Polyplexes [6, 9]—they employ biodegradable polymers that protect the DNA from the degradation mediated by DNAses. The use of cationic lipids or polymers permits the formation of complexes with the DNA (lipoplexes and polyplexes, respectively) that facilitates its cell internalization.

Dewadvantages of nonviral vectors, they have limited utility because of their difficult formulation for clinical application. Their efficiency in 'in vitro' experiments is much higher than the efficiency observed in 'in vivo.' Furthermore, they have sometimes induced the immune response in patients.

Since viral gene therapy has offered good transfer efficiency but with the potential risk of immune reaction, or even that the recovery of virus infectivity and gene therapy mediated by nonviral vectors does not offer real advantages, physical procedures for efficient and safe naked DNA transfer have been developed. The most important alternatives of these strategies are:

a. Electroporation consists of augmenting the cell membranes' permeability by employing electric pulses [6, 10, 11]. It increases the transfer efficiency but is more targeted to muscular tissue;

b. Sonoporation consists of the application of ultrasound on a biological tissue, mediating the formation of bubbles that create a stir able to destabilize transiently the cell membrane and facilitating the access of gene [6, 12];

c. Magnetofection based on the application of a magnetic field after the transfer of a gene linked to metallic particles in order to lead the product inside the tissue. This strategy has not demonstrated important improvement [13, 14];

d. Jet injection consists of the high-speed injection of particles. It is employed specifically in muscle tissue [15–17];

e. Hydrodynamic gene transfer is mediated by changes in cell permeability induced by the intravascular injection of DNA saline solution (hydrofection). This has proved to be one

of the most promising methods for naked DNA transfer and presents a great potential of clinical application in several different organs [18, 19].

1.2. Gene therapy in clinics

Since the approval of the first gene therapy clinical trial performed in patients in 1989, the number of these clinical trials, with little fluctuations, has increased constantly [20] achieving approximately 2600 in total (updated in November 2017) with a maximum rate of 169 clinical trials approved in 2015 (**Figure 1**).

The increasing use of gene therapy was possible, thanks to the development of gene constructs by employing different genes depending on the therapeutic application. Among the

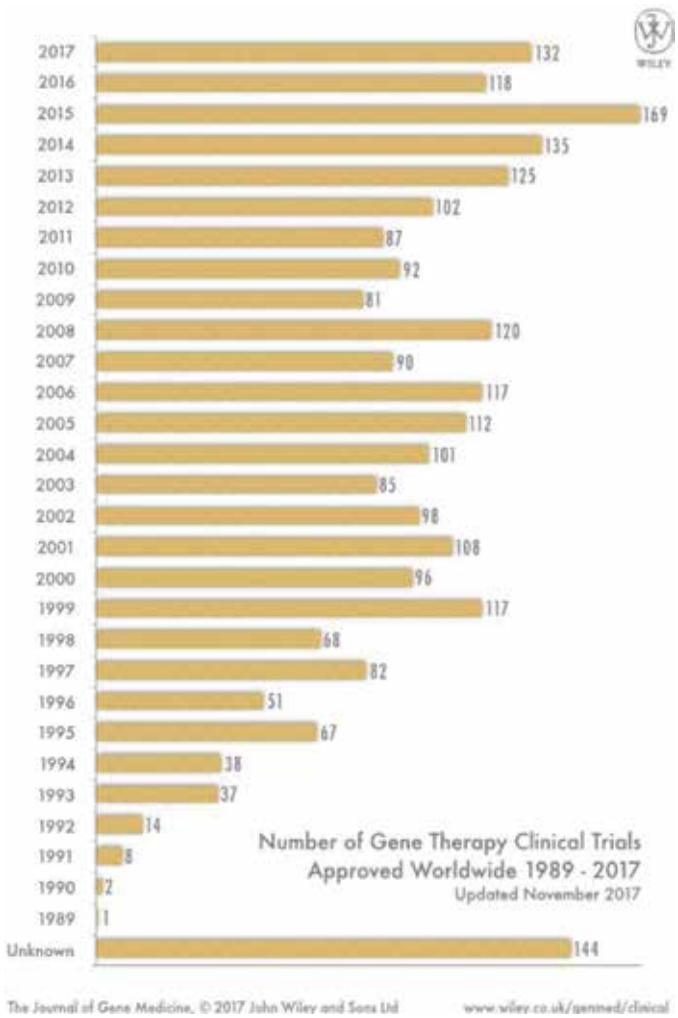

Figure 1. Gene therapy clinical trials approved in the world. In this figure, the number of gene therapy clinical trials approved in the world each year since 1989 until August 2016 is shown. Source: www.wiley.co.uk/genmed/clinical [21].

most employed, those modulating the immune response (by activation or repression) stand out from the others. In clinical trials, the most employed strategy for gene transfer has been viral gene transfer (around 70%). Adenoviruses (A) due to their capacity to carry large genes and express them transiently and retroviruses (R) because of their ability to integrate the gene within the host genome permitting its long-term stable expression (suitable for inherited deficiencies) are the most employed. The most employed strategies of nonviral gene therapy in clinical trials have been the lipofection and naked DNA (N), since they are the safest (**Figure 2**).

However, when clinical trials employing naked DNA for monogenic inherited diseases are searched, only six trials are found and when considering genes encoding cytokines, only one is found. Despite the different strategies to vectorize the gene and the types of treatment employed, only 0.1% of all clinical trials employing gene therapy reached phase IV (**Table 1**).

Among all the gene therapy procedures, the one that achieved the most positive benefit-risk balance has been the naked DNA hydrodynamic transfer. Given the potential interest

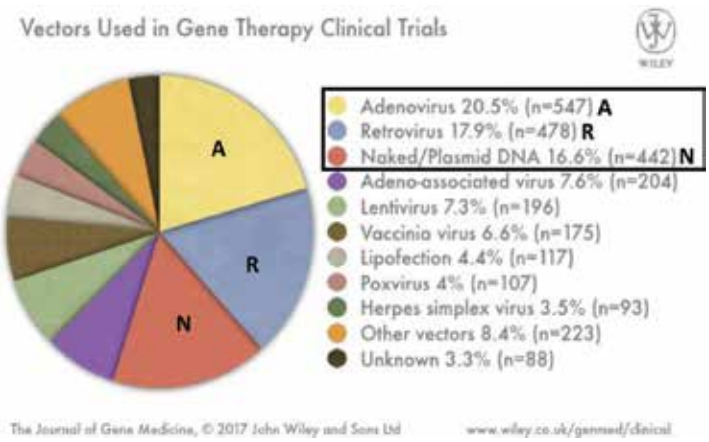

Figure 2. Vectors used in gene therapy clinical trials. Source: www.wiley.co.uk/genmed/clinical [21].

Phase	Number of clinical trials	Ratio (%)
I	1409	57.2
I/II	500	20.3
II	429	17.4
II/III	24	1
III	91	3.8
IV	3	0.1
Single subject	5	0.2

Source: www.wiley.co.uk/genmed/clinical [21].

Table 1. Phases of gene therapy clinical trials.

of hydrodynamic gene therapy and the wide range of application in clinics (especially in the liver), the translational process of the technique has been performed from the successful murine model to human liver segments. The swine model permitted adapting the procedure for 'in vivo' liver transfer. Different radiologic and surgical approaches performed to improve the liver hydrodynamic gene transfer 'in vivo' will be discussed in this chapter.

2. Hydrodynamic naked DNA transfer

2.1. Hydrodynamic methodology

The possibility of expressing heterologous genes with high efficacy after the delivery of naked DNA was firstly described during the mid-1990s [22, 23]. In 1999, Zhang et al. [24] and Liu et al. [25] introduced the hydrodynamic gene transfer procedure. This procedure consisted of the rapid injection of a large volume (2 ml in 5–7 s) of saline solution bearing the gene of interest through the tail vein in the mouse (20 g average weight). The possibility of transferring naked DNA efficiently aroused a great interest among researchers and clinicians since the hydrodynamic procedure permitted expressing high levels of a heterologous protein, employing a safer strategy than viral gene therapy. Different research groups focused their efforts on improving the technique in order to be safer, more efficient and reproducible [26].

2.2. Hydrofection mechanism

In **Figure 3**, the sinusoid circulation within the liver before and after the retrograde injection of a saline solution containing a plasmid is shown. The gene solution injected through the tail vein reaches the liver in a retrograde sense and increases the pressure inside the

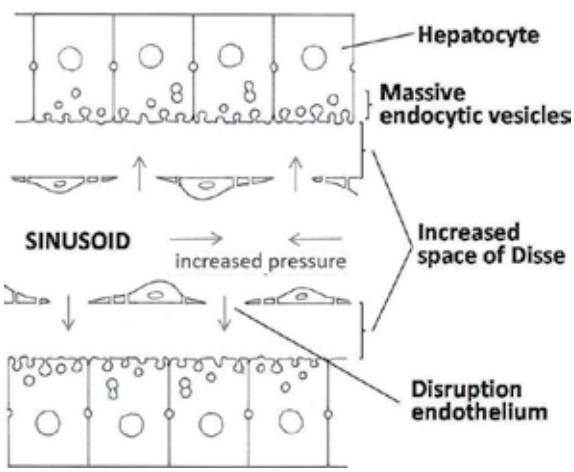

Figure 3. Schematic representation of sinusoid organization after retrograde hydrodynamic injection of gene solution. When a plasmid is injected, vessel pressure increases inducing the separation of endothelium cells. This permits the access of gene constructs to the Disse space and to the hepatocytes through the massive formation of endocytic vesicles.

vessel. This distends the wall mediating the transient separation of endothelium cells. When this occurs, the DNA leaves the blood vessels through the sinusoid pores and intercellular spaces and reaches the Disse space. From this space, the DNA can access the hepatocyte, the massive formation of endocytic vesicles playing a relevant role in this process. This DNA must reach the nucleus of the hepatocyte in order that the gene information delivered can be decoded. When this process takes place efficiently, the DNA is transcribed to RNA and this is translated to the protein, which is released into the bloodstream (in case of plasma proteins).

Employing this procedure, therapeutic plasma levels of alpha-1-protein were achieved for periods of more than 6 months in mice [27]. Nowadays, many gene therapy experiments for different pathology treatments are being studied in mice [28–30]. The possibility of achieving therapeutic levels of heterologous proteins after hydrodynamic human gene transfer in the murine model boosted the efforts of research teams to develop and adapt the procedures in larger animals aiming to translate it into the clinics, since the hemodynamic changes induced by the hydrodynamic injection are not compatible with its use in humans.

The perfusion conditions that permitted achieving the most efficient results in the mouse implied doubling the animal's volemia in a very short period of time. The procedure had to be necessarily adapted since larger animals would have not tolerated these conditions. The modifications of the procedure were directed to diminish the systemic hydrodynamic pressure. This was performed by transferring the gene of interest to the one and only target organ by image-guided catheterization procedures. Studies were performed in rats [31, 32] and rabbit [33] models but the results obtained were much less efficient than that observed in the mouse. The most recent efforts focused on developing models of liver hydrodynamic perfusion in pigs given their anatomical proximity with humans [34].

In the swine model, different strategies for minimally invasive gene transfer were designed through liver catheterization [35, 36]. Although these procedures proved to be safe, the efficiency achieved was not remarkable. Some authors highlighted the possibility that higher intravascular pressure within the liver could be required. For this reason, different strategies to block the venous backflow and employ more demanding perfusion conditions were studied. Levels of heterologous protein expression were not close to therapeutics in any case. After several works carried out by research groups around the world, no significant result was achieved [37–41]. However, given the huge interest of this procedure and its potential to be translated to human clinical practice, different groups evaluated minutely the molecular process of the transferred gene decoding in order to confirm or refuse this possibility. Evaluating at molecular level the detailed delivery, transcription and translation of a transferred gene permitted in identifying the step of the decoding process that limited the final efficacy in liver tissue and comparing this process in different animal models: mouse, pig and human. The best conditions of efficacy and safety for liver hydrodynamic gene therapy have been established in pig liver 'in vivo' (by catheterization and surgery) and the human liver 'ex vivo' (by catheterization in watertight segments). The methodology permitted comparing quantitatively the efficiency of different procedures of liver gene transfer. These procedures included partial and complete vascular exclusion aiming to pressurize the organ without affecting the systemic hemodynamics.

2.3. Therapeutic targets

Since gene transfer can deliver a gene functionally complete to the cell, it presents a great interest for the treatment of inherited metabolic diseases [42–45], such as alpha-1-antitrypsin deficiency [46], in which the entire functional gene could be implemented.

Gene therapy can also play an important role in the treatment of different acquired pathologies. Its application for modulating the immune response in different proinflammatory conditions, such as liver transplantation, has been studied by implementing genes of anti-inflammatory cytokines such as interleukin-10 (IL10).

3. Clinical translation of hydrodynamic gene therapy

Several animal models have been employed for hydrodynamic gene transfer. The murine model has resulted in the gold standard of the procedure since therapeutic levels of the protein encoded by the transferred gene have been achieved. The translational process has been carried out in rat, rabbit, guinea pig, dog, pig and human liver segments.

The murine model consisted of the rapid injection of gene saline solution in a volume equivalent to the animal volemia. This large volume facilitates the backflow of the gene solution and provokes its retrograde access to the liver. The high heart rate of the mouse permits the injection of such volumes with animal survival. Similar conditions to those employed for mice were carried out in rats, although different adaptations for diminishing the solution volume have been proposed in order to follow up the translation process. Other researchers [47] studied different strategies to improve hydrodynamic gene delivery efficiency by targeting the right lateral liver lobe of the rat through the portal vein branch. The need for outflow blockade in the target area was reported since the portal vein pressure was too low to avoid backflow. In another attempt to improve the efficiency of the procedure, the left liver lobe was targeted in the rat and outflow occlusion was performed to compare its effect to free-flow control rats [32]. It was reported that outflow blockade is demanding to obtain efficient outcomes in transgene expression. Larger animals do not have the ability to increase the heart rate as mice and doubling their volemia would be incompatible with survival. Thus, the hydrodynamic injection had to be adapted to reduce the final volume and minimize the systemic hemodynamic impact. These adaptations focused on targeting an organ. Regarding this fact, Eastman et al. injected a gene to a single liver lobe employing a balloon catheter and to the entire organ of the rabbit with hepatic venous occlusion and achieved protein plasma expression in 2 days. The safety of the liver hydrodynamic gene transfer was also assessed in dogs to prove its feasible application in large animals [48]. They performed four successive injections in four different main liver lobes. Authors observed no significant harmful effects and rapid recovery of animals. However, the results obtained were poor.

The following step for the clinical translation of the procedure was to test its potential use in anatomically more similar animals to human beings such as pigs and primates. The techniques for gene delivery that were employed should be applicable in human settings.

Yoshino et al. [35] and Aliño et al. [36] described the first attempts performed in a pig. The total volume employed was reduced by targeting an area of liver and compared different

catheter-mediated delivery strategies. These strategies included portal vein occlusion, left hepatic artery occlusion, portal vein and left hepatic artery occlusion and both vessels' occlusion with blood flow washout. Yoshino et al. injected the gene solution through the cava vein. The occlusion of portal vein and hepatic artery with the washout mediated the most efficient outcomes achieving disperse protein plasma levels for several weeks. For the first time, the procedure showed interesting results in pigs, for those proteins with low expression. In another work, hydrodynamic retrovenous gene transfer was performed in large and small areas of pigs' liver. Alino et al. [36] reported the presence of gene and protein expression in tissues, mainly within the perivenous area. Targeting smaller areas but employing same volumes of gene solution, higher plasma protein levels were achieved, much lower than those considered therapeutic. Fabre et al. [37] targeted the entire liver and isolated the hepatic segment of the inferior vena cava by clamping it suprahepatically and infrahepatically. Gene solution was transferred by a hydrodynamic procedure through two parallel syringes and, although the efficiency of gene delivery was much lower than the one observed in the mouse and rat, they confirmed the clinical feasibility of the technique as determined by systemic blood pressures, ECG, heart rate and so on.

Pressure reached within the liver during the hydrodynamic injection played an important role. For this reason, Fabre et al. [40] focused their work on pressurizing individual lobes of the liver by isolating them. Aiming to achieve localized high pressure without affecting the systemic circulation, they proposed individualizing the lobe by employing catheters with balloon and ligation. Although most of the authors suggested blood pressure to be the most important feature of hydrodynamic injection for efficient gene transfer, others have pointed other characteristics such as impulse [49] and flow rate [50, 51] to be relevant. However, nearly all authors agree to the need for isolating target areas or the entire liver to improve the procedure efficiency. This vascular isolation could be partial or complete.

Firstly, the implication of the complete vascular exclusion in the final efficacy of the procedure should be evaluated in order to determine its relevance. As previously reported, the complete liver vascularization of the pig can be occluded up to 20 min without neither hepatic injury nor systemic damage [52]. Considering this fact, Carreño et al. [50] described in pigs a surgical procedure to completely exclude liver vascularization 'in vivo' and perform hydrodynamic gene delivery, targeting the entire organ. A complete midline laparotomy was carried out, exposing all the abdominal organs. The clamping sequence was as follows: first, the hepatic artery, then the portal vein and finally the infrahepatic vena cava, to interrupt hepatic inflow. The suprahepatic vena cava was clamped last, to secure total hepatic vascular exclusion. Depending on the flow sense of gene transfer, three different models were designed. In model 1 the portal vein was clamped, and only a longitudinal incision was made on the anterior surface of the cava vein to insert the perfusion cannula. In model 2, the process was the same as in model 1 but with the clamping of the vena cava and perfusion through the portal vein. In model 3 (**Figure 4**), the gene solution was injected simultaneously through suprahepatic IVC (Inferior Vena Cava) and the portal vein employing two catheters connected by a Y connector and a high-volume pump. After solution perfusion, the liver was kept under total vascular exclusion for no more than 5 min to allow gene penetration into the cell nuclei.

In all three models, when suprahepatic IVC was occluded and liver vasculature was completely excluded, the systemic pressure decreased rapidly. However, 1 min after revascularization this parameter was entirely normalized and animals recovered in few hours. Due to the invasiveness of the surgical procedure that included a laparotomy, same authors designed

a technique for liver venous sealing mediated by image-guided catheterization [53]. Two strategies with different degrees of liver vasculature closure were proposed:

a. Inject the gene solution through a balloon catheter placed in a single lobe (**Figure 5**), and only target this part of the liver and

b. Place simultaneously three catheters with balloons within suprahepatic IVC, infrahepatic IVC and portal vein around the liver entry (**Figure 6**) in order to close its vasculature. The gene solution is injected through the catheter placed in suprahepatic IVC and the entire organ is targeted.

These three procedures, surgery and open and closed catheterization, proved to be safe. After gene transfer and animal awakening, their recovery was very fast and presented normal

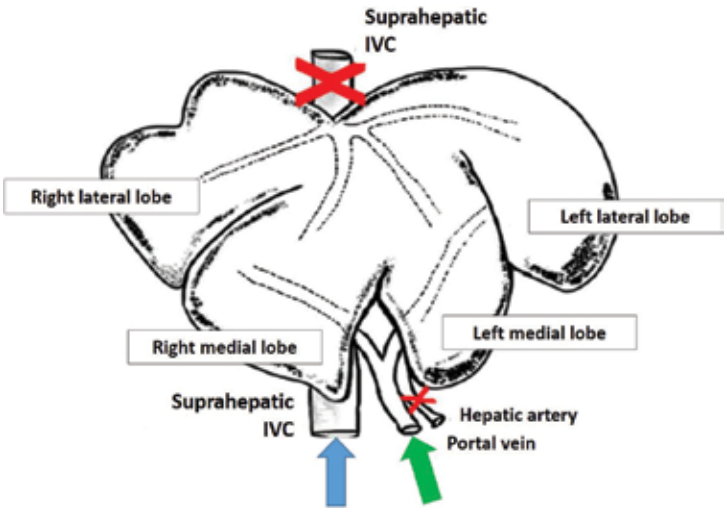

Figure 4. Schematic figure of liver simultaneous cava and porta perfusion with laparotomy surgery. Suprahepatic inferior vena cava and hepatic artery are ligated, and gene solution is transferred simultaneously by portal vein and infrahepatic inferior vena cava. Modified from [50].

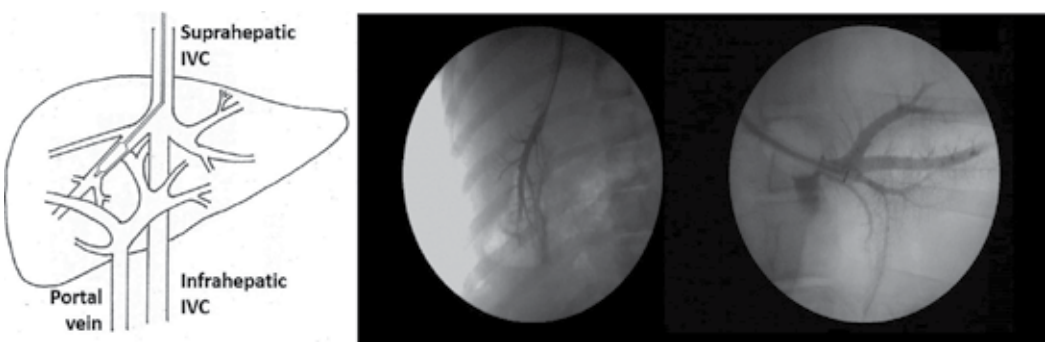

Figure 5. Single-lobe catheterization by balloon-catheter. Left panel is a schematic figure of catheter localization. Only the hepatic vein employed for gene transfer is occluded. Suprahepatic IVC, infrahepatic IVC and portal vein are not closed. Right panel shows two radiologic images of catheter position and iodinated contrast solution injection in single lobes defining the area affected by solution injected. The gene solution is injected through hepatic veins. The backflow is blocked by inflated balloon [54].

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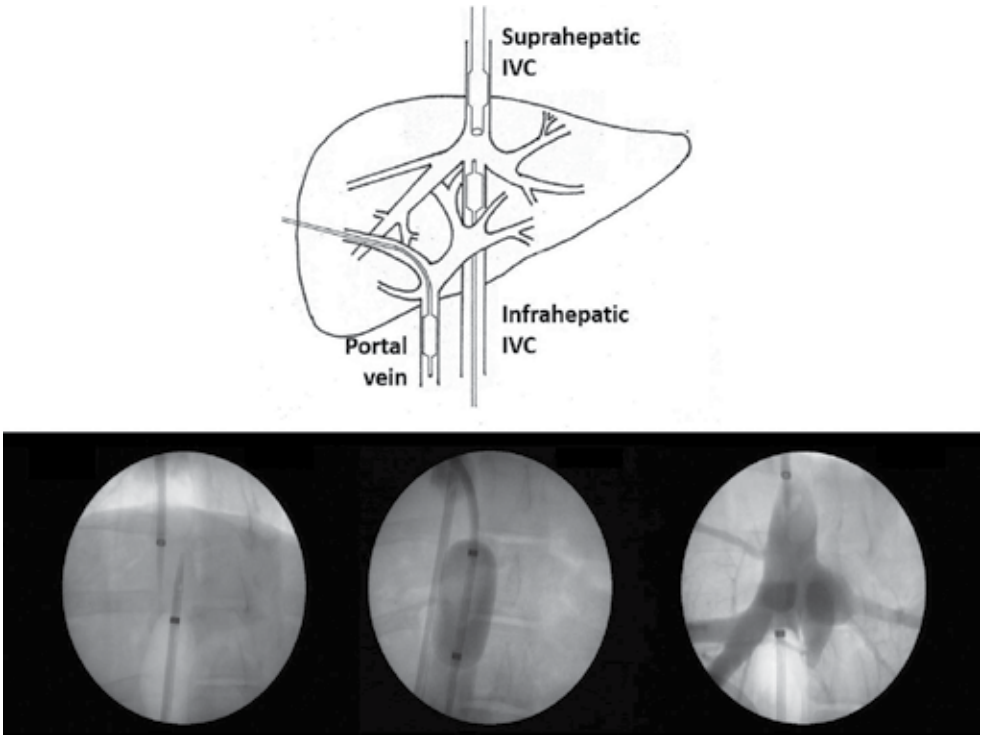

Figure 6. Whole liver catheterization by balloon-catheters. Upper panel is a schematic figure of catheters localization. Infrahepatic IVC and portal vein are closed at liver access by inflated balloon-catheters. Lower left panel shows a radiologic image of supra and infrahepatic IVC catheters position. Lower mid panel shows an inflated balloon-catheter placed at portal vein blocking its exit. Lower right panel shows the iodinated contrast solution injection in the entire liver. The gene solution is injected through suprahepatic inferior vena cava [54].

behavior few hours after the intervention. Furthermore, all of them mediated tissue expression of the protein encoded by the transferred gene. The rate of protein translation showed a direct relation with the degree of vasculature closure: surgery-mediated complete liver vasculature exclusion > catheterization-mediated venous vasculature closure > catheterization-mediated single lobe without organ vasculature closure.

Transferring the human alpha-1-antitrypsin, the single liver lobe strategy mediated 20,000 copies of protein per cell in the liver. Targeting the entire organ with the closure of suprahepatic IVC, infrahepatic IVC and portal vein mediated a higher translation rate up to 100,000 copies per cell. The complete exclusion of liver vasculature by occlusion IVC, portal vein and hepatic artery with surgical procedure increased this rate up to 400,000 copies per cell in the liver tissue. The highest rate of tissue translation achieved was only 10-fold lower than the one obtained with the successful gold standard procedure performed in the mouse. This suggests that the hydrodynamic procedure of liver gene therapy with vascular exclusion mediated by radiological and surgical strategies mediated efficient delivery with efficacious translation protein.

Once proved the efficiency of these procedures in pig and the confirmation of their safety for gene transfer 'in vivo,' the following step of translational process consisted of demonstrating the efficacy in human liver tissue.

In this sense, human liver segments proceeding from surgical resection in patients with cancer were injected with different genes to evaluate the potential transferability of this technique. Given their precedence, the vasculature of these human liver segments is entirely excluded so they are watertight and hence, pressurized. The gene is retrogradely transfected through a catheter placed in a hepatic vein (**Figure 7**) and the segment remains watertight for 5 min.

The first studies of gene transfer with human liver segments [56] used the eGFP tracer gene in order to easily determine its expression efficacy, and it was demonstrated that the gene could be efficiently delivered and the protein was produced within the liver tissue as observed by fluorescence microscopy. After confirming the feasibility of the technique in this type of tissue, genes with clinical interest were employed to define the translational potential to clinical real settings.

Sendra Gisbert et al. [55] transferred a plasmid bearing the human interleukin-10 gene (IL10). Interleukin-10 is an immunomodulatory protein with pleotropic effects with potential interest for the treatment of inflammatory diseases or for inducing tolerance in organ transplantation. The rate of tissue protein translation achieved was around 1000 copies per cell, this meaning the potential therapeutic production of protein (IC50 of IL10 for TNFa = 124 pg) if compared with other results of the same group.

Our group also transferred in similar human liver segments a plasmid with the same human alpha-1-antitrypsin employed in mice and pigs but modified. In order to permit differing endogenous and exogenous genes and proteins, a sequence of nucleotides encoding the flag peptide was added. Preliminary experiments demonstrate that the procedure is efficient and the use of a human gene in human tissue favors the production of protein. First, results prove a rate of tissue protein translation of 10^4–10^5 copies of hAAT-flag protein per cell, this accounting for up to 22% of all the hAAT proteins present in the liver tissue in 1 week.

The efficacy of gene transfer can be measured by different techniques and authors have studied many variables to present their results and evaluate how efficient a procedure is. This requires the use of a more detailed analysis that allows to identify the effectiveness of each of the stages of the process of delivery of the gene, its decoding of protein and its subsequent location.

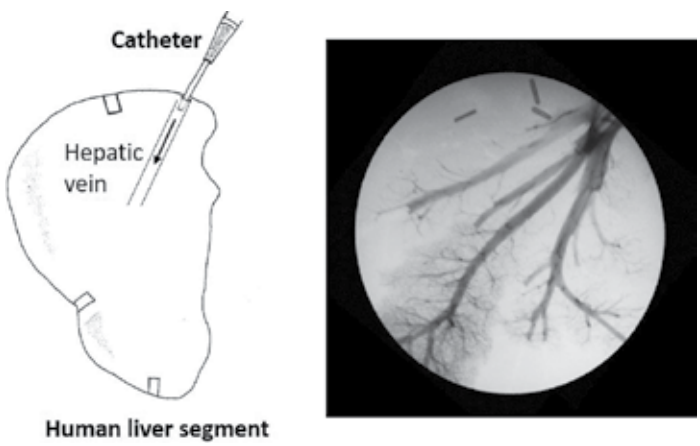

Figure 7. Catheterization of human liver segment. Left panel shows a schematic figure of a human liver segment with a catheter placed in a hepatic vein. Right panel is a radiographic image of a human liver segment injected with iodinated contrast solution through hepatic vein. Modified from [55].

The molecular quantitative evaluation of decoding is demanding for a correct interpretation of the process. Quantitative determination of the molecular process provides real data of delivery, transcription and translation indexes. It would be important that researches achieved an agreement in data quantitation and expression to be able to objectively compare results and define the better conditions for gene transfer. The units should be expressed in molecular units (as number of copies or moles) or other units of mass. It is also very important that the data are referred to a common circumstance, such as a standard or 'normalized cell.' The normalized cell is defined as 'typical mammalian hepatocyte with defined content of total DNA' (genome weight of each specific animal, for instance, human: 6.6 pg), RNA (20 pg) and protein (500 pg).

This strategy offers an objective analysis that permits expressing the data as the copy number of each molecular specie, considering the standard content of DNA, RNA and protein in a normalized cell. This offers a more comprehensive interpretation of the entire process and permits comparing the results among different works and research groups.

To sum up, the hydrodynamic procedure is an efficient strategy for gene delivery demonstrated by the levels of tissue protein that is observed. The more the vasculature is occluded, the better is the final protein expression. The surgical procedure permits, excluding liver, entire vasculature and mediates the higher expression rate. However, non-invasive image-guided catheterization permits good levels of protein production without the need of a laparotomy incision.

Acknowledgements

This work has been partially supported by Spanish Ministerio de Economía y Competitividad (SAF2011-27002, SAF2007-64492) and Grifols ALTA Award 2017.

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Section 7

Psychosocial Aspects Evaluation

Chapter 11

Neurocognitive Impairments and Depression and Their Relationship to Hepatitis C Virus Infection

Mihaela Fadgyas Stanculete

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74054

Abstract

The hepatitis C virus is a blood-borne virus with a direct cytopathic action. Chronic hepatitis C is a prevalent and costly disease. Studies have shown that it is a significant overlap between hepatitis C virus and mental disorders and that a substantial number of patients infected are suffering from mood disturbances and neurocognitive impairment. Recently, the neurocognitive impairments (attention, memory, and executive function alterations) were recognized as being independent of liver fibrosis and representing the direct effect of hepatitis C virus on neurons. However, until now impairments in neurocognition are not associated with viral replication or overall viral burden. Moreover, interferon alpha is still used to treat patients with hepatitis C. According to various researchers, 30–70% will develop significant psychiatric symptoms, leading to a premature discontinuation of therapy or noncompliance and worsening of quality of life. Several potential mechanisms may be implicated in the onset of a depressive episode following interferon alpha, the most important being the activation of immune-inflammatory pathways. This chapter will present the complex and striking relationships between hepatitis C virus infection and central nervous system symptoms. A variety of approaches, which integrate the extensive research data (including molecular, brain imaging, and neuropsychological findings), will be discussed.

Keywords: hepatitis C, cognitive impairments, psychiatry

1. Introduction

Hepatitis C virus (HCV) infects about 200 million people and is considered a public problem worldwide. Studies suggest that patients with HCV have a high burden of comorbidities such as psychiatric disorders, co-infection with hepatitis B and human immunodeficiency virus

(HIV), atherosclerosis, chronic kidney disease, mixed cryoglobulinemia, insulin resistance, and several cardiovascular diseases [1, 2]. The extrahepatic manifestation is secondary to HCV-related inflammatory responses and autoimmune reactions. According to a recent meta-analysis, the most frequent extrahepatic manifestation occurring in HCV-infected persons is depression, irrespective of alcohol and drug abuse or antiviral treatment [3].

The issue of a direct relationship between hepatitis C virus (HCV) infection and neuropsychiatric symptoms was raised for some years. Initially, the psychiatric and neurocognitive complaints were considered as the results of impairments in liver function. About 50% of the patients complain of chronic fatigue, deficits in attention, memory, learning, and depression [4–8].

Meanwhile, it has been shown that the reduction of global health-related quality of life (HRQoL) and development of psychiatric and neurocognitive impairments are not correlated with the level of hepatic alterations [9, 10].

Data point to an increasing evidence to support central nervous system (CNS) change in HCV patients. Several studies detected HCV in cerebrospinal fluid and brain. Further evidence was provided by studies using imaging techniques like magnetic resonance spectroscopy (MRS), positron emission tomography (PET), and single photon emission computed tomography (SPECT).

The mechanisms through which HCV entries and replicates in the brain are not fully elucidated yet, but evidences point to microstructural changes, and cerebral metabolite abnormalities [11–13].

Historically, the treatment of hepatitis C with interferon (IFN) was burdensome, complicated, and often was associated with neurocognitive abnormalities, depression, anxiety, and psychosis.

This chapter aims to analyze the current data about the relation between chronic HCV infection, depression, and neurocognitive impairments. Also, the effects of pharmacologic viral clearance on cognitive dysfunction and psychiatric features will be discussed.

2. Neurocognitive impairments and hepatitis C virus

The evidence of CNS infection is supported by the detection of replicative intermediate forms of HCV RNA and viral proteins within the CNS. Additional mechanisms, involved in neurological dysfunction, are possibly related to the consequence of circulating inflammatory cytokines and chemokines in the brain tissues through altered sites of the blood–brain barrier [14–17]. HCV ribonucleic acid (RNA) has been detected in peripheral blood mononuclear cells, cerebrospinal fluid (CSF), and the brain of chronically infected patients with neuropathological abnormalities. The majority of reports supporting HCV in the CNS have used PCR-based approaches to detect viral genomes in brain tissue and CSF. The presence of HCV in the brain was demonstrated using immunostaining and Western blot techniques. The presence of RNA negative strand intermediate is considered as a direct evidence of HCV replication. Until now, it is not clear which cells are involved. Some authors demonstrated that HCV infects microglia/macrophages, astrocytes, brain microvascular endothelial cells,

neuroepithelioma cells, and neuroblastoma cells. More recent studies showed that CSF was found to be HCV positive in more than 50% of patients with HCV [18–23].

A variety of mechanisms have been hypothesized to explain the biological abnormalities in the brain:

a. direct infection of the brain,

b. chronic neuroinflammatory response,

c. indirect stimulation of neurotoxic cytokine pathways, and

d. toxicity mediated by vascular damage.

2.1. Neuroimaging studies

The imaging techniques that have been used to determine the biological abnormalities in HCV patients were: magnetic resonance spectroscopy, positron emission tomography, single photon computed tomography (SPECT), magnetic resonance-perfusion weighted imaging, and diffusion tensor imaging (DTI).

MRS provides noninvasive measures to evidence the metabolite abnormalities concentration in specific brain regions: myoinositol (mI), choline-containing compounds (Cho), creatine and phosphocreatine (Cr), glutathione, and N-acetyl aspartate (NAA). These metabolites are sensitive to changes in neuronal and glial state and density.

In general, metabolites are reported as a ratio to creatine. The evidence of neuroinflammation in HCV-positive patients is underlined by choline/creatine ratios. The choline-containing compound (Cho) peak is considered a marker for cell turnover and membrane metabolism. They were significantly higher in the basal ganglia (BG) and white matter of HCV positive patients. This data was associated with elevated myoinositol/creatine ratios (a marker of glial density). Myoinositol is a cerebral osmolyte and considered a marker for gliosis. Increases are thought to reflect microglial activation and are associated with CNS inflammation. Choline and myoinositol were significantly higher in the BG. N-acetyl aspartate (NAA) is considered a marker for neurons/axons. NAA and N-acetyl-glutamate were also significantly higher in BG.

Alterations in brain metabolism and neurotransmission are presented in **Table 1**.

In spite of the fact that the results vary greatly in the areas of the brain most affected, HCV positive patients with mild liver disease are characterized on MRS by higher mI (or mI/Cr), higher Cho (or Cho/Cr), and often lower NAA (or NAA/Cr). Those results are considered to represent the results of neuronal dysfunction and immune activation of microglia cells.

The main limitations of these studies are represented by:

- small study sizes,
- heterogeneity and varying selection of patients, and
- differences in data acquisition and data analysis.

Author	Year	Technique	Findings	Journal
Forton et al.	2001	MRS	Elevated choline/creatine (Cho/Cr) ratio in the basal ganglia and frontal white matter	Lancet [24]
Forton et al.	2002	MRS	Higher choline in basal ganglia, white matter	Hepatology [10]
Weissenborn et al	2004	MRS	Decrease of the N-acetyl-aspartate (NAA)/Cr ratio in the frontal gray matter	Journal of Hepatology [12]
			no changes of the Cho/Cr ratio	
Bokemeyer et al.	2011	MRS	Increased Cho and myoinositol concentrations in basal ganglia and white matter	Gut [25]
			Increased Cr, NAA, and N-acetyl-aspartyl-glutamate in basal ganglia	
Nagarajan et al.	2012	Localized two-dimensional correlated spectroscopy (L-COSY)	Increased myoinositol and glutathione	International Journal of Hepatology [26]

Table 1. Neuroimaging findings.

Diffusion tensor imaging (DTI) is a technique of magnetic resonance imaging that provides metrics for the speed and direction of water diffusion along the white matter tracts in the brain. DTI is a sensible method for detecting microscopic differences in tissue properties. The common DTI measures are mean diffusivity (MD), fractional anisotropy (FA), radial diffusivity (Dr), and axial diffusivity (Da). Mean diffusivity (MD) is an averaged measure of speed of diffusion in the three main directions. Fractional anisotropy (FA) measures the degree to which diffusion is faster in one direction than others. FA is used to highlight the microstructural changes, but it seems to be not very specific to the type of changes. Reductions in FA and elevations in MD seem to indicate impaired white matter integrity. Microglial state is also assessed using the positron emission tomography (PET) ligand PK11195, which binds to the mitochondrial membrane translocator protein (TPSO) present in endothelial, astroglial, and microglial cells. It is considered a marker for microglial activation. The most important neuroimaging findings are presented in **Table 2**.

2.2. Cognitive impairments

Approximately more than 50% of patients with chronic HCV infection complain of:

- poor memory,
- impaired attention, and
- fatigue.

Despite its potential clinical significance, cognitive impairments are often missed in patients evaluated for HCV, unless the manifestations are overt or interfere with the functionality, leading to impairments in health-related quality of life. When the symptomatology is very

Author	Year	Technique	Findings	Journal
Bladowska et al.	2013	DTI	Decreased FA in all white matter areas measured	Journal of Hepatology [27]
Thames et al.	2015	DTI	Increased FA in striatum, thalamus, and insula	Neurology Neuroimmunology Neuroinflammation [28]
Grover VPB	2012	PET	Significantly higher binding potential in all subcortical areas assessed (caudate, thalamus, pallidum) but in no cortical areas	Journal of Viral Hepatitis [29]
Pflugrad et al.	2016	PET	No differences between patients with mild HCV and healthy controls	Journal of Viral Hepatitis [30]

Table 2. Neuroimaging findings in HCV patients.

severe, the patients could present word finding difficulties, anomia, and significant deficits in attention performance. In general, constructional abilities and nonverbal recall are intact in these patients. Many studies suggest that approximately 30% of patients with chronic HCV exhibit cognitive dysfunctions even in the absence of cirrhosis. It seems that the cognitive performances are unrelated to viral load or viral genotype. The imaging studies showed significant reduction in striatal and midbrain dopamine availability and reduced metabolism in limbic, frontal, parietal, and temporal cortices. Thus, a crucial role of impaired dopaminergic transmission in causing cognitive impairment in HCV-infected patients was suggested. Moreover, pathologic cerebral serotonin and dopamine transporter binding were observed.

Emerging lines of evidence suggest that the profile of neuropsychological dysfunction in HCV-infected patients is characterized by impairment in:

a. executive function,

b. sustained attention,

c. working memory, and

d. verbal learning and verbal recall.

Several cognitive impairments demonstrated in patient with HCV are presented in **Table 3**.

Author	Year	Domains	Journal
Weissenborn et al.	2004	Impaired executive function	Journal of Hepatology [31]
Karaivazoglou et al.	2007	Impairment of verbal learning and memory	Liver international [32]
Fontana et al.	2007	Impairment in verbal recall and working memory	Hepatology [33]
Lowry et al.	2010	Alterations in memory, sustained attention, and delayed auditory recognition	Journal of Viral Hepatitis [34]
Ibrahim et al.	2016	Worse performance in nonverbal reasoning, attention, spatial orientation, age identification, and working memory	Journal of Clinical and Experimental Neuropsychology [35]

Table 3. Summary of major cognitive dysfunctions observed in patients with chronic hepatitis C virus infection.

There is evidence that cognitive dysfunctions in HCV patients have some impact in the reduction of health-related quality of life, chronic fatigue, and impaired functionality. The literature demonstrates evidence of neurocognitive impairment in patients with chronic HCV infection. However, until now, it is not clear that these dysfunctions can be linked, wholly or in part, to the virus itself. The longitudinal evaluation of the cognitive functioning could provide valuable information regarding the persistence of symptoms after the clearance of virus in the periphery.

3. Depression and hepatitis C virus

Depression has long been recognized and associated with many chronic medical conditions. The occurrence of depression is higher in patients with chronic liver disease than that in the general population. The depression is a very common psychiatric comorbidity in HCV patients. The link between HCV and depression has been the focus of many investigations. Several studies have reported variability in the prevalence of depression among the HCV population. The prevalence of depression in HCV patients has been estimated to be 1.5–4 times higher than in general population. Moreover, the prevalence rate seems to be unrelated to liver disease severity and interferon treatment. However, psychiatric comorbidities are usually underdiagnosed or overlooked when patients seek primary care, even though depression affects overall disease progression in the HCV-infected population [36–40].

Understanding the depressive disorder comorbid with HCV may be critical for developing effective intervention strategies. Most patients with depression will suffer noticeable changes in social and physical activities, a loss of interest in work or leisure activities, or poor academic performance. Another important issue is that HCV infection is often associated with behaviors that are condemned by society (e.g., drug use, alcoholism, and high-risk sexual behaviors), promoting prejudice, discrimination, and abuse against patients. Also, these maladaptive behaviors could further exacerbate the depression.

The high prevalence of psychiatric comorbidities in HCV-infected patients has been typically associated with direct effects of the virus on the central nervous system or adverse effects of hepatitis C treatment. The high prevalence of psychiatric comorbidities in HCV-infected patients has been typically associated with direct effects of the virus on the central nervous system or adverse effects of hepatitis C treatment.

The comorbid depression in HCV patients could be:

a. depression that may be pre-existent,

b. a reactive depression to the diagnosis of HCV,

c. a biological effect of HCV infection, or

d. an α-interferon-induced depression.

Both biological and psychosocial factors are important considerations for the effective clinical management of HCV and the prevention of HCV disease progression.

3.1. Psychosocial factors involved in development of depression

It is very important to distinguish between psychological reactions to the knowledge that one has been infected with HCV and the direct effects of the virus itself. Learning that one has contracted HCV infection represents a significant life stressor and will produce emotional stress in most patients, and psychiatric disorder in many. The psychological reasons for the development of depression are illustrated in **Table 4**. The psychosocial factors involved in the development of depression are illustrated in **Table 4**.

Stigma negatively affects the HRQOL, mental health, and social life of the patients, and leads to difficulties with receiving or accepting treatment. Poor social and work adjustment, lower acceptance of the illness, and higher subjective complaints are other problems associated with stigmatization. Researches showed that women generally are prone to experience more stigmatization. The social stigma may cause some HCV individuals to refuse to disclose their HCV diagnosis. Furthermore, HCV-related stigma is an important stressor that leads to poor treatment adherence. In some cases, HCV-infected individuals tend to isolate themselves to prevent stigma-related negative attitudes. Low income is also a socio-demographic factor significantly associated with the appearance of depressive symptoms [41–46].

The most commonly used coping styles by HCV patients are:

- problem-solving behavior,
- distraction and self-revalorization,
- religiousness and search for meaning,
- cognitive avoidance and dissimulation, and
- depressive coping.

Illness perception
Risk of cirrhosis/cancer and other health-related worries
Fear of transmitting the disease
Concerns about the complications of disease/treatment
Functional disability
Impaired quality of life
Fatigue severity
Personality disorders
Low income
Social stigma
Coping styles
Social support

Table 4. Psychosocial factors associated with the development of depression in HCV patients.

Several studies have reported using inappropriate coping strategies in patients with HCV, which may negatively affect several aspects of their management.

Psychosocial interventions that include cognitive, behavioral and lifestyle strategies may influence the negative impact of HCV symptoms and treatment side effects on HRQOL.

3.2. Biological factors

Biological factors appear to play a significant role as well. Major depressive disorder is associated with the increased production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and interferon gamma (IFN-γ). Chronic HCV infection is also known to increase inflammatory cytokines like IL-1, IL-6, and tumor necrosis factor alpha (TNF-α). The inflammatory model of depression provides a possible link between the HCV infection and major depressive disorder. An increased macrophage migration inhibitory factor was also demonstrated in patients with major depression. Elevated pro-inflammatory cytokines have been found in patients with anxiety and depression symptoms and pharmacological agents who specifically inhibit inflammatory mediators seem to determine a reduction in depression and anxiety symptoms. The rise in cytokine levels is associated with fatigue, malaise, lethargy, and depression. Another effect of pro-inflammatory cytokines is the activation of the hypothalamic–pituitary–adrenal (HPA) axis, which represents the regulator of the stress response.

Many studies suggest that the activation of HPA pathways can modify monoamine expression in the CNS, and as a consequence, leading to symptoms of depression. It was demonstrated that the neurochemical imbalance of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) is linked to the development of depression. Studies in HCV-infected patients demonstrate impaired levels of dopamine and serotonin among distinct brain regions.

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IFN-α-induced depression may increase suicidality, impair quality of life, increase, and lead to noncompliance or even treatment withdrawal among patients with chronic HCV infection. Following IFN treatment of patients with HCV, up to 70% may develop depression. Several mechanisms have been proposed:

a. altered monoamine metabolism,

b. altered hypothalamus–pituitary–adrenal axis function,

c. increased rate of apoptosis, and

d. brain-derived neurotrophic factor (BDNF) reduction.

The predictors of development of depression during antiviral treatment are:

1. history of depressive disorder,
2. sub-threshold depressive symptoms,
3. female gender,
4. low educational level, and
5. high baseline serum interleukin 6 (IL6) concentrations.

Specific risk factors for IFN-induced suicide are still unknown.

Standard treatment for CHC was for a period a combination of pegylated interferon (pegIFN) and ribavirin (RBV), which was known to exacerbate fatigue and depressive symptoms. Interferon-based regimens are related to complicated dosing schedules, weekly administration of subcutaneous injections, and many side effects. Interferon alpha combined with ribavirin has been shown to be more effective than interferon alone on obtaining sustained virologic response. Moreover, it seems that SVR achieved with PEG-IFN-α and RBV combination therapy is durable over time [51].

Until now, we do not have sufficient data whether or not the cognitive impairments are irreversible in patients who have eliminated HCV after successful treatment. A study performed by Byrnes et al. concluded that HCV eradication was associated with an improvement in memory (visual and spatial) and verbal learning [52]. Another survey of 168 HCV patients receiving antiviral therapy with interferon and ribavirin evaluated 12 months after the termination of antiviral treatment concluded that in patients with a sustained viral response a significant improvement was observed in three out five cognitive domains (working memory, vigilance, and shared attention) [53, 54].

3.3. Direct-acting antivirals

Approval of direct-acting antivirals (DAA) against the hepatitis C virus has dramatically changed the management of HCV infection due to high cure rates and a favorable safety profile. It was reported that DAA in certain combinations are curing HCV infection in almost 100% of cases [55]. DAA are taken once-daily in oral combinations. Treatment duration has also been shortened considerably in comparison with interferon therapies, making treatment regimens more tolerable. Patient-reported outcomes (PROs) provide the patient's perspective on the physical, functional, and psychological consequences of treatment and the degree and impact of disease symptoms. Recent regimens are interferon-free, and in many cases, RBV-free, and involve a combination of DAA agents. Many studies showed a consistent improvement in the quality of life, fatigue, and work productivity during treatment in patients receiving IFN and RBV-free strategy. Newly approved oral anti-HCV drugs are very safe and effective, but unfortunately, they are very costly. DAAs do not seem to increase the neuropsychiatric risks to patients undergoing HCV triple therapy [56–60].

In the absence of the neurocognitive side effects of interferon, it should be expected a significant improvement in neurocognitive functioning if, as suggested, the impairments are directly attributable to HCV action on CNS.

3.4. Treatment of depression

Several studies have specifically investigated the treatment of depression in HCV patients. The literature suggests that depression, anxiety symptoms, and cognitive complaints are responsive to selective serotonin reuptake inhibitors (SSRIs) antidepressants. However, the neurovegetative symptoms seem to be less sensitive to SSRIs. Some evidence suggests that dual antidepressants neurovegetative symptoms can be better influenced with serotonin-norepinephrine reuptake inhibitors (SNRIs). Although the data are not strong, it does appear that SSRIs might be the first choice for the treatment of interferon-induced MDD and citalopram is recommended as first-line treatment for IFN-induced depression. Antidepressant medication should be continued for at least 12 weeks following the end of IFN treatment. Antidepressant therapy is also indicated for those patients with baseline depressive symptoms and those with a history of IFN-induced depression. Data showed that antidepressant pre-treatment with SSRIs lowers the incidence and severity of IFN-associated depression in patients with chronic hepatitis C infection. But we need to keep in mind that antidepressants are not recommended for all HCV patients, and the indication should be tailored to each patient [54, 61–65].

4. Conclusions

HCV infection causes multiple provocations to practitioners due to nontreatment and especially treatment-related psychiatric comorbidities. The evidence reviewed in this chapter strongly suggests that HCV patients should be carefully monitored for psychiatric side effects of treatment. The psychiatric comorbidities along with the cognitive dysfunctions affect the patient's care significantly and might influence the course of the disease. The mechanisms involved remain mainly not sufficiently understood. Psychological adjustment to illness is determined by a complex interaction of many factors. Psychosocial factors appear to be of significance, particularly concerning the coping mechanisms and perceived stigma. In the long run, the goal is to offer a multidisciplinary approach for optimal medical and psychosocial management of patients with HCV.

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Chapter 12

Psychosocial Aspects of Liver Transplantation and Liver Donation

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74551

Abstract

The construct of adjustment may help to understand the demands of end-stage liver failure (ESLF) and liver transplantation. Adjustment can be operationally defined on the basis of whether or not recipients and donors suffer from psychological problems and the ways in which they perceive their quality of life. For recipients of a transplant, evidence suggests that ESLF is related to the experience of psychological problems and poor quality of life, whereas transplantation is associated with less psychological problems and improvement in quality of life. Among donors, there is some evidence to suggest that organ donation surgery is associated with deterioration in quality of life and high levels of psychological problems. However, findings have been contradictory regarding the extent of these difficulties. Attempts to predict these outcomes are limited. More research is therefore needed. The construct of beliefs in general and the self-regulatory model of illness and qualitative research in particular could guide future attempts to explain these outcomes. Qualitative findings suggest that recipients and their donors experience ESLF and/or transplantation surgery or organ donation surgery in ways that are not identified by quantitative research. These findings can be used not only to develop ESLF-specific quality of life or emotional well-being questionnaires but also patient- or donor-derived interventions to improve poor outcomes.

Keywords: liver transplantation, liver donation, adjustment, quality of life, mood and anxiety disorders

1. Introduction

Two concepts including disease and illness can be differentiated. The concept of disease refers to changes that occur in the structure or functions of bodily systems, whereas the concept of

illness refers to patients' perception of their symptoms and their own and their significant others' reactions to these symptoms [1].

In medicine, the concept of 'chronic patient' is a relatively recent concept [2]. Approximately, in the last 3 decades, a considerable amount of attention is given to chronic physical illnesses for two main reasons. First, medicine can effectively control infectious diseases [2]. Second, the number of patients with a chronic physical illness is increasing. A chronic physical illness refers to a long-lasting and incurable physical illness, although patients may not experience the symptoms all the time [2]. Currently, in developed countries, common causes of death are chronic physical illnesses [3]. Healthcare professionals dealing with patients with a chronic physical illness need to pay attention to both concepts of disease and illness if the aim is to provide a high quality of care which is responsive to all the needs of the patients and their significant others.

The treatment of chronic physical illnesses aims to slow down their course as well as to reduce distress resulting from associated physical symptoms. In many cases, medicine is uncertain about the mechanisms of cause and cure of these illnesses [2]; as a result the growing number of patients with a chronic physical illness presents themselves as a big challenge to healthcare professionals. In general, treatment involves changes in lifestyle (such as dietary restrictions), dependence on medical technology such as the use of medication and artificial means to replace bodily functions.

The main characteristic of the ESLF is the liver failing to execute its main functions of digesting, metabolizing and storing the essential nutrients [4]. ESLF occurs due to a number of causes. Hepatitis, liver diseases, metabolic conditions and cancer of the liver constitute some causes of ESLF [5]. Cadaveric and living donor transplantations are the main choices of treatment. Transplantation not only aims to achieve maximal quality and quantity of life but also to minimize the effects of illness and its costs [6].

Cadaveric transplantation is preferred over living donor transplantation, but the former has a number of disadvantages including long waiting time and low chance of survival [7]. In addition, cadaveric liver transplantation generally requires inpatient treatment and care which may also decrease the chances for survival [7]. The waiting time of the cadaveric liver transplantation is generally long, but available cadaveric donors are scarce [7–10].

In living donor transplantation, a healthy individual related by blood or an individual who is considered by the ethical committee as suitable to donate, although not related by blood, provides a transplant. This form of transplantation has the advantage of decreasing the time that candidates wait for a transplant and increasing the survival rate [11]. However, adult-to-adult transplantation is a complicated procedure because approximately 60% of the liver of the donor, in other words the entire right lobe, is used [12].

Due to advances in liver transplant procedures and immunosuppressive medications, the prognosis following transplantation is good, and the survival rate after 1 year and 8 years of transplantation is approximately 85–90 and 61%, respectively [6, 13]. Some donors are likely to develop complications after organ donation surgery such as biliary problems, reoperation and persistent physical symptoms [9, 14–16]. Donor mortality ranges from 0.1 to 0.3% [17].

This means that transplantation has the possibility of endangering the health of donors. Therefore, in order to maintain their health, they are asked to go through an interdisciplinary

preoperative evaluation involving a series of medical as well as psychosocial assessments. This evaluation aims to ensure that they made autonomous and voluntary decisions to donate, and they can cope with the requirements and/or outcomes of organ donation surgery [18–20]. In general, medical inclusion criteria for liver donation include being between the age of 21 and 55, being within the normal weight range, the absence of any liver disease or any other significant disease such as cardiovascular diseases or diabetes and being free of any viral infection such as viral hepatitis or HIV [21, 22].

Recipients of a liver transplant and their donors are required to change their behavior or lifestyle to meet the demands brought by ESLF and transplantation or surgery for liver donation. Therefore, they both tend to face adaptational difficulties. The construct of adjustment may help to understand these difficulties. Adjustment can be defined in different ways depending on different assumptions. One way of defining adjustment involves whether or not the recipients or donors experience psychological problems (such as depression, anxiety or distress) or difficulties in overall functioning. Another way of defining adjustment is to do it in global terms by, for example, in terms of overall quality of life.

The impact of chronic illness in general and ESLF in particular goes beyond the patient himself or herself to all individuals who the patient is interacting with [1]. In general, a chronic illness can potentially influence various dimensions of life including interpersonal relationships, economic conditions and daily as well as social functioning [1]. Therefore, it is essential to understand the ways in which transplantation or surgery for liver donation influences both recipients and donors in order to formulate appropriate criteria for selecting suitable donors and promote donors and recipients' adjustment.

While reviewing the adaptational difficulties of recipients and their donors, it is important to review the difficulties experienced at pre-transplant and the ways in which these difficulties change across different time points following transplantation or organ donation surgery. Moreover, the adaptational difficulties will be reviewed on the basis of the construct of adjustment. For the purpose of this chapter, the construct of adjustment will be operationally defined on the basis of whether or not recipients and donors suffer from psychological problems and the ways in which they perceive their quality of life.

To that effect the search strategy aimed to identify all studies relevant to the experience of adaptational difficulties by recipients of a liver transplant and their donors. A number of databases were searched from 1985 to 2017. These databases included Medline, Embase, Psychinfo, PsycArticles and the Cochrane Library. A number of keywords were used. These keywords included chronic liver disease, ESLF, adjustment, quality of life, anxiety, depression, emotional well-being, mood disorders, psychological distress, psychological problems and psychiatric problems.

2. Recipients' experience

Both quantitative and qualitative studies have aimed to understand the adaptational difficulties experienced by the recipients.

2.1. Quantitative research

2.1.1. Psychological problems

Among candidates of liver transplant, reviews [23] have shown that the most common psychological problems at pre-transplant period include delirium, alcohol and substance misuse, anxiety and depressive disorders. In particular, the rates of depression, anxiety and delirium have varied from 4.5–64%, 20–50% and 50–56%, respectively [24–32].

Suitability of candidates with major mental illnesses for liver transplantation is subject to controversy. It has been argued that the presence of a major mental illness should not be an automatic exclusion criterion. Indeed evidence suggests that candidates with schizophrenia can be successfully transplanted [33]. It has been found that 27% of the sample had a severe personality disorder and 40% of this subsample were put on the transplantation list [34]. Therefore, specific exclusion criteria for those who suffer from a major mental illness may include poor compliance with medical and psychiatric follow-up appointments and poor quality of social support [33].

At post-transplantation, psychological problems experienced by the recipients include delirium, anxiety, depression, dysthymia, adjustment disorder, psychosis, post-traumatic stress disorder (PTSD) and substance related disorder [35–38]. Eighteen to twenty-seven percent of recipients report at least one disorder [38–40]. For example, it was reported that 23% of recipients experienced symptoms of PTSD, and among these recipients, 50% also experienced major depression [38]. However, the rate of depression has ranged from 5–46% across different studies [38, 41]. Nevertheless, the rate of psychological problems was the same as the general population [38].

Some studies have examined whether or not at post-transplant, the rate of psychological problems changes compared to that of pre-transplant. For example, while within 3 months post-transplant, the rate of these problems has been estimated to be 54% [36] at 1- and 3-year follow-up this rate has been estimated to be 7 and 2%, respectively [42]. Research has also shown that levels of different mood problems such as depression and anxiety have got reduced after transplantation [43]. In contrast another study found that there was no difference in terms of depressive symptoms prior and following transplantation [44].

Recipients also tend to experience different psychological problems at different time periods following transplantation. For example, it was found that recipients experienced depressive symptoms more commonly while they were in the intensive care unit, whereas they experienced anxiety symptoms more commonly after discharge from hospital [45].

2.1.2. Quality of life

Reviews [46] have shown that quality of life of candidates is poor at pre-transplantation. Indeed, the extent of impairment is greater than that of hospitalized patients with pneumonia, outpatients with rheumatoid arthritis, patients with minor nonacute conditions and the general population but similar to those of patients with peripheral vascular illness and osteoarthritis [47, 48].

After transplantation, systematic reviews and individual studies [44, 49–52] have shown that recipients have better quality of life. Studies indicate improvement in many areas including emotional, cognitive, social, behavioral, vocational, domestic and sexual areas [53]. A review showed that transplantation improved many dimensions of quality of life. These dimensions included physical health, sexual and social functioning, daily activities as well as overall quality of life [46]. Most positive changes were reported in physical, sexual and daily functioning and overall quality of life, whereas less positive changes were reported in psychological and social areas.

Prospective studies have also shown similar findings. One such study showed that recipients' general well-being was improved and the experience of physical symptoms (including tiredness, exhaustion and weakness) got reduced 1 year post-transplantation [54]. Similarly another prospective study reported improvements in cognitive areas and overall quality of life [55].

In contrast, evidence also suggests that observed positive changes in quality of life disappear when this is adjusted for those who died and that at a follow-up of 10 years, recipients' cognitive functioning and quality of life are poor [56, 57].

In addition, some systematic reviews and individual studies [51, 52] have shown that recipients have poorer quality of life in most dimensions of quality of life than healthy controls. In contrast, other studies have shown that quality of life of recipients is not different from or is higher than those of general population and patients with chronic liver disease at 1 year post-transplantation [58, 59].

Other studies have shown that high levels of psychological difficulties such as anxiety and depression reduce quality of life directly or as a mediator. For example, it was found that at pre-transplant, 31.1 and 25.8% of recipients were clinically significant for anxiety and depression, respectively, as compared to the rates observed in the general population (12.6 and 3.6% for anxiety and depression, respectively [59]). Those recipients with anxiety and depression within clinically significant levels also reported worse quality of life at post-transplantation. Similarly, following transplantation quality of life gets improved, and improvement in mood following transplantation is also related to improvement in quality of life [4, 43, 46, 54, 60, 61]. For example, one of these studies found that recipients without anxiety or depression symptoms at pre-transplant reported quality of life within the normal range at post-transplantation [60].

2.2. Qualitative research

Studies have shown that patients with ESLF experience their illness by going through two stages including 'becoming ill' and 'not living' [62]. Accordingly, the stage of 'becoming ill' includes interpreting the illness as an illness which develops insidiously, doubting the illness in the absence of experiencing its signs and managing the illness (such as by being positive, independent and supported by the family and friends) and managing its physical symptoms (such as tiredness). The stage of 'not living' includes losing independence due to deterioration in physical functioning, becoming disabled and wishing to return to a normal life by regaining independence. Other studies have provided specific information on the ways in which recipients of a transplant progress from physical, social and psychological dependence to independence [63]. The same study also showed that at pre-transplant period, recipients

recounted that their quality of life was poor and their physical problems prevented their independence, their social activity, the fulfillment of personal goals and management of psychological issues. At post-transplant period, recipients recounted that they wished to socially integrate and achieve control but significant others limited their independence by overprotecting them. A principled personality, optimistic outlook, incentives and professional support helped toward independence.

Candidates or recipients of liver transplant reported that they not only experienced negative emotions (such as fear, guilt, anxiety, frustration, embarrassment and uncertainty), mood fluctuations, lack of activity and energy and physical symptoms (such as pain and discomfort) but also negative social changes such as isolation, stigma, dependence on carers, carers' overprotection and restrictions in lifestyle [64–69].

Only one study examined the views of donors on the ways in which recipients evaluated their life as a result of the diagnosis of ESLF and transplantation [70]. Accordingly, donors felt that prior to transplantation in addition to experiencing social limitations, recipients experienced others both negatively (such as being frightened of getting infected by ESLF and others being insensitive) and positively (such as being supported by others). The experience of negative (such as feeling down, hopeless, like a loser) and positive feelings (such as feeling happy and relaxed) as well as improvement in life characterized recipients' experience according to donors. Improvement in life included not only physical and social improvements but also altering life perspective (such as appreciating that ESLF is serious and holding onto life).

3. Donors' experience

Both quantitative and qualitative studies have aimed to understand the adaptational difficulties experienced by donors.

3.1. Quantitative research

3.1.1. Becoming a donor

The experience of becoming a donor was characterized with ambivalence. There are two different types of ambivalence [71]. Residual ambivalence comprised uncertainty feelings and hesitation about the process of donation (such as being frightened of going through with donation) that continue to be present after medical assessments. Acute ambivalence refers to feelings of indecision present during the psychosocial assessment which prevent the prospective donor to give informed consent [18]. Acute ambivalence is uncommon (less than 2%) [72–74], whereas residual ambivalence is common (75%) [75–79].

Studies suggest that donors tend to make decisions that are not informed. A systematic review showed that a high percentage (89–95%) of donors felt they comprehended medical information provided by healthcare professionals regarding drawbacks and benefits of donation, although they reported that their needs for information and knowledge regarding the risks and possible complications were not met [80].

Although a small minority of donors (less than 5%) report to regret their decision to donate [81–84], the majority (80–100%) of donors report to be willing to donate again [77, 84–87]. Those donors who are hesitant or regret donating explain this on the basis of the specific characteristics of their situation (such as risky behaviors of the recipient) rather than the characteristics of the donation process (such as medical risks). Relatedly, donors who believe that the recipient is healthy are willing to donate again, whereas donors who believe that recipients risk their transplant are not willing to donate again.

3.1.2. Psychological problems

Compared to studies which examined the extent of psychological problems among candidates or recipients of liver transplant, not many studies have examined the extent of these problems among donors.

At pre- and/or post-donation periods, psychological problems that are experienced include low self-esteem, stress and low confidence [88, 89] and mood and anxiety disorders [37, 38, 90–92].

Although some studies suggest that donors' mental health gets improved at post-donation period [88, 93–95], other studies report that the extent of psychological distress is one in every four donors [85, 95, 96].

3.1.3. Quality of life

As in the case of candidates or recipients of liver transplant, the findings regarding to quality of life of donors have been mixed both at pre-donation and post-donation.

Before liver donation, evidence has suggested that quality of life of donors is low [97]. Yet many studies have suggested that the levels are better than that of general population [87, 92, 98, 99], whereas other studies have shown that donors report poorer quality of life based on mental dimensions [100] as compared to healthy controls.

After donation quality of life has been found to be high among donors [15, 94], and physical and mental aspects of quality of life are equivalent to and even higher than that of general population [81, 84, 86, 87, 90, 100–102]. Recent systematic reviews [103] have shown similar findings.

Evidence suggests that prior to organ donation, quality of life of donors is good, but following donation quality of life gets reduced particularly with regard to physical aspects and activities of daily living [99]. Compared to general population, evidence suggests that prior to donation, quality of life of donors is equal to and in some cases higher but following donation the physical but not mental dimensions of quality of life deteriorate, and this level returns to starting levels at 6-month to 1-year follow-up [87, 100]. More specifically, in one of these studies, donors returned to work at 1 year post-donation, but their levels of physical functioning contrasted with those of mental functioning [87]. With regard to social aspects, most donors do not report any changes in their relationship with recipients or report that their relationship gets improved post-donation [84, 87]. However, closer relationships including relationship with the spouse get worsened [81, 101, 104].

Relatedly, studies show that donors rate their physical health as fair to poor or worse following donation [77, 95, 101, 105]. More specifically, it was shown that quality of life was worse at 2-year than 5-year follow-up [106]. Donors also suffer from debilitating symptoms including pain around the scar, fatigue and poor body image [84, 87–89, 94–96, 105, 107]. In particular, difficulties in quality of life are related to financial difficulties, negative changes in employment status or social relationships as indicated by reviews [15].

As in the case with recipients of transplant, reviews [15] show that donors who report poor quality of life also report psychological problems.

3.2. Qualitative research

More qualitative research has been undertaken to examine the experience of donors than that of recipients.

A number of qualitative studies have explored the donors' views on becoming a donor. Accordingly, donors perceive the process of becoming a donor as an automatic response and as an opportunity to help the loved one [67, 108, 109]. The donors felt that they had no choice and decided to be a donor by prioritizing the recipient's life, viewing transplantation as the last chance for the recipient and her family and feeling obligated to save the recipient [110]. More specifically, this study showed that donors decided on becoming a donor by going through five stages [110]. The first stage, recognition, involves learning of liver transplantation from recipients, family, doctors or media; the second stage, digestion, involves realizing the seriousness of liver transplantation and wanting to save recipients from suffering and avoiding the guilt; the third stage consists of making a decision; the fourth stage, reinforcement, involves the donors reinforcing themselves psychologically; the final stage, resolution, involves preparedness and acceptance of donation. Relatedly, it was also reported that donors give three types of consent [111]. 'Unconditional consent' is a voluntary consent to save family members' life; 'pressured consent' is a consent whereby the donor feels pressurized to become a donor but he/she feels frightened. 'Ulterior-motivated consent' refers to the situation when the donor has a hidden motive.

Relatedly, other studies have shown that donors consider donation to cope with guilt regarding their own health and to reduce the responsibility for the ESLF of the recipient [112]. In the same study, donors recounted that they would only donate to certain family members or close friends [88]. By contrast, in another study donors recounted that they would donate to people who were related by blood as well as to anybody whom they felt close to regardless of whether or not they were related by blood [113].

Only one study explored donors' beliefs of the ESLF of the recipients, their transplantation and their own organ donation surgery [113]. This study found that donors' beliefs could be viewed in a number of groups including beliefs about recipients ESLF, beliefs about being a donor, beliefs about surgery for organ donation and beliefs about organ donation. Beliefs about recipients' ESLF included diverse explanations for ESLF (such as spontaneous failure of the liver, worry, stress, senseless drug use, blaming oneself and physicians) and physical symptoms (such as cramps, itching, weakness, developmental slowing down). Beliefs about being a donor consisted of reasons for donating (such as being related by blood, saving a life, doing the right thing, being healed), barriers to being a donor (such as pregnancy, obesity, other people being senseless and selfish), ways of managing these barriers (such as getting

significant others' consent and acting on one's gut feeling) and factors helping toward donation (such as the feeling that one does not have any responsibility). Beliefs about organ donation surgery included physical effects (such as pain, opening of stiches, putting on weight). The views that it is necessary to encourage organ donation and to raise people's awareness made up beliefs about organ donation.

In other qualitative studies, donors reported various feelings related to being a donor including not only negative emotions but also positive emotions. The former included feeling frightened, sad, anxious, angry and disappointed as well as feeling of being a failure, whereas positive emotions included feeling motivated and certain [109, 114, 115]. There was also the feelings of disappointment and anger toward medical system and insurance and the views that donation was not valued, that one is not supported and is not taken seriously by the medical staff [115]. Another study found that when the transplant did not fail, donors felt happy for having saved life. When the transplant failed, donors comforted themselves by the fact that they did everything they could [108].

On the other hand, a recent study found that donors experienced not only emotional changes but also changes in character. The former consisted of both negative (such as feeling angry, hopeless, down and helpless) and positive emotions (such as feeling appreciated, reputable, conscientiously comfortable). Changes in character were characterized by both worsening of (such as changing into an aggressive person) and positive changes in character (such as turning into a believer and stronger) [116].

The relationship of the donor with the recipient has been idealized [109], and difficulties about accepting recipients' ESLF have been experienced [114]. Research has also shown that there is a special bond between the recipient and the donor [116], in that the donor and the recipient become closer and donation is considered as a "proof of love" and the scar as a symbol of a special experience shared by the recipient and the donor only [117]. Moreover, the latter study also found that donation enhanced the positive or conflicting characteristics of the donor recipient relationship and there was not any deterioration in this relationship. Donors sometimes minimized the negative characteristics of this relationship and emphasized the improvements [117]. Similarly, another study reported that the extent of marital breakdown was lower than the general population. In the case of no marital breakdown, marital relationship has become stronger because of donation. In the case of marital breakdown, causes were independent of transplantation or donation process [108]. By contrast, another study reported that donors recounted mixed relationships. These included not only a continuum of feeling supported by significant others/doctors and not feeling supported by mothers or spouses but also formation of a special bond and worsening of close relationships [116]. Relatedly, it was reported that donors tend to postpone their personal needs such as emotional needs associated with rehabilitating oneself [108].

4. Correlates

A small number of quantitative studies have examined the effect, of a number of factors on outcomes among recipients of a liver transplant and their donors. For example, it was found that that 51–58% of the variance in quality of life was explained by a number of factors [60]. After

transplantation among recipients, employment, age, and depression predicted physical aspects, whereas anxiety and depression predicted mental dimensions of quality of life. Transplant-related factors such as rejection of the transplant, the number and length of hospital stays, effectiveness of the medication and complications did not predict anxiety symptoms. However, more patients suffering from anxiety and/or depression went through re-transplantation.

Studies have shown that the experience of feelings including ambivalence about donation, hesitation and uncertainty are important predictors of poor adjustment and quality of life at post-donation period among donors [76, 86]. Moreover, it was also shown that donors who were concerned about their own health, finances and close relationships at pre-donation period had a history of psychiatric illness or present psychiatric illness and held a graduate degree reported poorer quality of life, although donors' medical complications were unrelated to their quality of life [86].

5. Conclusion

To date, there are numerous studies among candidates or recipients of a liver transplant and their donors on their adjustment. Evidence suggests that ESLF is associated with adjustment difficulties including experience of psychological problems and poor quality of life among candidates or recipients of a liver transplant. However, findings have been contradictory regarding the extent of these difficulties partly due to different approaches that studies have taken to defining and measuring psychological problems and quality of life. Transplantation is associated with less psychological problems and improvement in quality of life, with more improvements in physical functioning and less improvements in psychosocial areas. However, although it can be argued that quality of life improves after transplantation, the ways in which this improvement continues over time are not clear. Some studies show that quality of life remains similar during follow-up, whereas other studies show subsequent deterioration. In studies which examine quality of life across different time points following transplantation, recipients with high mortality rates need to be accounted for to avoid bias.

There is also some evidence to suggest that contrary to recipients of a transplant, organ donation surgery is associated with deterioration in quality of life, particularly in physical functioning among donors and experience of psychological problems and poor quality of life among donors. However, findings have also been contradictory regarding the extent of these difficulties partly due to different approaches that studies have taken to defining and measuring psychological problems and quality of life.

As mentioned above, contradictory or inconsistent findings may be due to methodological problems. More specifically, studies have mainly used generic measures of quality of life [81, 85, 101]. Such measures may not be specific and sensitive enough to understand adjustment-related issues among recipients of a transplant or their donors. Moreover, studies which examined the long-term implications of liver transplantation and donation have assessed recipients and donors at different times after surgery [19, 84].

Evidence also suggests that high levels of psychological problems such as anxiety and depression negatively influence quality of life directly or as a mediator among recipients of a liver

transplant and their donors. One explanation for this evidence is that high levels of these problems impair quality of life directly or as a mediator by, for example, maintaining the sick role [60]. Another explanation is that anxiety and depression may reduce compliance with treatment, and this in turn reduces quality of life [61].

Despite numerous studies on the extent of psychological problems and quality of life, attempts to predict these outcomes have fallen short. There is little evidence to conclude from quantitative studies that particular factors predict outcome. Therefore, more research is needed. The construct of beliefs could guide future attempts to explain these outcomes. A review on adjustment in end-stage renal failure (ESRF) [118] shows that although the variance explained in outcomes by beliefs is small, beliefs have been more consistent in predicting these outcomes than other variables such as social support. One exception for these small effects is the beliefs postulated by the self-regulatory model of illness [119, 120] which is developed on the basis of interviews with patients suffering from different types of chronic physical illnesses. This model includes beliefs about identity, cause, consequences, timeline and cure or controllability of a particular chronic physical illness. Future research may examine the ways in which these beliefs predict these outcomes.

An alternative approach to better understand these outcomes is to be guided by qualitative studies. In terms of beliefs, only one qualitative study [113] examined donors' beliefs about ESLF, transplantation and organ donation surgery. More qualitative research is needed, in particular about recipients' beliefs about ESLF and transplantation.

Overall, qualitative findings suggest that candidates or recipients of a liver transplant and their donors experience ESLF and/or transplantation surgery or organ donation surgery and the process of organ donation in ways that are not identified by quantitative research. God's will, blaming oneself, blaming physicians as causes for recipients' ESLFl, doing the right thing, being healed as reasons for being a donor, the views that others are frightened of getting infected by ESLF and insensitive, experience of positive emotions, ways of improving, worsening aspects of character and close relationships are among findings which extend quantitative findings. These findings can be used not only to develop ESLF-specific quality of life or emotional well-being questionnaires but also patient- or donor-derived interventions to improve poor outcomes.

Conflict of interest

There is no conflict of interest.

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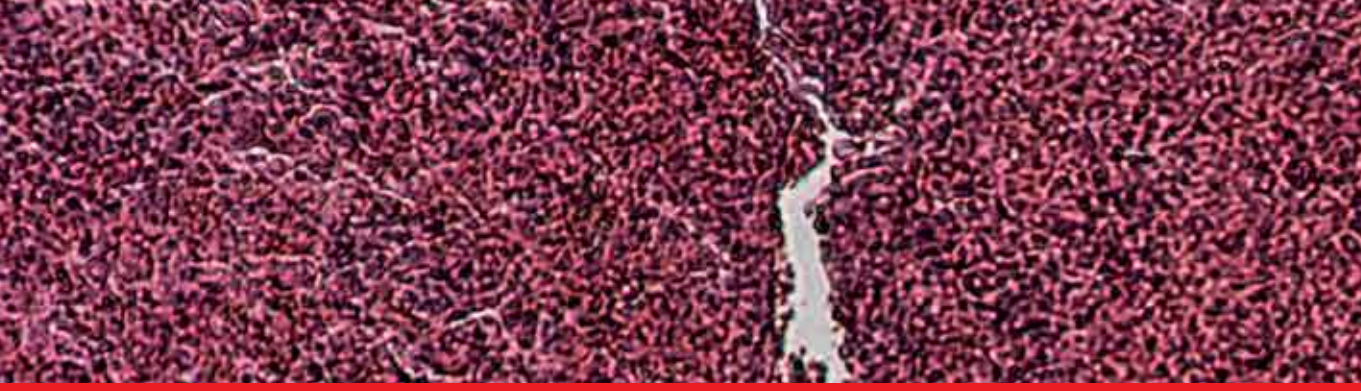

Edited by Luis Rodrigo

Hepatology is the medical specialty that studies the normal functioning of the liver and its diseases. It has experienced a steady progress in recent decades, as well as occurred in other medical specialties. It deals with the acute and chronic inflammatory processes of the liver, among which is the viral hepatitis. Recently, very effective drugs have been introduced in this field that achieves the elimination of the hepatitis C virus in the great majority of patients. Nonalcoholic steatohepatitis has increased markedly worldwide especially in Western countries in relation to overweight, diabetes, and other metabolic conditions. Cirrhosis and its complications are better managed, and patients live longer, thanks also to the earlier detection of hepatocarcinoma and the generalization of the use of liver transplants. This book deals with all of these interesting topics, thanks to the excellent collaboration of a great group of specialists that have collaborated with their knowledge and expertise in this edition.

Published in London, UK

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ISBN 978-1-83881-472-4