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Organ Donation and Transplantation

Current Status and Future Challenges

Edited by Georgios Tsoulfas

ORGAN DONATION AND TRANSPLANTATION - CURRENT STATUS AND FUTURE CHALLENGES

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Meet the editor

Dr. Georgios Tsoulfas received his medical degree from the Brown University School of Medicine and completed his general surgery residency at the University of Iowa Hospitals and Clinics as well as a transplant research fellowship at the Starzl Transplant Institute, University of Pittsburgh. He then completed a 2-year transplantation surgery fellowship at the Massachusetts General Hospital, Harvard Medical School, and then joined the Division of Solid Organ Transplantation and Hepatobiliary Surgery at the University of Rochester Medical Center as an Assistant Professor of Surgery. He has currently moved back to Greece, where he is an Associate Professor of Surgery at the Aristotle University of Thessaloniki. He has published over 70 papers in peer-reviewed journals and in PubMed as well as 17 book chapters. He is a reviewer of over 40 International Journals and is an Editorial Board member of several others.

He is currently the Vice Chair of the International Relations Committee of the American College of Surgeons and the World President of the International College of Surgeons.

Contents

Preface

One of the most interesting and at the same time most challenging fields of medicine and surgery has been that of organ donation and transplantation. It is a field that has made tremendous strides during the last few decades through the combined input and efforts of scientists from various specialties including surgeons, hepatologists, nephrologists, immunologists, ethicists, and infectious disease specialists. What started as a dream of pioneers has become a reality for the thousands of our patients whose lives can now be saved and improved. However, at the same time, the challenges remain significant and so do the expectations, for what was once an experimental treatment could eventually become the future of medicine, as it involves most organs including the heart, lungs, eyes, liver, kidney, pancreas, and small intestine.

This book with the contribution of an excellent group of world authorities in the field of organ donation and transplantation presents the challenges involved including the ethical, legal, and medical issues in organ donation and the technical and immunological problems facing experts involved in the care of these patients. In addition to the knowledge shared, the authors provide their personal clinical experience making this book an extremely useful tool for every scientist and physician practicing in the field of transplantation. The book is divided into a section dealing with donation-related issues and challenges and another one where the different types of transplantation are presented. The chapters include information on the current state and different types of donation, the challenges identified in increasing donation, and the potential solutions. Chapters in both the organ donation and the transplantation section provide us with a combination of the technical and surgical aspects, as well as a glimpse of the opportunities offered by molecular and basic science in achieving progress in the field of tolerance.

We can learn from the history of transplantation and, at the same time, tackle the remaining critical questions, such as achieving tolerance, which represents the Holy Grail of medicine.

Overall, this book represents a true tour-de-force of a variety of topics having to do with organ donation and transplantation. It should be stressed that the intended audience are scientists, physicians and surgeons of different specialties, which all have in common an interest in transplantation, improving the lives of their patients.

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Organ Donation

Chapter 1

Organ Donation

Young-Nam Roh

Additional information is available at the end of the chapter

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Abstract

Organ transplantation is the only way of giving the gift of life to the patients with organ failure; however, the inadequate supply of organs, especially from deceased donors, has created a wide gap between organ supply and organ demand. Many organs from deceased donors are still not being used worldwide because of lack of information, education, and social system. Effective systems such as opt-out, donation after circulatory death, and donor action programs are needed to promote deceased donations. Counseling on organ donations must be an essential step of families of brain-dead patients. Standard practice should include that physicians call an Organ Procurement Organization coordinator before meeting with the families of potential donors. Tight screening for potential organ donor in intensive care unit, decoupling, and professional counseling are key components. The authorities have to consider the establishment of an opt-out system, and social systemic efforts are needed.

Keywords: organ donation, deceased donor, promotion

1. Introduction

Progress in transplantation science and medicine has been impressive in the last decades. Nevertheless, transplantation activity is constrained by the shortage of organs. How can we maximize the utilization of organs that are abandoned from the deceased donors? The process for organ donation is a complex one involving medical, psychological, ethical, and social scientific aspect. Public opinion on organ donation and social maturity is also important factors for a stable and sustainable social system for organ donation. This chapter describes the essential knowledge, principles, and considering factors for the promotion of organ donation.

2. Living and deceased donations

The demand for transplants continues to increase with the increasing aging population and prevalence of renal failure. Thousands of patients on the wait list die annually, and the wait for an organ transplant has significantly increased due to the wide gap between organ supply and demand. Transplantation has become a consolidated therapy to extend or improve quality of life, an activity that constitutes less than 10% of the global transplant needs [1].

Living and deceased donations are two sources for organ transplantation. Each organ donation has its advantages and disadvantages. The advantages and disadvantages for kidney transplantation from living and deceased donations are listed in **Table 1**.

There are also ethical issues associated with each donation. In living donations, it is the safety of the healthy individual undergoing the surgical removal of an organ. This is associated with long-term consequences and affects donors' quality of life. Another important ethical concern is the motivation of the donor. The decision to donate is a psychologically complicated one. Living donors can be impacted by a feeling of moral obligation, not just pure altruism. In addition, there are issues surrounding the commercialization of organ donation and donor rewards. Deceased donations also have important ethical issues. In particular, who should be the one to decide on the donation in the absence of a declared opinion. Does the family have the right to decide? Deceased donations can also result from moral obligation. Financial and non-financial incentives for the families can also affect deceased donations.

Living donor kidney transplantation	
Advantages	Longer graft survival than deceased donation
	Short cold ischemia time
	Planned surgery
	Possible pre-emptive transplantation
	No waiting time
Disadvantages	Requires that the donor undergo major surgery
	Long-term donor safety concerns
Deceased donor kidney transplantation	
Advantages	No harm to the donor
	Possible options for patients without a living donor.
Disadvantages	Shorter graft survival than living donation
	Long cold ischemia time
	Long waiting time on list
	Requires an unplanned surgery

Table 1. Living versus deceased kidney donation.

The medical safety associated with living kidney donations is an ongoing issue. The premise of living donations of the kidneys is that the removal of one does not impair survival or long-term kidney function of the donors. Data have shown that live kidney donations are safe in northern European populations who underwent nephrectomy [2–5]. Nevertheless, Ellison et al. [6] identified 56 live kidney donors in the OPTN database who were subsequently listed for a kidney transplant. The rate of ESRD in donors (0.04%) is comparable to the rate in the general US population (0.03%). In a meta-analysis evaluating reduced renal mass in humans, Kasiske et al. [7] demonstrated that living donations were free of progressive renal dysfunction or an increased incidence of proteinuria. The data indicated little long-term medical risks in healthy donors after unilateral nephrectomy. However, it is recommended that before the donation, the donor receives a complete medical and psychosocial evaluation, provides informed consent, and is capable of understanding the information presented to ensure a voluntary decision.

Although living and deceased donations are important sources of organs for transplantation, a proportion of organs from deceased donors worldwide are not being used due to a lack of information, education, and social system. The use of organs from deceased donors could be significantly increased with the implementation of public education and social systems. Unlike the practical problems observed in living donors, the ethical issues associated with deceased organ donations occur post mortem and can be solved by social agreement and systemic supplementation. In addition to the efforts to increase living donation, a social infrastructure, including education and the creation of laws, should be established to promote deceased donations.

Most of the progresses made in modern transplantation were to overcome the organ shortage (**Figure 1**). Medical and surgical progresses include ABO-incompatible transplantation, en bloc transplantation, and using expanded criteria for donors. On a social level, progress includes the legalization of donations after circulatory death, an opt-out system, and donor action program. The establishment of these systems is needed to promote deceased

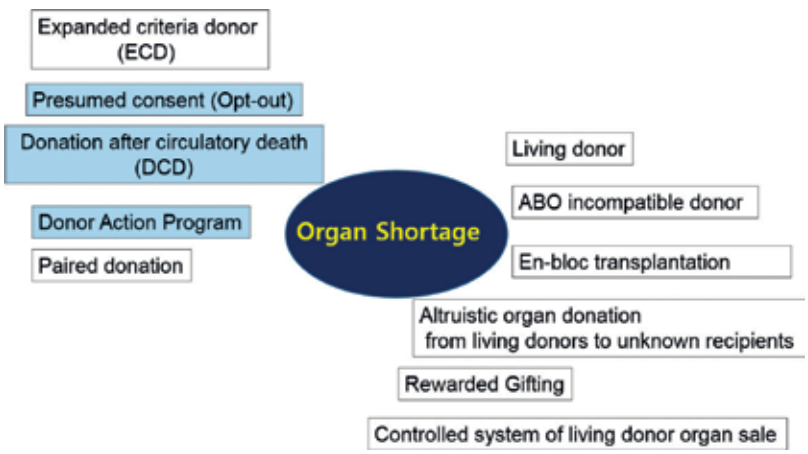

Figure 1. Measures used to overcome organ shortage.

donations. However, organ donation also needs to be socially accepted, and public opinion should change before the change of social system.

3. Deceased donation: Donations after brain death versus Donations after cardiac death

Organ donation has traditionally been possible only after brain death. It now includes donations after cardiac death (DCD), which is increasing in European countries, North America, and Australia. However, the majority of deceased donor organs continue to be from donations after brain death (DBD). DCD are from donors who do not meet the criterion for brain death, and whose cardiac function stopped before the organs were procured. The cessation of cardiac function could have occurred spontaneously or initiated deliberately. There are two types of DCDs, controlled and uncontrolled. In controlled DCD, the donor is withdrawn from life support and his or her family has given written consent for organ donation in a controlled environment. The clinical steps for controlled DCD are shown in **Figure 2**. In uncontrolled DCD, the donor died in the emergency department or elsewhere in the hospital before consent for organ donation was obtained. Catheters are placed in the femoral vessels to cool organs and infuse perfusate until consent can be obtained.

DCD now accounts for 17% of the 31,812 donors reported to the Global Observatory on Organ Donation and Transplantation in 2015 [1]. DCD is used in a limited number of countries, because of legislative and ethical obstacles, lack of technical expertise, and/or insufficient organizational capabilities [2, 8]. There are also differences in DCD practices, including differences in legislative and ethical frameworks, patterns of end-of-life care, and approaches for the treatment of patients with cardiovascular arrest outside of the hospital [9]. Although transplant outcomes from organs obtained from DCD donors are appropriate overall, they need improvement [9]. It is generally accepted that DCD can substantially increase the availability of deceased donor organs with optimal results.

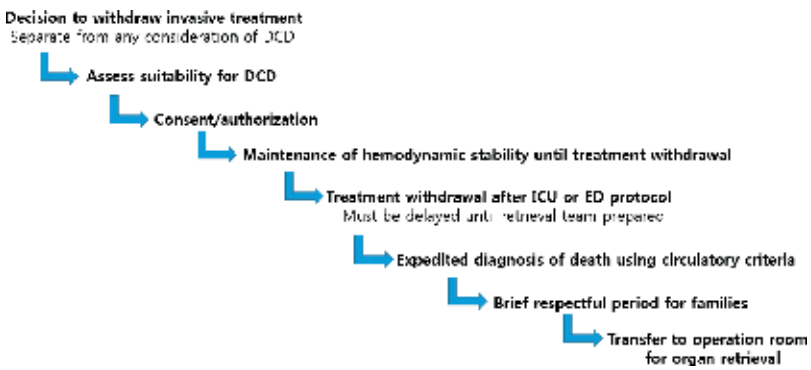

Figure 2. Clinical steps for controlled DCD.

4. Practical issues with organ donation after brain death

The clinical course of patients with severe brain injury varies depending on the degree of injury and the clinical decisions made by the primary physician. The latter are impacted by legislative and ethical frameworks, as well as patterns of end-of-life care. Organ donation is one of the options in end-of-life decision, which must be considered in every patient who may become brain dead (**Figure 3**). Organ donation counseling is an essential step that should be incorporated in end-of-life decisions.

Although the consent rate for organ donation in Europe is 50–80% with approximately 85% of families being requested to donate, only 50% provide consent. Other studies have confirmed these findings [10–14]. It is important to identify potential cases of brain deaths and obtain informed consent for organ donation from the families of the patients. Because most countries have an opt-in system, voluntary consent is considered an essential factor in organ donation. Only a small portion of these brain-dead donors are being used for solid-organ transplantation, primarily because of the low percentage of families who consent to donation [15]. Several studies have evaluated the factors associated with these types [1–4, 12, 14–16], which are listed in **Table 2**.

How to ask for an organ donation correctly is another important practical issue. The physician should call an Organ Procurement Organization (OPO) coordinator before meeting with the family of a potential donor and it must be a standard practice. Including an OPO coordinator in conversation is critical to successfully counsel families. Studies have shown that the time spent with an OPO coordinator is strongly associated with a family's decision to donate organs [15]. Incomplete or inaccurate information about the donation process may limit consent. Furthermore, the early involvement of an OPO coordinator is the best way to deliver complete and accurate information to families. Discussion of common fears and misinformation about organ donation should be part of the organ donation request process during counseling. Important questions families typically have regarding organ donation focus on the process, physical impairment during organ recovery, and the way the organs are used.

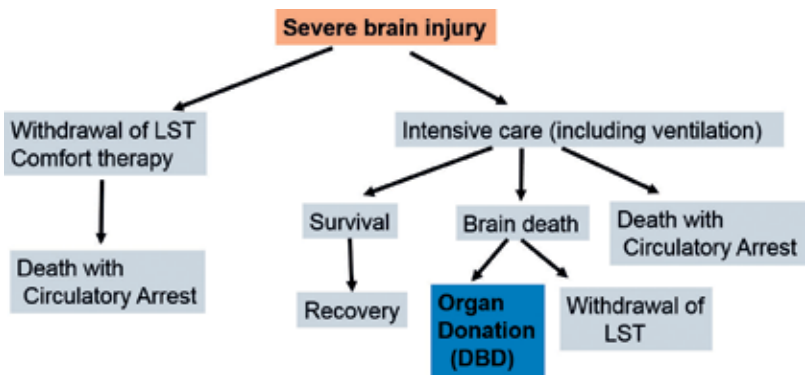

Figure 3. Clinical pathways of potential brain-dead donor. LST, Life-Supporting Treatment; DBD, Donation after Brain Death.

Patient factors
Age
Religion
Cause of death
Wish to terminate life support
Wish for organ donation
Health-care factors
Request factors
Timing and preparation for decision
Decoupling
Time to decide
Accurate information before decision
Behavior of care professionals
Care for patient and relatives
Supportive communication
Critical events before request
Respect for patients
Care professional's attitude toward organ donation
Family factors
Prior knowledge and opinion
Family culture
Religion
Education
Information about brain death
Information about organ donation
Opinion about who has to decide
Decision making
Emotional stress and grief
Family relationship
Agreement among relatives
Economic status
Financial incentives

Table 2. Factors affecting deceased donation.

In addition, incentives for organ donations are a topic of interest. Most physicians cannot deliver enough information about these topics to the families. The early involvement of OPO coordinators is easy, and a definite solution for this problem has been recommended in many studies. A physician must be accompanied by an OPO coordinator before beginning family

counseling, and precise information with supportive care must be given if the families need more information (**Figure 4**). Common reasons families refuse organ donation include the following:

1. Protecting and respecting the body
2. Fear that the surgery will disfigure the body
3. Belief that their loved ones have already experienced enough trauma
4. Concerns about the wholeness and integrity of the dead body
5. Wish to keep the body intact
6. Observation of a lack of respect for the deceased by the hospital staff
7. Gift of life is frequently considered by the relatives to be a sacrifice
8. Financial incentives do not influence the decision

OPO coordinators can counsel families on these specific topics. Families are often concerned about the physical impairment and pain sensation associated with preserving the donor's body or thinking that the donor will feel the pain. In addition, a significant portion of families believes that the surgery causes excessive physical damage. These are significant concerns associated with decision making in families. Therefore, it is important that the medical staff or a transplantation coordinator offers specialized information about this subject during counseling. Efforts to address families' concerns are an important step toward gaining consent to donate. OPO coordinators can provide the right information to families and address negative perspectives on organ donation (**Figure 5**).

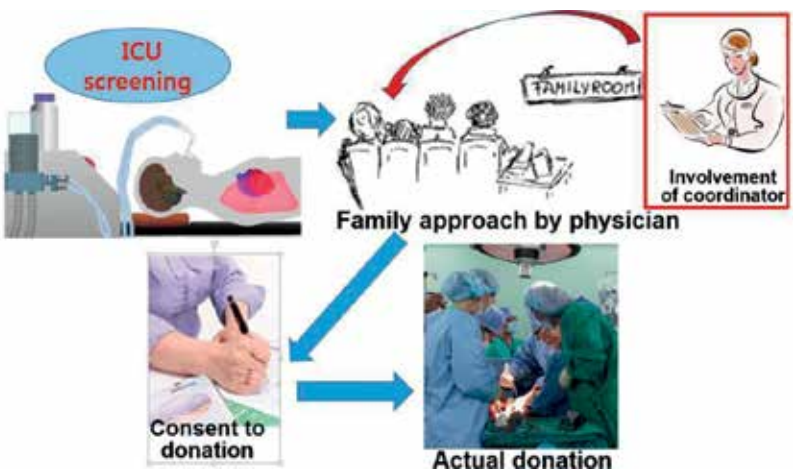

Figure 4. Early involvement of OPO counseling.

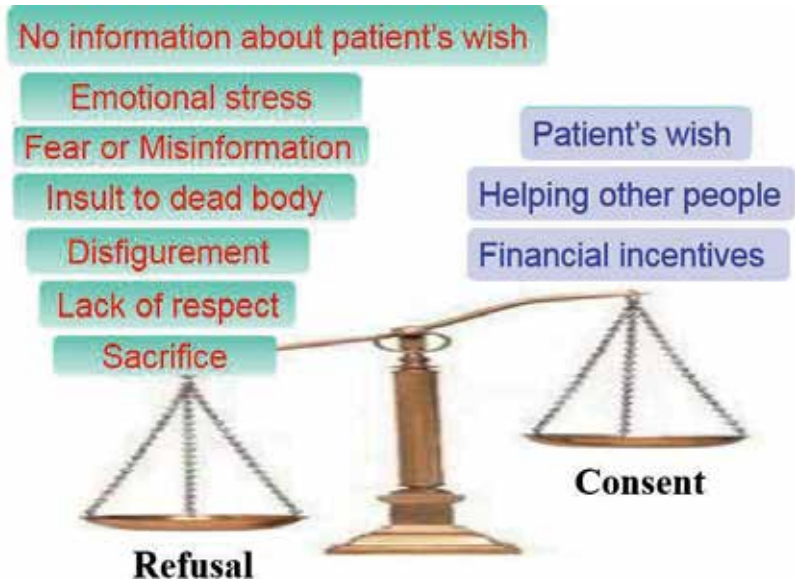

Figure 5. Key negative perspectives on organ donation.

The decision to donate is often forced on families during complex clinical situations, at a time when they may be shocked and stunned, and ill-equipped to make a decision [16–19]. It can be difficult to accept the death of a loved one, and many family members are not prepared to understand the medical concept of brain death because of emotional stress. In addition, one of the most stressful situations is when a family member has to make this type of decision without his or her previously specified opinion about organ donation. Even when counseling is done correctly, nearly half families refuse to donate. However, some of the families refuse to donate to avoid the request as a nonresponse. Frutos et al. suggest discussing organ donation as an option more than once with relatives who initially refuse or are unsure [20]. Relatives should also have the opportunity to spend time with the donor and say their final farewell. More than one-third of relatives regret declining to donate soon after the funeral [18].

Emotional upheaval in acutely bereaved families and lack of clarity on brain death cause dissonance and distress that adversely affect decision making in families and grieving over time [21–23]. Several factors have been shown to affect decision making in family members [12, 14, 15]. The complex situation and emotional stress make it difficult for families to understand the nature of brain death and accept the actual death of their loved one. This ultimately impacts the decision-making process regarding organ donation. Multiple factors negatively affect the decision to donate and lead to time delay for the final decision. A final decision may require several hours to days. This time delay, though justifiable, can be associated with the refusal to donate or failure of a successful donation. In one study, researchers reported that a delay in decision making does not reflect a negative attitude about organ donation, but a reasonable and necessary amount of time for deliberation [24]. Therefore, the medical attendant and OPO coordinator should continue their efforts to maintain organ viability and consider extended repetitive counseling to encourage donation.

Figure 6. Principle of decoupling.

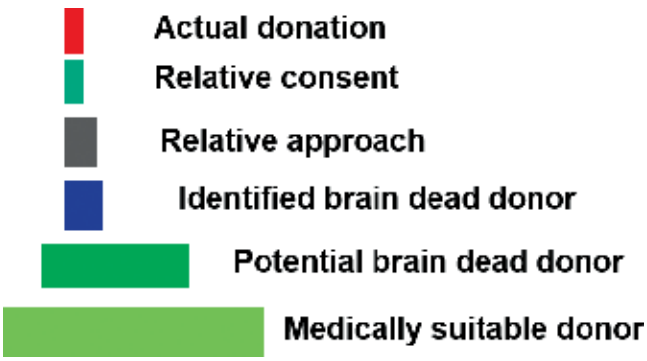

Figure 7. Multiple steps for organ donation after brain death in intensive care unit.

Decoupling is one of the best principles in which making a donation request is delayed until the family understands that brain death is the same as death and has the opportunity to realize that their loved one is dead (**Figure 6**). This principle of waiting to discuss organ donation until the family is ready to make end-of-life decisions is important to correctly timed request. The principle of decoupling is a well-known way to increase the consent rate for organ donations [25]. In a study by the Kentucky Organ Donor Affiliates in 1989–1990, researchers reported that the consent rate increased from 18 to 60% if there was a separation between when death is pronounced and the approach for organ donation [25]. However, decoupling frequently becomes impossible when the hemodynamics of a potential donor worsens. The patient's attending physician may feel an ethical conflict about providing active or invasive life-support care that seems to have no therapeutic benefit on the patient's recovery and appears to have significance solely for maintaining organ quality, especially when the family's opinion about organ donation is not specified yet. This frequently occurs in the emergency department or the intensive care unit [26]. In addition, decoupling is sometimes not consistent with the current recommendation of early referral to the OPO coordinator [26]. If we profoundly believe that there is value in organ donation, a more flexible high-dimensional strategy is needed when a potential donor is progressing to circulatory death.

Identifying a potential brain-dead donor is the fundamental step for a successful donor action program. The typical steps of actual organ donation in the intensive care unit are illustrated in **Figure 7**. OPO coordinators or transplantation teams typically identify only a small portion of potential brain-dead donors. If the OPO coordinator approached the families and appropriate counseling was performed, the families consent is an invincible one. A tight screening system must be established to increase the rate of identification of potential donors in the intensive care unit. The generally accepted criteria for potential deceased donors are shown in **Table 3**.

Every ventilated patient with
Glasgow coma scale of <5
Brain death test being considered
Do-not-resuscitate or comfort care being considered
Withdrawal of life support being considered
Family initiates conversation about donation
Within 1 h of every cardiovascular death

Table 3. Criteria for referral of a potential donor.

5. Social system for organ donation

Strategic efforts by the government and local authorities, as well as individual efforts by medical personnel, are necessary to promote organ donation. These include the revision of laws, simplifying the required procedures for receiving consent, expansion of the donor card system, adoption of a presumed consent concept known as an opt-out system, and the establishment of a DCD system. The strategic processes put in place in Europe and the United States have resulted in a progressive and gradual increase of organ donation [27–30].

Despite the effectiveness of these strategies, public acceptance of organ donation is essential before these measures can be implemented in other countries. The establishment of social systems for organ donation depends on public consensus. There are currently two moral values on organ donation: deontologism versus consequentialism. This means where we put our maximum value of some behavior, as it were, the legitimacy of process or the benefit of consequence (**Table 4**). Many procedural details in organ donation and recovery have points of conflict, which can be solved with social agreement.

Asking families for organ donations to families is generally regarded as a stressful task by primary physicians. Only a small portion of potential donors are being asked about organ donation as an option of end-of-life decision, and it is decided according to the primary physician's point of view or belief. Despite the important role of the medical staff in recommending organ donation to families, imposing this burden on physicians alone may not be adequate. If we, including local authorities and the general population, agree on the importance of organ donation, its promotion would not be the sole responsibility of individual medical staffs. The authorities have to consider establishing an advanced system that links potential donors to organ donations, known as an "opt-out" system. Many valuable lessons can be learned from the efforts of European countries to adopt it [28].

Religious beliefs were found to be important. Officially, nearly all religious groups support organ transplantation as long as it does not impede the life or hasten the death of the donor [31]. However, only a small portion of the public knows about the stance of their religion on organ donation. More active involvement of religious bodies is needed to raise the public's awareness and encourage organ donation.

Deontologism	Consequentialism
Moral principle	*Moral principle*
Duty or obligation-based ethics	Outcome-based ethics
Action or process is more important than the consequences.	A morally right act is one that will produce a good outcome.
A moral obligation may arise from rules	The end justifies the means
Donation system	*Donation system*
Donor's will	Social need
"Opt-in" system	"Opt-out" system
Informed consent	Presumed consent
Explicit consent	Implicit consent
No donor incentives	Donor incentives
Individual decision	Social campaign
Donor management after consent	Donor management before consent
Volunteering	ICU screening and family approach
Resuscitation for organs forbidden	Resuscitation for organs allowed
Femoral cannulation after consent	Femoral cannulation before consent

Table 4. Moral dilemma surrounding organ donation.

Asian countries have delayed the creation and adoption of social systems for organ donation. Despite the socioeconomic development of several Asian countries, the number of organ donations per million is extremely low, compared with western countries. In addition, most of the data on organ donation consent after brain death are largely based on findings from Western populations. The current opinion on organ donation after brain death is unclear in Asian countries. Traditionally, the body of a loved one should not be tampered with after death in Asian cultures, especially in Korean, Japanese, and Chinese. This belief originates from the Confucian tradition, and it is believed that this tradition may be the main reason for the low consent rate of organ donation in Asian countries. In addition to cultural differences, it is thought that widely differing opinions, perceptions, and concerns may be related to low frequency of organ donation in Asia. However, these factors are not well studied. The general opinion on brain death and organ donation appears to be quite positive in Asia [24, 32]. The perception of brain death as death is widely accepted [32]. However, there were several perceptional barriers against organ donation in Asia [32]. Evidence-based strategies focused on these barriers should be established to increase the rate of organ donation effectively.

6. Summary

- Living and deceased donations are two sources of organs for transplantation. Each type of donation has its advantages and disadvantages.
- Many organs from deceased donors are still not being used worldwide because of lack of information, education, and social system.

- Effective systems such as opt-out, donation after circulatory death, and donor action programs are needed to promote deceased donations.
- DCD is developed in a limited number of countries, because of legislative and ethical obstacles, lack of technical expertise, and/or insufficient organizational capabilities. It is generally accepted that DCD can substantially increase the availability of deceased donor organs with optimal results.
- Counseling on organ donations is an essential step for stable end-of-life decision of families. Standard practice should include that physicians call an Organ Procurement Organization (OPO) coordinator before meeting with the families of potential donors.
- Delays in deciding on organ donation do not reflect a negative attitude, but a reasonable and necessary time for families to deliberate.
- Decoupling is important to properly timed organ donation requests. However, a more flexible high-dimensional strategy is required when the potential donor is progressing toward circulatory death.
- A tight screening system must be established to increase the rate of identification of potential donors in the intensive care unit.
- The authorities have to consider the establishment of an opt-out system.
- More active involvement of religions is needed to encourage organ donation. The participation of religious societies in public campaigns would also be helpful.

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Chapter 2

Organ Donation and Transplantation: "Life after Death"

Kanmani Job and Anooja Antony

Additional information is available at the end of the chapter

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Abstract

Organ donation is defined as giving an organ or part of an organ to be transplanted into another person. Organ transplantation is the only option to save lives in patients affected by terminal organ failures and improve their quality of life. However, there is a disparity exists between the supply and demand of donated organs, leads to a loss of many lives. The number of organ transplantation have gradually increased in the last two decades and provide excellent results in children and young adults, and are challenging by the growing proportion of elderly transplant patients with co morbidity. The results of organ transplantation continue to improve, as a consequence of the innovations and the improvements in peri-operative management. This chapter describes organ donation and transplantation and its trends and challenges.

Keywords: organ donation, motivation, psychosocial

1. Introduction

Organ donation is defined as giving an organ or part of an organ to be transplanted into another person. Organ transplantation is the only option to save lives in patients affected by terminal organ failures and improve their quality of life. However, there is a disparity exists between the supply and demand of donated organs, leads to a loss of many lives. The number of organ transplantation have gradually increased in the last two decades and provide excellent results in children and young adults, and are challenging by the growing proportion of elderly transplant patients with co morbidity. The results of organ transplantation continue to improve, as a consequence of the innovations and the improvements in peri-operative management.

Organ transplantation currently depends on the availability of human organs. Their scarcity means that there is a waiting list of almost 63,000 in the European Union, and over 100,000 people in the United States according to the recent survey. The process of obtaining organs for donation and transplantation purely depends on the resources of health services and by health professionals' performance in potential donor identification and management tasks. However, in accordance with the current legislation it is mainly subjected to a personal or family decision, strongly mediated by psychosocial processes. Therefore, the need to analyze and intervene both in the practices of the professionals involved in the process of organ generation and in the attitudes of the general population need to stressed and addressed [1–5].

2. Organ transplantation and organ donation: an overview

Organ transplantation involves the surgical implantation of an organ or section of an organ into a person whose own organ is failing. The donor organ may come from both deceased individual as well as from a living donor. The patients psychological and behavior aspect as well their emotional response and mental health and adherence to medical regimen should be assessed before and after organ transplantation. The living donor's psychological response towards organ donation (most commonly for kidney and liver segment transplantation) is an important aspect to consider in the transplantation process.

Organ donation is defined as "giving an organ or part of an organ to be transplanted into another person" (Organ procurement of Transplant Network (OPTN), 2015), organ donation has the potential to save lives. The organs donated from one single donor can save up to eight lives. Organ transplantation may be one of the options left to sustain someone's life. However, the disparity that exists between the supply and demand of donated organs, leads to a loss of many lives. Based on recent OPTN data, approximately 21 people will die each day while waiting for a transplant in the United States (US). Currently, 123, 358 people are awaiting organs and on the transplant list in the US with this number growing and the number of donated organs declining.

Asian Indians are more likely to have higher rates of having obesity and diabetes when compared with other Asian subgroups which make them at an increased risk of needing a donated organ [35]. These conditions can lead one to develop coronary artery disease and hypertension which then can lead to chronic kidney disease and other chronic illnesses. Patients who suffer from chronic kidney disease need regular dialysis which can ultimately lead them to organ transplantation to improve one's quality of life. Also, conditions such as diabetes and obesity can be detrimental to one's life and can lead to fatty liver disease which can lead to chronic liver disease requiring liver transplantation if the liver decompensates.

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The development of organ transplantation in the second half of the 20th century has been a remarkable achievement. Recently; organ transplantation is one of the most effective options for those with an end-stage organ failure. Its success has been basically dependent on public awareness, support and active participation. Without these factors, the efficiency of organ transplantation and the consequent saving or extension of lives would have undoubtedly suffered adversely.

The number of patients in need of organ transplantation has increased at a rapid pace; in contrast, the number of available organs has increased only slightly. Expanded criteria for donor selection, such as older age, have resulted in more people who meet the criteria for brain death becoming organ donors although fewer organs are transplanted from each donor. Improvements in automobile and highway safety, as well as increased enforcement of gun control laws, have also contributed to a plateau in the number of young, healthy donors. Public education efforts that encourage organ donation may be effective in getting more people to sign organ donor cards, but most individuals who do so will never be in a position to become organ donors.

Faced with increasing numbers of patients who need transplantation, deaths on the waiting list, and a fixed number of available organs, some transplant programs are working to increase the number of transplants from living donors. Although living donation has always been an option for some types of transplants, many programs have been reluctant to promote it, as living donation requires invasive surgery on a healthy person with associated risks of morbidity and mortality. For example, since dialysis is an option for patients with end-stage renal disease, surgery on a healthy donor may be difficult to justify, despite the dialysis patient's diminished quality of life.

The most important in organ donation is to maximize the psychological status and well-being of the donors before and after transplantation has become the foremost goal of all transplantation centres. The psychological issues that mainly concern with the living organ donation includes prevention of psychological harm, ensuring the donors are fully informed and decide to donate without coercion, monitoring donor psychosocial outcomes are intimately linked to the factors that historically served as barriers to use of organs from living donors. These barriers can be overcome by the motivating of the public and creating awareness and responsibility among oneself.

Organs that can be transplanted from the living donor includes one kidney, part of intestine, pancreas, islets of Langerhans, bone, part of liver, one testis, bone marrow and blood. The organ that can be transplanted from the deceased donor are heart, kidney, pancreas, stomach, hand, skin, blood vessels, lungs, liver, intestine, testis, cornea and heart valve.

Types of organ donation

1. **Autograft:** Transplanting a person's tissues from one site and use it in another site of his body and is called autograft. For example, removal of skin from the legs and using it for damaged skin face or other exposed part.

2. **Allograft:** Transplant of an organ between two genetically non identical individuals, it is called allograft. Due to the genetic difference, the donor's organ will be treated as foreign by the recipient and will try to destroy it. This is called s rejection.

3. **Isograft:** Transplant of organ/tissue from a donor to genetically identical recipient is called isograft. There will not be any immune response hence no transplant rejection.

4. **Xenograft:** Transplantation of organ/tissues forms one species to another species. For example, the heart valve of pig is transplanted successfully to human.

5. **Split transplant:** An organ like liver retrieved from the deceased donor can be divided between two recipients, usually an adult and a child.

6. **Domino transplant:** When the lungs are to be transplanted, surgically it is easier to replace them along with the heart. If the recipient's original heart is healthy, it can be transplanted into another recipient in the need of one.

7. **ABO incompatible transplantation:** The immune system of young children aged below 12 months might have developed fully. They can receive organs from incompatible donors.

Types of donors

1. **Live donors:** A living person, mentally and physically healthy can donate one of a paired organ, part of an organ or a tissue. The organs donated are kidneys, part of live, one of the lung, part of small intestine, skin, bone marrow, one of the testis and one of the ovaries. Live donor can either be related or unrelated.

2. **Unrelated donors:** For altruistic reasons, a person can donate one of his organs to an unrelated donor. According to TOHO act, the unrelated donor should be known to the recipient and have some obligation to him. It has to be established that there is no monetary transaction between them. But in many other countries, even a stranger can donate one of his organs to a needy person on altruistic grounds.

3. **Deceased donors:** Organs are harvested from brain dead person whose respiration and circulation are maintained artificially. Brain dead has to be certified by a team of doctors nominated by Government I every organ retrieval centers.

4. **Paired exchange:** When a living donor is not compatible with the related recipient, but may be compatible for another recipient. That second recipient related donor is compatible to the first recipient, then permission can be granted for transplantation. The surgery for all four donors and recipient are conducted simultaneously and anonymity is kept until after the transplant.

5. **Spousal donation:** A spouse can donate an organ to the partner. It has to be recorded that the couple is legally married.

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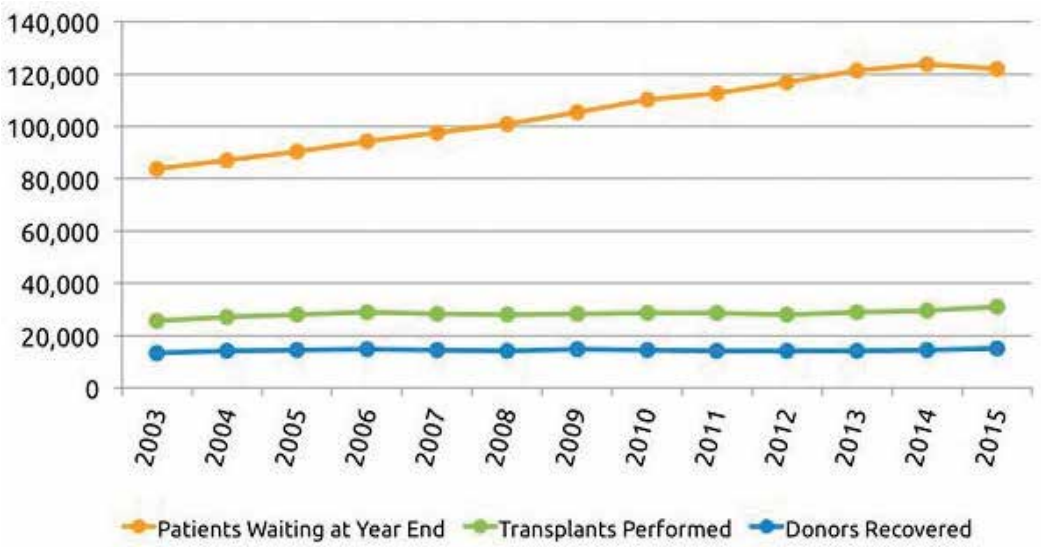
3. Current scenario: trends



Despite advances in medicine and technology, and increased awareness of organ donation and transplantation, the gap between supply and demand continues to widen. Each year, the number of people in the waiting list is increasing in both donor and transplant. The donation statistics according to OPTN Annual report shows that in 2016, total of 41,335 organs were donated. It can be either deceased or living and four out of five donations came from deceased donors and four out of ten from living donors. According to the report by OTPN 2018, 115,033 people need life-saving organ transplant, of those 74,926 people are the active waiting list candidates.

The real reason behind a living person's interest in donating one's organ is important to determine but it is often difficult. Now days, money has become the motivation for donation. The relationships also have played a great role in increasing donation rates. The shortage of available organs can be reduced if; people choose to donate their organs after they die. If more people did that the issue regarding organ shortage can be minimized.

The trend is expected to accelerate each year. Many organ procurement and the Joint Commission on Accreditation of Healthcare Organizations actively participate to increase the donation rates. The organizations take various to steps against traditional social taboos.

The approach, known as "donation after cardiac death" (DCD), usually involves patients who have suffered brain damage, such as from a car accident or a stroke. After family members have made the difficult decision to discontinue a ventilator or other life-sustaining treatment, organ-bank representatives talk to them about donation. Sometimes, the donor is suffering from an incurable disease also end up with the decision of organ donation.

According to U.S Department of Health and Human Services, more than 1,23,000 men, women and children currently needed life-saving organ transplants every 10 minutes and another name is added to the national organ transplant waiting list. In 2014, more than 8500 deceased donors made possible approximately 24,000 organ transplants. In addition, there were nearly 6000 transplants from living donors. In India, nationally with a population of 1.2 billion people, the statistics stands 0.08 persons as organ donor populations. Mrithasanjeevani, Kerala network of organ sharing which began in 2012, also states that the need for organ transplantation is high as the patients in waiting list is increasing day by day who requires organ transplantation.

The need for organ has gone up substantially all over the world. India also suffers from acute organ shortage with little to no solution for this issue. It is estimated that every year 1.5 lakh people suffer from renal failure out of which only 3000 people get donors. Similarly, every year around 2 lakh people die of liver failure or cancer and rarely get any help in the form of organ donors. It is the same for heart patients, for every 50,000 heart attack patients there are only 15 hearts available for transplant. Therefore, there is an urgent need for widespread campaigns to spread awareness about organ donation in India and to bridge the gap between supply and demand. The numbers that are mentioned here are estimates and real numbers could be far more than this, it is scary because this means very few people get relief and get a second chance in life.

The main reasons for organ shortage in India are mainly ignorance and lack of knowledge. People are not well informed enough about the benefits of organ donation. Today social media and so many other forums can promote the positives of organ donation and how it will save so many lives if more people register themselves for organ donation. The reason for organ shortage is myth and superstition. Many people do not want to donate their organs even after death because of so many myths and superstition they are instilled with. People with existing medical condition or old people, who wish to donate, do not donate thinking they are not fit or eligible. Almost everyone can donate some part or the other unless you have any extreme medical condition.

The need for organ donation is necessary because out of the 1.5 lakh people who need kidney in India only 3000 people receive them, only 1 out of 30 people receive kidney and 90% of people in the waiting list die without getting any donor. Around 70% liver transplants are dependent on a live donor but 30% dependent on cadaver (corpse) donations. Hence, there is an urgent need to increase the organ donation rates and give a person a second chance in their life.

4. Challenges in organ donation

As far as the challenges concerned it includes mainly donor's motives for donation, the predominant ways in which donors arrive at the decision to donate, and the donors' psychological status and its relationship to their fitness as donors.

4.1. Pre-donation challenges

4.1.1. Donor's motives

Most donors are likely to be motivated by multiple factors. These factors include intrinsic factors (e.g., desires to relieve the suffering of another or to act in according to the religious convictions) and extrinsic factors (e.g., the social pressures or perceived norms) that may operate simultaneously. The particular combination of motivational forces will also differ depending on whether and how the donor is related to the recipient.

Among living related donors, it has long been assumed that family members or emotional partners are motivated primarily for saving the lives of their loved ones. Such motives are indeed the most commonly expressed feelings, as noted in a variety of studies over the past 30 years. Among nondirected living donors (individuals donating to unrelated patients whom the donors did not select)(NDLDs), it was identified as the altruistic/humanitarian motives, along with beliefs that the donor's self-worth would be improved, and feelings of moral and religious obligation or self- identity.

4.1.2. Donor's decision-making

The motivation for the organ donation is purely on the donor's decision of organ donation and it may be influenced by many factors including the relationship to the recipients. Decision-making swiftness may indicate the type of decision being made. There appear to be two decision-making approaches that include the moral decision making and the rational decision making. "Moral decision-making" involves awareness that one's actions can affect another; ascription of responsibility to oneself; acceptance of the social/moral norm governing the behavior; and taking action consistent with that norm. Because moral decision-making does not involve the costs and benefits of a given behavior but, instead, is based on perceived norms governing that behavior, it is likely to lead to non- deliberative, instantaneous decisions. In contrast, "rational" decision-making includes various steps that focus on gathering relevant information, evaluating alternatives, selecting an alternative, and implementing the decision.

4.1.3. Support

It includes mainly the assessment of the donor's available physical, financial and emotional support. It is necessary to identify whether the donor have someone to provide care in the recovery period, have sufficient financial support and so on. This important to avoid distress if the donor develops any complications. Finally, does the donor have the support of significant others for being a donor, or is he or she choosing to donate over the objections of persons who have a legitimate interest in the outcome of an autonomous decision.

4.1.4. Family attitudes toward donation

Spouse and family attitudes about donation should also be explored. Collateral interviews with significant others is necessary, especially those who will be providing tangible support to the donor during the recovery period, should be conducted whenever possible. Conflicts

between potential donors and significant others should be addressed and, ideally, resolved prior to surgery itself in order to avoid conflicts later. Family members should provide a good understanding of the donor's wishes and motives, even if they agree to disagree to the donor's decision.

4.1.5. Behavioral and psychological health

The behavioral and psychological health of the donor should also be considered before donation. It is important to identify donor's lifestyle is sufficiently healthy to reduce unnecessary risk for both donor and recipient. Many potential donors may have some unhealthy behaviors, such as moderate obesity or smoking. It is necessary to identify that there is sufficient time for the donor to reduce risks (e.g., lose weight, stop smoking). Moreover, it needs to be taken care of that the donor is emotionally stable to cope with stresses which may come up before, during, and after the donation. Hence it is important to identify psychological and behavioral status of the donor or else it may affect the quality of life.

4.1.6. Donor-recipient relationship

The relationship between the donor and recipient is a complex matter. Even when both parties are agree for donation and transplant, family dynamics may be complicated, and other family members may assertively involve themselves in the decision-making process. The donor may have unrealizable expectations that transplant will alter his or her relationship with the recipient. The health care team should not expect an ideal relationship in which all interactions between donor and recipient are harmonious. However, obvious tensions and overt psychological issues should be addressed. Joint interviews, involving both donor and recipient, should be avoided early in the evaluation process in order to preserve privacy and give the potential donor the opportunity to express reservations or "opt out" gracefully.

4.1.7. Diversity issues

Non directed donors may have diversity concerns that may affect the organ donation. The potential donors should be assessed for comfort with donation to recipients of different genders, races, religions, sexual orientations, nationalities, ages, underlying diseases, and lifestyles. Donors who express objections, fears, or concerns about who might receive their organ may need to be deferred until they can receive counseling.

4.1.8. Psychological status of potential donors

The potential donor's psychological status is of greatest concern for donation and transplantation. Concerns have been particularly high in case of unrelated donation (either directed to a specific patient, or NDLD): the willingness or desire to donate to a stranger has been historically viewed with suspicion and as likely to reflect significant psychopathology. There is no doubt that some potential donors will be psychologically poor candidates to serve as donors.

4.1.9. Post-donation challenges

The donors' perceptions of their physical functional, psychological, and social well-being were found to be either nonsignificantly different from or significantly better than levels reported in the general population. The post challenges mainly includes recipient death or graft loss, donor medical complications, donor history of mood or other psychiatric problems, and poor donor relationships with recipient or family. The other factor is that it may affect the donor's quality of life if any complication arises.

The post transplantation challenges are many which include minimizing rejection risks, immunosuppression, organ shortage, handling of the stressors of transplantation, psychosocial adaptation and psychological disorders and so on.

4.1.10. Minimizing rejection risks

The twin conditions of antibody sensitization and antibody-mediated rejection remain challenging and frustrating to treat. The recent drugs which are used to desensitize patients or reverse antibody-mediated rejection, especially chronic antibody mediated rejection is totally unsatisfactory. Development of therapies those are more effective and less toxic should be made available. Recent regimens used for antibody desensitization and reversal of antibody-mediated rejection include plasmapheresis, immunoglobulin (IVIG), and rituximab, an anti-chimeric, anti-CD20 antibody. Recently, the proteasome inhibitor Velcade has also been reported to reverse refractory antibody rejection. Eculizumab, a humanized anti-C5 monoclonal antibody appears to protect the renal allograft despite the presence of donor-specific antibodies (DSA). None of these agents have been tested in rigorous studies.

4.1.11. Immunosuppression

This is one of the major challenges after organ transplantation. Many studies have suggested that most of the late graft loss occurs because of immunologic reasons, frequently antibody-mediated. So the approach of minimizing immunosuppression is necessary with the present drugs to reduce toxicities may actually be helpful in the long-term survival of the graft. The toxicities are minimized by allowing more grafts to be rejected by immune mechanisms. Hence, development of effective agents that lack long-term toxicities so that we can maintain optimum immunosuppression over the long-term.

4.1.12. Stressors after transplantation

In the perioperative period, the focus is on the patient's physical recovery, with possible rejection episodes and other medical complications causing anxiety and emotional strain. Within the first days after transplantation, a postoperative delirium can occur. The patient can present with symptoms of mental confusion, language disturbances, and occasional hallucinations and delusions are often a frightening experience to patients and their families. Acute brain dysfunction can occur in intensive care patients and patients after surgery. The corticosteroids which are administered for immunosuppression cause these problems. Some of the

patients experience problems in accepting the new organ from another individual and suffer with feeling of guilt towards the donor which, in turn, can increase psychological stress and nonadherence [6–11].

In the long-term postoperative period, medication side effects and associated comorbidities become central stressors impeding patient's life quality. Most common comorbidities seen are infections, diabetes mellitus, hypertension, lipometabolic disorders, adipositas, cardiovascular diseases, oncological diseases, osteoporosis, and chronic kidney failure [12, 13]. Furthermore, psychiatric symptoms (e.g., depression, anxiety, agitation, psychosis) and neurological symptoms (e.g., sleep disturbances, cognitive impairment, delirium) can occur as neurotoxic side effects in patients receiving immunosuppressive drugs.

Faced with the multiple health risks, patients often continue to experience anxiety and worries regarding possible retransplantation, serious comorbidities, and death. Even patients in good physical health are confronted with severe challenges, for example, regaining their previously lost or restricted social roles as family members and partners (including sexual activity) and returning to work or taking up other meaningful activities. Financial constraints and legal disputes with health or pension insurance agencies constitute other possible sources of psychological strain.

5. Psychosocial adaptation and psychological disorders

After the transplantation, the psychosocial burden more severe in preoperative period than postoperative period. Nevertheless, patients themselves have to demonstrate considerable coping skills. In the best case, transplant patients learn to adapt to their new situation, often by reevaluating life goals and by focusing on more positive consequences, for example, personal growth. On the other hand, unsuccessful readjustment can lower the quality of life and psychiatric morbidity. The most common psychological disorders among patients before and after transplantation are affective and anxiety disorders.

The literature review shows that prevalence of depression in 20–25% of cases before and after kidney transplantation. Less information is available concerning patients receiving other organs. Prior to and following lung transplantation, depression seems to be prevalent in approximately 30% of patients. Hence these show that the depression is a major challenge after transplantation. These issues can be reduced by personal and social resources (resilience factors), that is, favorable coping skills, self-efficacy, sense of coherence, optimism, and social support.

6. Factors affecting donor's motivation

There are many factors affecting donor's motivation which includes feelings of love and responsibility, spiritual motives, and greater success rate of organ donation.

6.1. Feelings of love and responsibility

Motives for donating organ to their relative patients were that they tended to do something for their loved ones. In fact, they feel responsible for their problems. They do not treat others' problems with indifference and attempted to do whatever they could for resolving the problems experienced by transplant recipients. It is considered as their own responsibilities to help them to get rid of their problems. The feel like they are the ones who need to support their patients.

6.2. Close and constant companionship

Another factor affecting the participants' feeling of responsibility for donation to their family members was close and constant companionship with recipients. This close and constant companionship made the participants to clearly understand the recipients' conditions and hence, it had resulted in their decision on organ donation in order to alleviate recipients' problems. This close and constant companionship with patients help family members understand patients' problems well and increase their degree of commitment to do something for patient's pain and discomfort. They also noted that this had made them experience deeper shared emotions with their patients and hence, required them to feel responsible for minimizing their patient's problems.

6.3. Inability to tolerate recipient's discomfort

Another motive for organ donation was one's difficulty in tolerating recipient's discomfort. Love for their sick family members had made the participants feel responsible and decide on doing something for solving their patient's problems. Their patient's pain, suffering and discomfort cause a great inconvenience and irritation which lead them to the decision of organ donation. They hoped that organ donation alleviate their patient's problems [14–34, 36].

6.4. Spiritual motives for donation

Religious beliefs played a significant role in motivating to organ donation. Some of them believed that donation was a way for expiating their past sins. They referred to faith in God, reliance on Him, and hope for a successful transplant as the important motives for organ donation. Some of them even accused themselves of causing their family members to develop organ failure and believed that donation was a way for alleviating their feelings of guilt. Such a practice was particularly common among the parents of sick children. Some of them considered donation as a God-approved practice, and noted that God has helped them donate their organs. They noted that they donated their organs for gratifying God and believed that he sees it and help them in all bad situations.

6.5. Greater success rate of organ transplantation

The category is the greater success of organ transplantation. In other words, obtaining information and realizing the greater benefits of organ transplantation had motivated the participants to

opt for organ donation. Some of them reported that they had never thought about donation until obtaining information from their patient's physicians. However, after obtaining adequate information, they had made an irreversible decision about organ donation. Accordingly, a major motive for organ donation was the lower likelihood of organ rejection.

7. Measures to overcome challenges for organ donation

The decisions regarding organ donation based on the personal beliefs (religious, cultural, family, social and body integrity) levels of knowledge about organ donation and previous interaction with the health care team. Many maintained positive attitudes to organ donation despite significant reservations about the organ donation process. Resistance to organ donation found to be less in the case of living donation for family.

There are some religious beliefs that can have both positive and negative influences, these often stemmed from uncertainty or misrepresentation of religious edicts. One solution would be to actively engage religious leaders in the transplant community, especially when it has been reported that, across the major religions, there are very few cases where organ donation can be seen to be inconsistent with religious beliefs. Religious leaders should be made available in hospitals and other transplantation setting to assist families in making decisions regarding organ donation and potentially to remove the misperceptions. Staff members who are involved in approaching families to request consent for donation should be part of the awareness programs and resources about religious concerns. Similarly, cultural sensitivity to issues such as apprehensiveness to discuss death among certain groups or individuals and the importance to many of death rituals may improve dialog regarding organ donation.

Studies have shown that engaging some minority groups in the health care system and creating a sense of belonging and ownership can improve compliance with organ donation. As a consequence, more efforts should be made to create positive interactions within the health care team members, especially for minority groups, to improve the organ donation rates. Although many of the studies have showed that higher socio-economic status and education were associated with a stronger willingness to be an organ donor. Some of the strong reservations held, even among those with generally positive views towards donation, such as concerns that agreeing to donation would discourage doctors from caring so much about saving their lives in case of an emergency or that it would result in the premature removal of their organs or indeed prevent them from having an open coffin at their funerals, are examples of very real barriers that can be readily addressed through information. Through a proper awareness and motivation the donation rates can be improved which can save many lives.

7.1. Psychological care

Psychological consultation is essential for all disease stages enabling patients to better cope with their extraordinarily stressful situation. A need for psychological care was found in up to 50% of transplant patients. Educational and supportive therapies are of utmost importance but also cognitive-behavioral interventions including relaxation techniques can also

be considered. Less common methods like hypnotherapy and "Quality of Life Therapy" have also been utilized for overcoming the challenges.

Moreover, family members as well as caregivers of transplant patients show increased psychological strain before and after transplantation. Family counseling, and psychotherapeutic support, can help reduce psychological strain, thus also maintaining the valuable social support provided by care givers and family members of the transplant patient. Henceforth, the family and care givers should also be considered in psychosocial evaluation to overcome the problems.

7.2. Alternative methods to increase donation

In view of ethical, legal and political issues, it was deemed important to obtain some opinion about alternative methods to increase organ donation rates. Financial incentives were given to increase organ donation. Many in both donor and non-donor groups were given a reasonable incentive. Education and dissemination of information about donation and transplantation was important to increase organ donation rates. There was nearly universal agreement that implied consent (presumed consent) should not be tried. The use of financial incentives was not markedly opposed (some accepted the idea of funeral expense reimbursement), although there was not strong support either. In general, methods to increase organ donation had not been well thought out by either donors or nondonors indicating, perhaps, that the assumption of altruism or motivation is the best way to increase the donation rates.

8. Responsibilities of nurses in organ donation and transplantation

Organ and tissue transplant nurses need comprehensive and scientific knowledge. They include the evaluation and management of deceased donors, transplant recipients, potential donors or live donors, teaching and counseling of transplant recipients and live donors related to self-care management, healthy life and a peaceful death when this is imminent. This is important in order to improve the posttransplant quality of life.

Nurses have important role in the development of a successful transplantation program. They are key members of the team that works to deliver care to patients and relatives, through the use of technological, logistic and human resources, with a view to coordination, care, education and research on organ and tissue donation and transplantation. Therefore, the nurses need adequate knowledge on the principles of good ethical principles and should have resources available for them to assess patient's risks and social issues related to organ transplants and donation. The researchers hope that the future studies will encourage further researches on the role and responsibilities of nurses.

9. Conclusion

The organ donation decision is a complex one, based strongly on personal beliefs. There are some factors, such as religious and cultural beliefs, that are seemingly intractable and are often

cited as reasons for a refusal to donate. In this chapter, it is shown that these have often been found to be tied in with more complex issues such as a distrust of the medical system, misunderstandings about religious stances and ignorance about the donation process. Interventions to better engage the community, including disadvantaged and minority groups, to foster trust and provide information represent promising opportunities of promoting organ donation in the future.

Donor motives directly contribute to their decision to donate, is not uniform and is influenced by multiple factors. Majority of the donors were relationship oriented donor, whose major motives were desires to relieve the suffering & save the life of their loving ones. Creating awareness to the organ donation will directly influence the donor motives and willingness. By deriving the motives many more intervention to improve the willingness to be a living organ donor can be evolved. Recruitment of living donors represents a medical and moral responsibility. The possibility of organ removal from healthy donor to a recipient needs great inner motivation. Saving one's life is divine.

The psycho social assessment must be made as a routine part of the nursing process. These assessments are meant to identify patients at risk for poor outcomes, provide guidelines for their management and improve the post-transplant quality of life [6]. "Because donated organs are a severely limited resource, the best potential, recipients should be identified. The probability of a good outcome must be highly emphasized to achieve the maximum benefit for all transplants" (OPTN/UNOS Ethics committee General Considerations in Assessment for Transplant Candidacy White paper-2010).

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Chapter 3

Procurement of Abdominal Organs in Multi-Organ Donation in Deceased Donor

Bulang He, Xiuwu Han and Michael A. Fink

Additional information is available at the end of the chapter

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Abstract

Organ procurement is an essential step for organ transplantation, from which a quality organ is received for subsequent transplantation. As the demand for organ transplantation continues to grow, multi-organ donation including the heart, lung, live, pancreas, kidneys, and small intestine from one potential donor is always a priority to meet the demand. The donor is generally rigorously assessed for suitability of organ donation prior to proceeding to organ procurement. The quality of the organ from multi-organ procurement is usually satisfactory without jeopardizing its transplantation. In this chapter, the surgical technique for procurement of the organs from the abdomen is described. Some alternative techniques have also been discussed. Some pictures are inserted to facilitate understanding of the surgical procedure.

Keywords: abdomen, multi-organ procurement, liver, pancreas, small intestine, kidney

1. Introduction

Organ transplantation is a definitive treatment for patients with end stage of organ failure. It is one of the greatest advancements in medicine in the twentieth century. Organ transplant is a life-saving treatment and improves the quality of life. Organ donation and procurement are essential steps for performance of the organ transplantation. Organ donors are classified as live organ donors, donation after brain death (DBD), and donation after circulatory death (DCD). Rigorous assessment of a potential donor is mandatory prior to proceeding organ procurement. For DBD and DCD donors, procurement of multi-organs is always the priority to ultimately benefit multiple recipients without jeopardizing the graft function [1, 2].

The surgical technique for abdominal multi-organ procurement is based on the anatomy and has been evolved over the decades as a result of the increased demand for organ transplantation [2–5]. In this chapter, the surgical technique is described with some pictures inserted to facilitate understanding of the progress of the surgery.

2. Preparation in theater

It is mandatory for the surgeon in charge to check the documents: certification of brain death of the donor, the consent form for organ and tissue donation, signature of the hospital delegation, and the patient serology test. The team time-out should be carried out. The communication is confirmed between the surgeon and anesthetist for administration of medications at various stages. One dose of intravenous antibiotics is given intravenously, as well as 1 g of methylprednisolone prior to surgery. One dose of mannitol 20 g is given after completion of abdominal dissection. Heparin 25,000 IU is administered following cardiac team dissection prior to the cold perfusion.

If the pancreas is retrieved for organ transplantation, 100 ml of half-strength betadine (5%) is injected through the nasogastric tube that is then clamped off during procedure to retain betadine solution in the duodenum.

Preparation and drape: under sterile condition, the surgical area from the lower part of the chin to the proximal one-third of the thigh is prepared. The hairs are shaved. The dressing and central venous catheter or femoral catheter is reorganized to ensure the surgical field is clean and neat for preparation and drape.

3. Surgical procedure

3.1. Dissection of the organs with normal circulation (warm dissection)

3.1.1. Open of the abdomen and chest

An incision is made from suprasternal notch to the point just above the symphysis pubis (**Figure 1**). The abdominal cavity is entered first, and a Balfour retractor is placed to have adequate exposure of the abdomen (**Figure 2**). The round ligament of the liver is divided and tied as well as the falciform ligament of the liver. Examination of intra-abdominal organs is carried out to exclude potential malignant disease. The quality of the liver is usually assessed at this stage, and liver biopsy should be taken if any concern is raised. The biopsy is taken by using F18 Trucut needle from the left and right lobe. An additional wedge biopsy is also taken for the frozen section and histopathology examination. Following that, the soft tissue over the sternum is cut opened by diathermy along the line for sternotomy. A tunnel is created behind the sternum by insertion of a pair of long Metzenbaum scissors to ensure that the posterior side of the sternum is clean from soft tissue attachment. The sternum is sawed open with bone wax applied to the cut surface. Hemostasis is reassured by using diathermy to all active bleeders. A sternal retractor is placed to open the chest. The pleura and pericardium are remained intact at this stage (**Figure 2**). If the donor had previous sternotomy for cardiac surgery, then the wires

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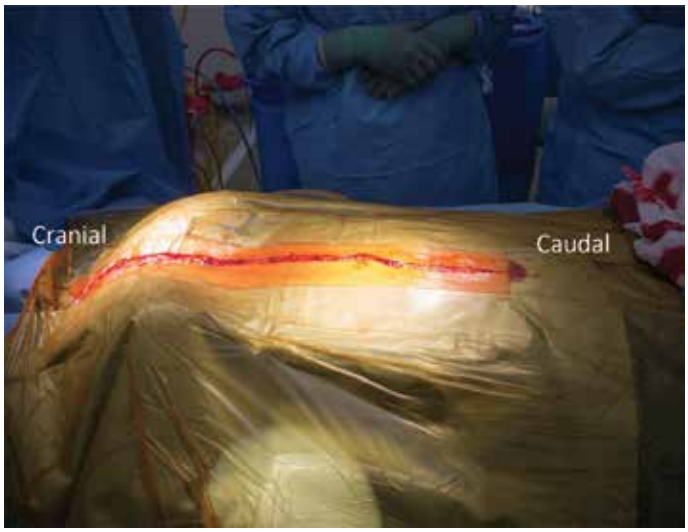

Figure 1. Incision for multi-organ procurement.

Figure 2. Retractors placed for exposure of abdominal organs.

over the sternum are removed, and a resternotomy saw is used for precise cut with caution. A surgical pack is placed over the chest wound to give the cardiothoracic team a nice clean field for subsequent heart and lung dissection. Now, the concentration is directed to the dissection of abdominal organs.

3.1.2. Dissection of the distal aorta

The retro peritoneum is incised along the white line of Toldt from the cecum to the hepatic flexure. The dissection is continued along the retroperitoneal avascular plane superiorly and

medially to mobilize and retract ascending colon medially. The right ureter is easily identified and preserved with the surrounding tissue. The dissection is continued by a Cattell-Braasch and Kocher maneuver. The inferior duodenal fold is divided allowing broad exposure of the inferior vena cava (IVC) and aorta (**Figure 3**). The left renal vein should be well visualized at this stage, and superior mesentery artery is palpated at its origin from the aorta. The distal segment of the aorta is dissected, and inferior mesentery artery is ligated and divided allowing the distal segment of aorta exposed adequately. A size 2 Dacron tie is placed around the aorta at the level proximal to the bifurcation of the common iliac arteries, which is used for ligation of distal aorta prior to cold perfusion (**Figure 4**). The second size 2 Dacron tie is placed around the aorta a few centimeters proximally that is used for tying the cannula after its insertion to the aorta for cold perfusion. At this stage, the inferior mesentery vein (IMV) is readily visualized along the edge of dissected mesentery of sigmoid colon lateral to the proximal jejunum. If the portal system perfusion is required, then a segment of IMV can be dissected, and a 2/0 tie is encircled for cannula placement at later stage immediately prior to aorta cannula insertion (**Figure 4**). Currently, most transplant units do not perform in situ portal perfusion but give 500 ml of cold UW perfusion to the portal vein on the back table when packing the liver.

3.1.3. Dissection of the liver

Following dissection of the aorta, the attention is directed to the liver. The left triangle ligament of the liver is divided to free the left lobe of the liver. The hepato-gastric ligament is divided, but it is usually checked for left accessory hepatic artery that usually arises from the left gastric artery. It should be preserved if present. The common bile duct is identified and transected at the level close to the duodenum (**Figure 5**). The portal vein is then visualized. At this stage, it is usually checked whether there is a right accessory or replacement of hepatic artery posteriorly to the portal vein by palpation via the omentum of foramen (Winslow). It

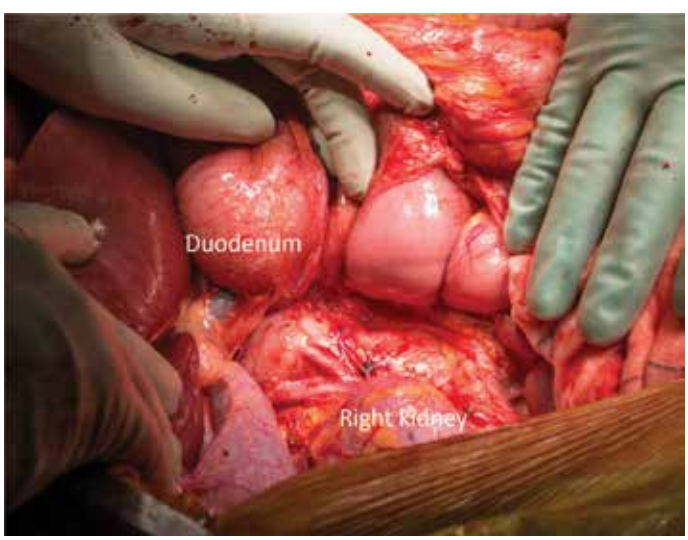

Figure 3. Cattell-Braasch and Kocher maneuver for exposure of retroperitoneal structure.

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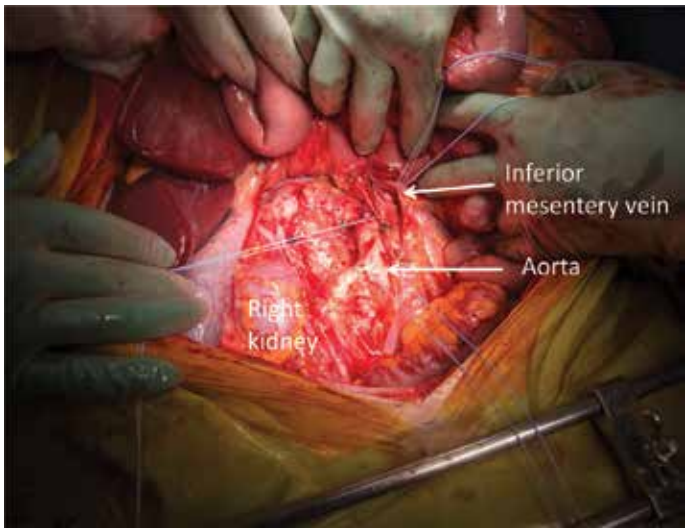

Figure 4. The distal aorta and inferior mesentery vein dissected.

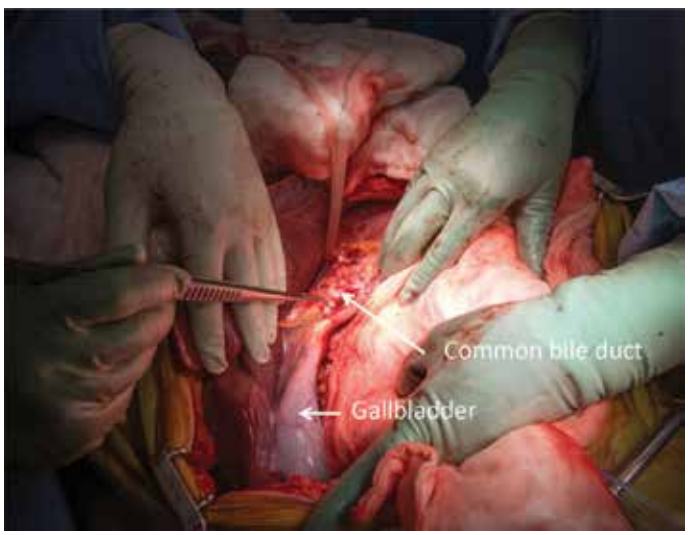

Figure 5. Transection of the common bile duct.

usually arises from the superior mesentery artery. The hepato-duodenal ligament is divided. The right gastric artery and veins are tied and divided. The proper hepatic artery as well as the gastroduodenal artery is identified. A short length of gastroduodenal artery is dissected (**Figure 6**). Dissection of proper hepatic artery is continued toward celiac trunk. Then, the splenic artery and left gastric artery is seen, and a short segment is dissected, respectively, which is readily for transection after cold perfusion during procurement. The left gastric vein may be encountered, which is ligated and divided. The liver dissection is now completed.

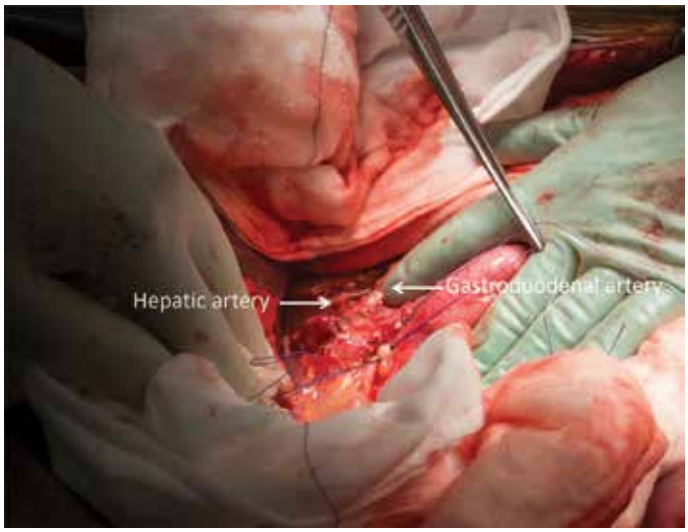

Figure 6. The proper hepatic artery and gastroduodenal artery dissected.

3.1.4. Pancreas dissection

The superior edge of the pancreas is partly exposed after dissection of proper hepatic artery. Then, the gastrocolic ligament is divided and ligated between the stomach and transverse colon. So, the anterior surface of the pancreas is visualized. The division is continued along the greater curvature of the stomach to its fundus, and gastrosplenic ligament is divided. The texture of the pancreas is properly assessed for suitability of transplantation. The spleen is always remained with the pancreas for procurement as a handle to prevent manipulation over the pancreas and reduce the risk of pancreatitis. The transverse mesocolon is divided as well as splenic-colon ligament on the left and duodenocolic ligament on the right side, and the inferior edge of the pancreas is exposed. The dissection is continued along the sigmoid colon and ascending colon allowing the colon retracted inferiorly or outside of the abdomen (this part of dissection can be done after cold perfusion). The superior mesentery vein (SMV) and superior mesentery artery (SMA) are identified inferiorly to the pancreas and anteriorly to the third part of the duodenum, which are slung with a 2/0 Vicryl tie, respectively, and subsequently tied and divided during pancreas procurement after cold perfusion.

3.1.5. Dissection of the small intestine

In rare circumstance, procurement of the small intestine may be required as part of multi-organ donation. In such case, the abdominal multi-organ procurement should be performed by the team who performs intestinal transplantation. After the laparotomy, the intestine is examined and wrapped in a surgical pack. The entire large intestine is dissected and placed caudally outside of the abdomen. The ileal branches of the ileocolic artery are preserved. Proximally, the small intestine is divided at the jejunum 5–10 cm post Treitz, and distally the small intestine is divided near the ileocecal valve by GIA stapler. A mark suture is placed at the jejunal end for orientation of the intestinal graft at transplantation. The small intestine is lifting upward, and the

mesentery is dissected posteriorly along the avascular retroperitoneal attachments. A short segment of the superior mesentery artery and vein is dissected inferiorly to the pancreas and anteriorly to the fourth part of the duodenum by dividing some small arterial branches or venous tributaries. Care must be taken to avoid injury to the inferior pancreaticoduodenal artery, which arises proximal to the origin of the middle colic artery and supplies pancreas head [6, 7].

3.1.6. Dissection of the aorta under the diaphragm

The last dissection before the cold perfusion is to dissect supra celiac aorta under the diaphragm. The hepato-esophagus ligament is divided, and the esophagus is retracted left laterally. The aorta pulse is palpated, and the overlayer crus is divided to expose the aorta. A thin fascia layer is incised, and the aorta is freed from the surrounding attachment. A nylon type is slung over this segment of the aorta that is subsequently tied when the cold perfusion is commenced to limit the perfusion to the abdominal organs (**Figure 7**). Care must be taken not to injure the aorta in particular when trying to free the posterior part of the aorta for bringing through the nylon type. This part can be done later if the risk of bleeding is predicted. Alternatively, the segment of thoracic aorta in the left chest can be dissected and slung with a nylon type.

At this stage the warm dissection to the multi-abdominal organs is completed. The gallbladder is incised open and flushed with normal saline until the outflow at the common bile duct becomes clear. The cardiothoracic team is informed for scrubbing and proceeds to heart-lung dissection.

3.1.7. Insertion of the perfusion cannula into the aorta for cold perfusion (in situ cooling of the organs)

A cold perfusion line is set up at this stage, and a large cannula is connected to the perfusion line. The air bubbles are removed from the line.

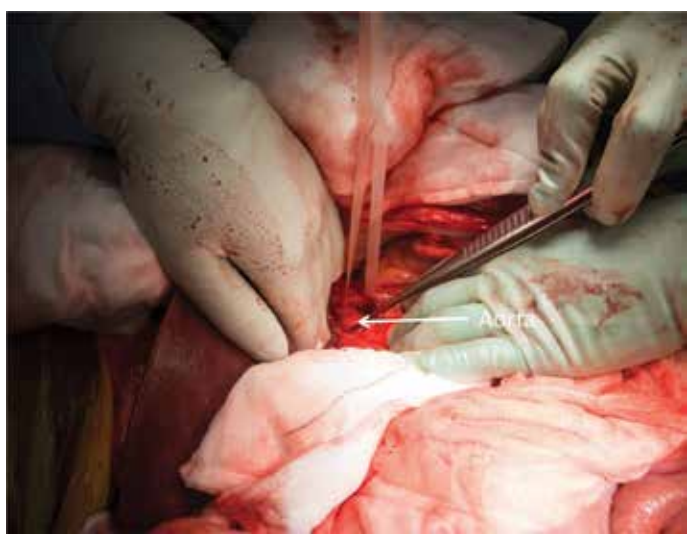

Figure 7. A segment of the aorta under the diaphragm dissected and slung with a nylon tape.

After heart-lung dissection, Heparin 25,000 IU is given intravenously. The distal part of the aorta is tied by pre placed Dacron tie just above the bifurcation of the common iliac arteries. A vascular clamp is placed onto the aorta about 5 cm proximally to this tie to block the blood flow to this segment of the aorta where an arteriotomy is made. The cannula is inserted via arteriotomy and is tied by a pre placed Dacron tie. The cannula is secured and the vascular clamp is removed. At this point, the cold perfusion is commenced simultaneously with cardiothoracic perfusion. The IVC is divided just below the right atrium above the diaphragm for draining the blood and perfusion fluid. Attention is made to leave adequate length of IVC with the liver at suprahepatic end. Alternatively, incision of IVC can be made at the lower part just proximal to the level of confluence of common iliac veins. A suction tube can be placed into the vena cava for adequate drainage of the perfusion fluid. At least, three sets of suction line are used to have proper evacuation of the blood and fluid during perfusion period. At the same time, the ice slush is poured into the abdominal cavity over the liver, pancreas, kidney, and intestine for immediate topical cooling of the organs. Usually, 2 L of Hartman fluid is used for initial flush of the blood followed by 4 L of UW preservation solution. The organs are checked in the meantime to ensure that the progression of perfusion is adequate. The in situ cold perfusion takes about 20 min.

3.2. Procurement of abdominal organs (cold procurement)

3.2.1. Procurement of the small intestine

After completion of the cold perfusion, the small intestine is harvested first. The superior mesentery artery and vein are divided below the origin of inferior pancreaticoduodenal artery, and the entire small intestine is removed and placed in ice-slush filled basin for package and transportation. The bowel contents are remained in the intestine and are managed at the time of transplantation. The small intestine can be harvested en bloc with liver and pancreas, and separation is performed on the back table [6, 7].

3.2.2. Procurement of the liver

Following procurement of the heart and lung by cardiothoracic team, the liver is taken first for the abdominal organs if the small intestine is not for procurement. The gastroduodenal artery is tied and divided. The portal vein is divided at the level of 1 cm proximal to the junction of splenic vein and superior mesentery vein if the pancreas is retrieved for transplantation. Otherwise, the division can be at the level of the confluence of splenic vein and SMV. The splenic artery is divided 0.5 cm from its origin arising from the celiac trunk as well as left gastric artery. However, if the left accessory hepatic artery is present arising from the left gastric artery, then the left gastric artery is preserved on the celiac trunk for its continuity to the left hepatic accessory artery. Along the celiac trunk, the celiac complex and lymphatic tissues are divided at the left side of aorta, and the aorta is exposed. An aortic patch is excised around the ostium of the celiac trunk. If the right accessory or replacement hepatic artery is present, then the SMA is better included in the aortic patch with celiac trunk. If the pancreas is also harvested for transplant, then the SMA is divided just above the level where the accessory hepatic artery arises. So, both liver and pancreas are suitable for transplant without jeopardizing its vasculature. After that the attention is directed to the IVC. The anterior wall of the IVC is incised transversely above the level of renal vein. Both entrance of left and right renal vein

is checked, and the IVC is transected. The IVC is lifted anteriorly by insertion of a finger from the suprahepatic end, and its posterior side is dissected to reach the suprahepatic end. The posterior side of the IVC is dissected superiorly to the level of division just below the atrium. Care is taken during IVC dissection without jeopardizing the quality of renal vein for kidney transplantation. The right hepatic triangle ligament is dissected, and part of the diaphragm is taken with the liver during the procurement. The liver is now removed freely from the abdomen and immersed in the ice-slush filled basin (**Figure 8**). One technical point is that the liver laceration is easy to occur at the location where the adhesion band is present. Therefore, gentle handling of the liver is emphasized during the dissection and procurement of the liver at all times to avoid the potential injury.

3.2.3. Procurement of the pancreas

The pancreas can be retrieved in favor with liver as an en bloc as described in a separate paragraph. Here, we describe a technique for pancreas retrieval as a subsequent procedure following the liver procurement. A 6/0 Prolene suture is placed at the transection of the port vein and splenic artery, respectively, as a mark during the liver procurement if known the pancreas is also procured. A segment of duodenum is routinely procured with the pancreas as exocrine drainage. A GIA stapler is used to divide the duodenum from the stomach distal to the pylorus. Care is taken to ensure that the NGT tube is positioned proximal to the pylorus without being caught in the GIA stapler. A reload GIA is needed to divide the distal part of the duodenum at the level of the fourth part of the duodenum or at the beginning of the jejunum. The distal part of the SMA and SMV is tied, respectively, by pre placed 2/0 Vicryl tie and divided. The transverse mesocolon is divided to free the inferior edge of the pancreas. On the left the dissection is continued to the splenic flexure and on the right to the duodenocolic ligament, which are divided together with the root of mesentery. So, the pancreas with attached duodenum is now free from its attachment. Then, the pancreas is lifted

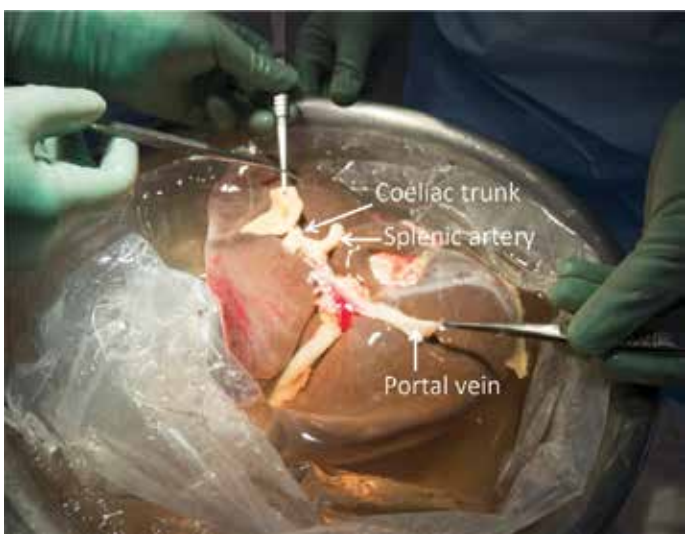

Figure 8. Liver graft harvested and immersed in ice slush.

anteriorly by holding the spleen, and the dissection to its posterior attachment is performed from the tail toward the body of the pancreas. At this point, the SMA is excised with a small aortic patch, and the pancreas is removed from abdomen and placed in the ice-slush filled basin. Care must be taken during excision of the SMA without jeopardizing the renal artery.[2]

3.2.4. Procurement of the liver and pancreas as an en bloc

The liver and pancreas are preferred to have en bloc procurement to minimize warm ischemic injury to these organs. Following cold perfusion, the proximal and distal part of the duodenum is transected by using GIA stapler at the level distal to the pylorus and the fourth part of the duodenum, respectively. The distal part of the SMA and SMV is tied by pre placed 2/0 Vicryl tie and divided. The Transverse mesocolon is divided, and the division is continued on the left to the splenic flexure and on the right to the duodenocolic ligament, which are also divided and the colon retracted inferiorly. The spleen is lifted anteriorly as a handle of the pancreas for dissection posterior to the pancreas. The SMA is excised at the inferior edge with a small aortic patch that is extended superiorly to include the celiac trunk on the same aortic patch. The left gastric artery is divided. The attention is directed to the IVC. The IVC is divided just proximal to the level of the entrance by the renal veins, and the dissection to its posterior is continued superiorly to reach the suprahepatic end of the IVC. At this stage, the liver and pancreas are free to be removed from the abdominal cavity and immersed in an ice basin for separation from each other on the back table [2].

3.2.5. Procurement of the kidneys

The kidneys are the lastly procured abdominal organs. Therefore, care must be taken during cooling and procurement phase to ensure that the kidneys are properly cooled by placing enough ice slush at its surrounding, in particular at its posterior and lateral sides [8].

The right kidney is retrieved first. The bowel is placed superiorly, and the kidneys, the vena cava, and the aorta are left in situ. The perfusion cannula is removed, and the aorta is transected at this level. The superior end of the aorta is also transected at the level where the SMA aortic patch is excised. The left renal vein is excised with a small patch from the vena cava and dissected away from the aorta. The anterior part of the aorta is split open as well as the posterior part of the aorta. Care is taken to keep an equal aortic patch with each side of the renal artery. The renal arteries are inspected at this point and are remained on its patch. The IVC is transected at the level of the confluence of common iliac veins. The right ureter is divided at the level of iliac vessels. The ureter is lifted anteriorly, and its posterior is dissected toward the kidney along the plane anterior to the major psoas muscle. Care is taken to retain plenty of surrounding tissues with the ureter. Then, the kidney is lifted anteriorly within Gerota's fascia together with the ureter, and its posterior side is dissected from the psoas muscle. The dissection is continued posteriorly to the IVC, and the right half of the aortic patch until the kidney is free from the attachment. The right kidney is then removed as a pack from the abdomen and placed in the basin filled with ice slush (**Figure 9**). Care is taken to ensure that the dissection is along the surface of the psoas muscle and spine. So, the injury to the renal artery and renal vein is prevented. The left kidney is procured in the same method as the right kidney.

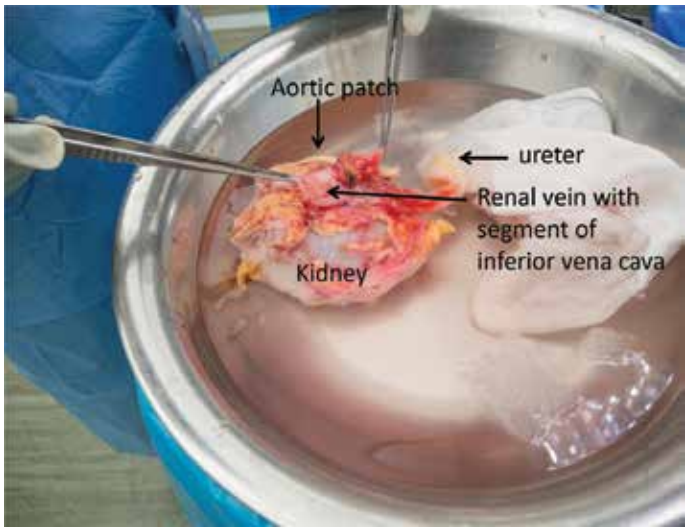

Figure 9. Right kidney harvested and immersed in ice slush.

3.2.6. Procurement of the kidney on en bloc

Alternatively, the kidneys can be procured on en bloc [5] and separated in the ice basin on a back table. The technique is faster to remove the kidney from the abdomen and minimize the risk of warm ischemic injury. In particular, it is preferred for procurement of the kidneys in DCD donors. The bowel is retracted anteriorly and superiorly. The kidneys, the aorta, and the vena cava are left in situ. The aorta and vena cava is transected at the level of its arteriotomy for the cannula. The right ureter is identified and divided at the level crossing over the iliac vessels as well as the left ureter. The ureter is dissected toward the renal hilum with plenty of surrounding tissues to prevent the injury to its blood supply. The dissection is continued superiorly along the plane posterior to Gerota's fascia but anterior to the psoas muscle and spine until reaching the superior end of the aorta and vena cava. Both kidneys are procured and placed in an ice basin on the back table. During the dissection, the assistant holds up both kidneys with ureters anteriorly to facilitate the dissection.

The kidneys are separated on the back table. The left renal vein is excised with a patch at its entrance to the IVC. The anterior side of the aorta is cleaned and incised along its midline allowing the equivalent patch to each side of the renal artery. Similarly, the posterior side of the aorta is incised along the midline. The ostium of the renal artery is inspected as well as the numbers of the renal artery are checked. The kidneys are now separated from each other. The kidney is inspected as described above and packed for transportation.

3.2.7. Procurement of the iliac vessels

A segment of the iliac arteries and veins are harvested as long as possible toward the distal part of the femoral artery and veins. The iliac artery and vein can be taken separately or together as one bundle. Care is taken to avoid injury by over pulling on the vessels. The vessels are stored in a sterile jar with UW solution and parked with two more layers of sterile bags for transportation.

The iliac arteries and veins are routinely retrieved for vascular reconstruction during liver and pancreas transplantation. In pancreas transplantation, the common iliac artery, the external iliac artery, and the internal iliac artery are used as a "Y" graft for reconstruction to the stumps of the splenic artery and SMA and then anastomosed to the recipient common iliac artery at the transplantation. Generally, one set of iliac vessels is sent with the liver, and another set is sent with the pancreas.

4. Organ package and the transportation

All the organs will be further inspected on the back table to ensure its quality and suitability for transplantation. Communication with the transplant surgeon at the recipient end is strongly encouraged to achieve the best outcome for organ transplantation [9].

4.1. Liver

The liver is inspected in the basin. Five hundred milliliter of UW solution (4°C) is perfused to the portal vein system. The common bile duct is flushed with 200 ml of UW solution by using a syringe. The liver is placed into the first sterile bag filled with 700–1000 ml of UW solution, and the bag is tied. The first bag is then placed into the second sterile bag filed with 1 L cold normal saline and tied. The second bag is placed into the third sterile bag and then tied. The liver in the three-layered bag is then placed in the Iskey filled with ice blocks for transportation to the recipient hospital with the document secured in the Iskey (**Figure 10**) [7].

4.2. Pancreas

The pancreas is inspected to ensure its quality for transplantation without injury from the procurement and then is packed by the same method as for the liver. It is placed in the Iskey with retrieved vessels for transportation to the recipient hospital. Often, the pancreas is allocated with the left kidney to the same recipient for simultaneous pancreas-kidney transplantation (SPKT). Care is taken to avoid manipulation over the pancreas. Perfusion to the pancreas on the back table is not recommended to avoid perfusion injury and pancreatitis.

Figure 10. Package of the liver. The first bag filled with UW solution.

4.3. Kidneys

Each kidney is inspected by dissection open the perinephric fat. The quality of perfusion is checked and the mass lesion is excluded. The kidney is placed in the first sterile plastic bag that is filled with 500 ml of UW solution and tied. The first bag is placed into the second bag that is filled with 1 L cold normal saline and tied. The second bag is placed into the third bag and tied. The kidney is placed in the Iskey and buried in the ice blocks for transportation with the document enclosed.

5. Conclusion

Multi-organ procurement is essential for organ transplantation, and proper training is mandatory. It usually involves a few transplant units for organ allocation prior to confirmation of the surgical time. The communication between all parties is very important to minimize the ischemic time and achieve the good outcome for organ transplantation. It should be bore in mind that the anatomical variation may be encountered during the surgery, and care must be taken to avoid any damage to the vessels and organs, which may jeopardize the organ transplantation. Establishment of a surgical protocol is encouraged to achieve a national-wide standard and consistence for organ sharing among the transplant units.

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Conflict of interest

There is no conflict of interest.

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Chapter 4

The Society, the Barriers to Organ Donation and Alternatives for a Change

Félix Cantarovich

Additional information is available at the end of the chapter

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Abstract

Objectives: To evaluate the current possibilities that transplantation offers to the patients on waiting lists, and to propose alternatives to modify this medical and social crisis.

Introduction: Persistent organ shortage remains a problem. Put simply, patients are dying because people have not understood that donation means to prolong lives. Ignorance and prejudice and mainly fear of dead and mutilation are major causes for inadequate society's response.

Conclusion: The stagnant shortage of organs shows that current educational strategy has not improved people's behavior. Renovate the current message might be a way for a change. New catch phrases like: "After death, our body is a unique source of health"; "Organ donation is not a gift. It is to sharing of a new life for one and all". "Throughout life, we are all potential recipients of a transplant"; could be included in educational programs; in addition, instructive action on this subject addressed to the youth, starting in the schools, could be another positive contribution for a solution to this crucial crisis for the possibilities of welfare of the Society.

Keywords: modifying educational programs, organ donation, organ transplantation, dying in waiting lists, social behavior, school curricula

1. Introduction

Transplantation of organs and tissues has succeeds to associate life and death for the benefits of society. Current evidence suggests that transplantation medicine might be a crucial health guarantee for society. However, the paradoxical shortage of donated organs limits this possibility.

This extraordinary progress of medical sciences offered to the people by health care organization, has generated the need for novels methods and State actions to be able to implement it without any restrictions for the benefit of society.

Certainly, this advance in medical practice should generate new health programs different from those recognized until now. The vital requirement to transform death into life, which is what organ transplants symbolize, requires the end of somebody life. Therefore, the knowledge and acceptance of the metaphor, "transforming death into life", should be recognized by the State and by the people.

It is important that society knows the benefits that organ transplants mean for the security systems. This reality is evidenced in the significant budgets differences of a kidney transplant compared to a same period of patient in hemodialysis.

To achieve this goal, it is essential that people should understand currently the essential solution to solve the inexorable evolution of patients suffering from a terminal organ failure and that our body after death is a unique and irreplaceable source of health.

This possibility depends of people's legal organ donation either during their lives or that at the time of death of their loved ones. Unfortunately, this people's option remained for decades in a partial response.

Organ shortage is a social, psychological, ethical, moral and political problem, causing unjustifiable damage to public health.

As a consequence of organ shortage, patients on waiting lists are "unfairly" dying everyday. In a way, the unjustifiable truth of today is that society is denying another human the chance to live.

Regarding the notion of people "unfairly" dying while waiting for an organ, it is also true that thousands of people are dying everyday because of socio-economic inequalities [1]. However, the reality of this is that such deaths are a consequence of multiple and complex problems, including economic, political, social inequality and corruption. All of these problems are extremely difficult to take care of. In contrast, organ shortage solution fundamentally depends on the change in the current social behavior toward organ donation. This alternative could be achieved if the strategies to modify frequent people's attitude toward organ donation are evaluated and revised.

This social paradox urges a rational response. The puzzle to answer is what is the motive of this crime of "lese majesty" that humanity is committing against itself?

The reasons most responsible for this negative behavior toward organ donation are basically ignorance and misinformation [2–6].

Worldwide, approximately 47% of people who are asked to donate organs and tissues gave their consent despite the fact that in opinion polls, between 75 and 90% of the population is in favor of the donation.

At this moment, more than 125,000 people in the United States are in need of a life-saving organ transplant. And 64% of them are currently on a waiting list—to which roughly one person is added every 10 min—unfortunately, only about half of them will actually receive, the transplant they need this year [7].

A critical analysis of the reasons for the uncertain people behavior toward donation suggests as a valid alternative, that the current message to society has not been able to develop a positive change in this current social conduct [2].

As a proposal, we have suggested a change of the classic slogan "Donate is a gift of life" for "Donate is to share life". In addition, we have proposed the following ideas as useful supplements to modify current behaviors toward donation:

"During life we are all potential recipients of a transplant".

"All monotheistic religions accept organ donation and transplantation".

A social education that allows a real knowledge of this problem will be a challenge to facilitate people understand a human right acquired by the Society: to give or receive donation of organs and tissues during life.

We suggest the aforementioned slogan: "Our body after death is a unique and irreplaceable source of health" as a valid challenge in search of a social change.

It is interesting to note that current people's feelings regarding organ donation does not fully agree with the concepts stated by UNESCO emphasizing that attitudes of society toward organ donation do not conform to the principles of social behavior [8].

It would be important to make people aware, as the main protagonist of the lack of organs, of these significant notions required today by society.

At present, time should ensure the possibility that anyone who requires a transplant can receive it in necessary time. The social security of modern states should use maximum resources in the achievement of educational programs that have analyzed and proposed solutions to the real barriers that generally inhibit the will to donate, in the search of achieving a positive change in the current indefinite social behavior.

In addition, the training of young people through the incorporation of topics on donation and transplants in curricular programs, periodically carried out in schools, colleges and universities, may be another way to develop a change in people's attitudes toward organ donation.

2. The underlying reasons for organ shortage

Ignorance and prejudice continue to be the general causes of society's lack of response to the social need of organ donation; particularly with respect to the deceased donor [3]. Several possible explanations for this behavior have been suggested:

1. People are only partially aware of how accepted, organized and frequent transplantation of organs and tissues is today.
2. People do not consider that they themselves may need a transplant during their life.
3. Society is not cognizant that the body after death offers a unique source of life.

4. Medical teams are largely untrained in organ donation because of insufficient education on this matter [4–7].
5. The decision about organ donor after death, or the response of relatives to donation requests, awakens primal fears such as:
   a. The instinct of self-preservation [9].
   b. The Freudian notion that nobody thinks about his or her own death until a loved one dies [10].
   c. The ancient idea that the integrity of the body is mandatory for the pathway to eternity [11].
   d. The fears concerning a diagnosis of brain death [12, 13].
   e. Beliefs that the organs will first go to the rich and only then to the poor [14–16].

People are not informed about the beneficial impact of transplantation on health budgets, that is, transplantation can reduce the cost of diseases, which would otherwise have to be treated by expensive long-term therapy [17, 18].

The media has featured stories about criminal "organ commerce" [19].

These myths, misinformation and prejudices are barriers that weaken the instincts of solidarity and altruism, arousing selfishness and uncertainties.

Regarding the aforementioned negative factors on donation, it is important to emphasize the importance of two of them: fear of death and respect for the integrity of the body that are not essentially linked to ignorance and/or bad information. Surveys on the behavior of university students regarding the implication of fear of death as an inhibition factor in the acceptance of organ donation and transplants were investigated in different studies. These surveys showed that negative attitudes toward organ donation were associated with higher fears of death and dying of the self and less strongly with higher fears of the death and dying of others. A study showed that students without donor card and with reservations about donation scored significantly higher fear of physical destruction. Possible implications of these findings for medical education and future research are suggested. It was mentioned that given the urgency, attitude toward organ donation should be considered a civic responsibility transforming an unfortunate yet inevitable event into something that positively affects someone else [20–22].

Body integrity, it also remains a central issue for negative behavior toward organ donation. Fear of mutilation is the fear of losing any part of our body structure, the idea of having limits in the mobility of our body or of losing the integrity of any organ, part of the body or natural function. These ideas can generate ethical and moral inhibitory behaviors regarding the treatment that our bodies or those of loved ones receive at the time of death. These reflections regarding the conservation of the body's image play a significant role in the decision of families toward donation [23].

Understanding that those factors can limit the potential supply of available organs for transplantation, the suggestion that our body after death as a unique and irreplaceable source of

health should be considered an educational challenge in the search to modify socio-psychological behaviors deeply structured in people's mind. Educational procedures searching the best way to spread this notion should be deeply analyzed by experts in social, psychological and religious questions.

Public education, mainly through the media, non-governmental organizations and lectures by experts, has been the main strategy to change social attitudes toward organ donation. It is important to highlight that the results of these educational endeavors have not been completely satisfactory, as the crisis remains almost unchanged. Although the public is more aware about transplantation issues, there remains a shortage of donated organs.

The increasing number of patients on waiting lists and their daily mortality is a clear expression of the insufficient impact of social education programs to date. The consequence of this inadequate social response is that at least 22 unreasonable deaths occur everyday on organ donation waiting lists [24].

Highlighting the serious situation, Matas and Hays considered that the United States educational policy has had little effect on organ donation [25]. For that reason, the author suggested that making organ donation financially neutral for donors should be supported as one solution to this serious problem. However, it should be considered that this strategy might generate a new type of social injustice and inequality. In addition, it should be noted that there has been several criticisms of the educational social programs on organ donation. Many authors believe that the current methodology is useless and is a needless economic investment [26, 27].

Paradoxically, none of these authors suggested thoughtful modifications to the educational methodologies in order to achieve a more positive social response to organ donation.

2.1. Potential reasons for the present crisis

2.1.1. The message to the public has been inadequate

From the earliest times of the transplantation era, the philosophy used for educational purposes has relied on the concept that organ donation is an expression of altruism and solidarity, a "gift" that will save or improve someone's life. In fact, several surveys have shown that, in general, people are open to donate their organs (or those of a family member) after death. However, when facing the moment of grief, a high percentage of people fail to remember this commitment and consequently the "gift of life" is questioned and does not come to fruition.

The inadequate societal response to the persistent lack of donated organs encourages the following conceptual changes in the philosophy of the organ donation message:

1. Organ shortage is a health emergency.
2. Our body after death is a unique source of health for everyone.
3. Sharing our bodies after death should be acknowledged as a tacit social agreement for a common welfare.

4. Organ donation is not giving life, it is sharing life.
5. Throughout our lives we are all potential organ and tissue recipients.

Successful implementation of these ideas requires acknowledgment by state health and education institutions, scientific societies, organ sharing organizations and representatives of monotheistic religions.

2.1.2. The message has not been effectively transmitted

Undoubtedly, a scientifically programmed and continuously disseminated MEDIA campaign will have an important influence on the improvement of social behavior toward transplantation and donation.

Nevertheless, on the other hand, the MEDIA can transmit prejudicial and negative information and the myths most commonly perpetuated as:

1. Premature declaration of death.
2. Transference of personality traits from donor to recipient.
3. Criminal black market for organs.
4. Corruption in the medical community.
5. Organ allocation systems, which allow celebrities to get transplants first.

A recent study has shown that mass media can generate a conflicting image of organ donation. The inadequate information is responsible for a negative attitude on the part of many potential donors. Investigations on the effects of MEDIA on donation have shown that when people are not in favor, they often mention the negative effect of television programs [28]. The myths transmitted were more believed by the viewers, than by those who did not see these programs. The same phenomenon occurred regarding the donation between spectators and non-spectators.

Although these are not the only myths that the general public believes to be true, the media is a powerful support for them. A well-programmed and persistently disseminated media campaign might have an important influence on improving society's knowledge of organ donation and transplantation.

It has been proposed that the ability of the press to correct negative misinformation might be fundamentally improved with the collaboration between specialists in transplants and journalists in the drafting of news or recommendations on donation and transplantation to society [29].

The decision of an organ donor is one of the most important and significant behavior of a current world citizen.

A public debate regarding organ donation can inform and stimulate many people to be donors.

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Proposed solutions:

1. Legal measures
2. Financial incentives
3. Expanding donors
4. Education

1. Legal measures

Laws about organ transplantation have been passing attempts to provide a better system of organ donation and distribution and to encourage individuals to volunteer as organ donors.

In USA, in 1968, The Uniform Anatomical Gift Act was the first effort at providing a national organ and tissue donation policy. The act created a uniform legal procedure for persons who wish to donate organs and for hospitals and medical institutions that want to accept them. A 1986 federal law requires all hospitals participating in Medicare or Medicaid to implement a "required request" policy. Hospitals are required to discuss with potential donors and their families "the option of organ and tissue donation and their option to decline" [30].

Consent and altruism remain core values of organ donation. The term consent is typically defined as a subject adhering to an agreement of principals and regulations.

Two types of legislation concerning organ donations are at present valid worldwide:

1. Informed consent or "opt in" (only those who have given explicit consent are donors or the expressed voluntary of their families in case of death).
2. Presumed consent or "opt out" (who has not refused to express their consent to the donation is a donor).

People have thus to register if they do not want to donate their body.

Presumed consent is one of a number of different varieties of consent. The paradigm of consent in biomedical ethics is to express consent. It appears in the Uniform Anatomical Gift Act's framework for organ donation as well as in rules of voluntary, informed consent in both therapy and research involving human participants.

On the other hand presumed consent has always been perceived as the "best" system for society in terms of organ donations.

However, in both systems, the family has something to say, especially for the deceased who did not sign anything while alive.

Proposals to introduce presumed consent should have full knowledge of their organization and results in the countries in which this legal instrument is in force. In practice, the citizen is informed that his non-registration as a non-donor means that he is a donor. The experience has shown that, in general, the presumed consent has not nullified the will of the families.

In Spain and Italy, it is considered that the increase in donation is the exponent of a better institutional management rather than the result of the presumed consent law.

In France according to the 1976 "Caillavet Law", a person is presumed to have consented to organ donation if he or she has never explicitly said otherwise to close relatives. But even for organs and tissue, where there is no such legal confusion, the "Caillavet Law" has not been applied uniformly in all French hospitals because there was no centralized administration in France to coordinate people's wishes.

France has modified its law on organ donation. All people are donors at death unless they record their refusal in an official registry. The new law provides for the procurement of organs, even against the wishes of the family.

Spain is routinely cited as a successful example of presumed consent. But in Spain, the next-of-kin still has veto power. Most of the growth in donation rates there happened well after the passage of presumed consent legislation.

Some people do not trust the government or the health care system. Many of them are afraid that that signing a donor card may make physicians give up on them too soon, especially if the hospital is likely to lose money on their care.

This legislation, if enacted, must take into account this relatively frequent fear of the people.

Changing the law will not be sufficient. As the experience with presumed consent in Western Europe shows, education of the public and constant training of hospital personnel are essential to achieve the improvement of the structured medical organization for the increase of organ procurement.

Another possibility for increasing donors has been developed with the modification of it acceptance criteria. This means the alternative to consider the use of donors with different medico-surgical conditions that may diminish part of their chances of long-term success.

On the other hand, improvement in the detection, prevention and better therapeutic of the pathologies leading to terminal organ failure, provides alternatives by reducing the number of requiring organs for transplantation.

It is complex to establish whether the presumed consent can justify various social behaviors in countries with or without that type of legal measure.

The medical-social people behaviors are adapted to the degree of education on the subject, the quality and formation of the responsible medical organizations and the economies of each one of the countries.

In particular, the participation of a medical group trained in excellence in the psychosocial methodology of approaching families at the time of the grief of the loss of a loved one, is undoubtedly a factor of principal importance for a positive result regarding the family response [31].

Some critics claim that presumed consent is a "fiction" [32]. However, the conception of presumed consent as tacit and silent overcomes the notion of being a fiction. Sometimes it can be

a concretely effective consent, according to the characteristics and organization of the institutional resources, as well as to the competence, understanding and motivation of the people.

With some notable exceptions, previous studies consider that, in practice, the laws of presumed consent have not achieved more important levels in the behavior of people toward donation [33]. In addition, it has been suggested that these results are related to the fact that, despite the law, the consent of the family is usually required [31, 32].

Finally, with respect to the effectiveness of laws concerning the mode of consent, we believed that hardly a law can change the moral and ethical behavior of the people.

2. Financial incentives

Financial incentives are considered any material gain or value obtained by those who consent directly to the process of obtaining organs, whether to the donor, the succession of the donor or the family of the donor.

The arguments in favor of financial incentives for organ donation are based on the hope that such a system will increase the supply of organs safeguarding the basic ethical concern of saving lives.

A set of reimbursement of funeral expenses has also been suggested as a direct "milder" means of incentive for donation.

Finally, a form of "insurance for the donor" has been suggested, for which an individual agrees in advance of the donation, with a payment to their beneficiaries that will only take place after the donation.

The concept that financial incentives can offer a possible solution to the shortage of organ donors in progress has been considered and debated among experts in the field of transplantation, ethics, law and economics [34].

The essential conception of altruistic donation, unchanged in general in the last 50 years, has not been able to overcome the constant lack of organs, with a critical permanent increase in mortality on the waiting list. This constant reality has motivated the justification by different authors to invoke a fundamental change of the current altruistic criterion: financial incentives to facilitate organ donation.

It has been specifically pointed out in this regard that the current system generates financial gains for all concerned: doctors, coordinators, social workers, hospitals, pharmaceutical laboratories, etc. Consequently, it has been described as unjust and insensitive to the families of the donors and a source of basic distrust on the part of the public, that the donor and the family are the only ones that do not directly benefit from the donation process, which therefore, some type of compensation must be defined [35].

Finally, those who promote financial incentives for organ donation conclude that their motives are ethical because they are based on concern for patients and saving lives and not only on abstract theories and issues without concrete answers.

Pilot programs are proposed to evaluate the potential effects of financial incentives for organ donation instead of rejecting this proposal on the basis of theoretical disadvantages not yet tested [36]. This means that it is time to use incentives to reward people who are willing to save the life of a stranger through donation.

Those who support the establishment of economic incentives consider that our current transplant system is inadequate for the task of increasing the volume of organs necessary to save lives. Altruism is not enough, incentive pilot trials are needed [37].

On the other hand, opposed to financial incentives base their objections on the argument that the altruistic system has not been correctly promoted.

It is pointed out that there would be a decrease in respect for life and the sanctity of the human body, and a loss of the personal relationship that currently exists in the donation process [38].

Great concern has also been expressed regarding a potential phenomenon of rich versus poor. Ironically, this type of incentive would be mainly aimed at racial communities of significant poverty [39].

Economic necessity should not be linked in a coercive way to consent to obtain organs. This money would be better spent more on education for medical communities regarding the need for organ donation through the current system to make the society understand the fundamental benefit for its future of donation and organ transplantation.

Beyond that the proposed incentives can be negative for potential donors, it has been argued that the financial gain of the family of the donor has not resolved at all the economic problem motivating the acceptance of economic incentive to donation.

On the other hand, the relative failure of the medical community to participate in the donation process will not be improved by the incentives directed to the potential donor [40].

In the discussion of the problem of acceptance of economic incentives for organ donation, it is convenient to mention the Iranian program of transplants. In this country, a system for payment of organ donation coordinated by the government has been implemented with significant results. Actually, a candidate for transplantation in Iran can get a kidney from a cadaver, living relative, or a living stranger.

However, in contrast to most countries, 76% of kidneys come from strangers; only 12% of kidneys are from deceased donors.

This significant difference makes it necessary to consider the obvious poverty-donation relationship, which notwithstanding any image of responsibility on the part of the state, does not excuse an unavoidable presumption of social injustice, ethically not compatible with the basic principles of ethics in organ transplants.

Those who oppose the financial incentives for organ donation predict the possible loss of control of this process by the government bureaucracy and the "organ traffickers" with a tremendous increase in the cost of administrative requirements [39].

Until it is available through universally accepted surveys as accurate and representative, the feasibility and effect of financial incentives for organ donation remain questionable.

3. Expanding donors

One important factor in the number of transplantations, currently performed, is the growing acceptance of marginal grafts, which are defined as organs at increased risk for poor function or failure that may subject the recipient to greater risks of morbidity or mortality [41].

The persistent "organ shortage" remains with an increase of 8% organ transplants per year. The annual growth of patients on the waiting list is 22% and its mortality is 18%.

This reality has conditioned a modification of the classic acceptance criteria for an organ donor. Currently, donors regarded as "expanded criteria donors" with potentially suboptimal organs have been included.

Simultaneously, the number of cadaveric organ donors has remained relatively static, with only a 4% increase per year. Most of this incrementally small increase has been through the use of "expanded" donors, reflected by the fact that the uses of donors older than 50 years old increased by 24% per year while those younger than 50 years increased by only 1.5% per year [42].

As it was mentioned, to increase the potential donor supply, the implementation of presumed consent and financial incentives for donation have been proposed, nevertheless public attitude toward presumed consent would probably not be acceptable. On the other hand, there has been resistance to financial incentives to the donor family because of the perceived danger of this escalating to the selling of organs as currently taking place in Southeast Asia and India [42].

Efforts to expand the donor pool are therefore limited to expand the criteria for the use of "suboptimal" organs.

Mainly, suboptimal donors are considered when donors are less than 5 years old or older than 65, donors with moderate decrease in renal function, donors with antibodies positive against hepatitis C, donors with type 2 diabetes or with moderate arterial hypertension and particularly the use of donors with cardio-circulatory death [42].

Essential characteristics of these suboptimal donors:

Older donors: the general refusal to use kidneys from older donors is due to the normal structural changes in the aging kidney.

However, these changes may not occur in all donors. For this reason, it has been considered that the older donor should be evaluated individually for its renal function at the time of death. Renal biopsy can be used in donors older than 50 years or with a history of significant hypertension. An organ that presents a glomerular sclerosis less than 20% and with mild interstitial fibrosis is acceptable to be implanted.

It is important in these cases to implement the system called Old to Old, it does means, old donors kidneys for old donor receptors.

It is very important in these kidneys the evaluation of the cold ischemia time (CIT). Kidneys with CIT of more than 48 h have a graft survival of 38% compared to kidneys with CIT less than 48 h, which had a very acceptable graft survival of 76% at 1 year.

Nefrotoxic injury with medication or rejection may also limit the long-term final result of these organs [43].

The hypertensive and diabetic donor: patients who received cadaveric kidneys from donors with a history of either diabetes or hypertension ("non-ideal") were compared with recipients of "ideal" organs. Although the overall graft survival of the non-ideal organs was somewhat less (69% versus 74%), these differences were not significant. Again, these kidneys should be evaluated on an individual basis by biopsy and donor history [42].

The donor with hepatitis C: the use of the hepatitis C (HCV) + donor organ has been controversial and was a subject of debate. Nevertheless, today advances in the treatment of hepatitis C have change acceptance criteria for these donors.

Prevalence of HCV positivity in organ donors has been reported to be between 2 and 6% with contradictory data with respect to the risk of transmission of HCV from positive organ donors [44].

Non-heart beating donors (NHBD): the use of NHBD has been increasing all over in recent years. In controlled and uncontrolled trials, delayed graft function is 60–80% of cases. Nevertheless, no matter this consequence of longer periods of warm ischemia, long-term results and graft survival rates are excellent [45].

Until other options such as xenotransplantation or tissue engineering become realistic, the challenge for the millennium will be to identify which donor organs previously considered suboptimal can be safely used to expand the organ donor pool.

Paired kidney donation: increased living donation (LD) rates are determined by less invasive approaches to donor nephrectomy and by the excellent long-term results.

In recent years, a number of strategies have been introduced to expand living donation programs beyond the classical direct donation, to overcome immunological barriers of blood group or HLA sensitization of recipients.

New strategies in LD include paired kidney exchange. In order to overcome the sometimes difficult barriers of incompatibility in blood groups or the hyper immunized receptor, the pair kidney donation technique has been a significant advance.

The procedure consists of combining the pair of incompatible donor recipients with each compatible member of different pair. Other alternative programs are: altruistic donation, altruistic donor chains and list exchange programs, and desensitization of hyper immunized patients and transplantation across the blood-type barrier [46].

The transplant community is challenged to address the ongoing crisis in organ transplant access. A discarded organ may be a missed opportunity to save a life. While careful judgment and prospective monitoring is crucial, a blanket "no" to these organs will help neither you nor your patients [47].

4. Education

The teaching of basic concepts regarding transplanting and organ donation has been insufficient.

An efficient education on the need to modify the current reluctant negative behavior toward organ donation by society constitutes a potential possibility to improve this urgent medical-social

crisis. Education and information will increase the value of altruism by protecting the population from exploitation, increasing the meaning and value of organ donation [48, 49].

Organ donation and transplantation education at all levels of society have been a matter of interest for decades. Nevertheless, the results observed to date must be considered inadequate because organ donation and procurement have not improved worldwide. With regard to medical education on transplantation, multiple polls show a severe lack of knowledge [3–7].

When searching for new alternatives to modify ancestral prejudices and barriers inhibiting the use of the body after life, priority should be given to youth education starting with children in schools.

The rationale of this proposal is that young people, particularly children, are free of prejudices, and are able to easily learn new ideas, sometimes much more easily than adults. Modern psychology suggests that childhood is the best developmental stage to start prevention programs against harmful prejudices. In addition, new notions learned by children at schools can be a way to offer clear and unprejudiced knowledge to their families [50, 51].

The central task of education is to implement facilities to learn; it should produce learning people. The truly human society is a learning society, where grandparents, parents and children are students together.

At present, young people have not been sufficiently informed of their future organ transplant needs and their potential role in the development of educational programs.

No one has yet realized the wealth of sympathy, the kindness and generosity hidden in the soul of a child. The effort of every true education should be to unlock that treasure [52].

Organ and tissue donation can also involve children. Because of its sensitivity, this topic requires careful decision-making. Children have the ability to carefully reflect on this subject and enjoy participating in family discussions about it [53].

Shoenberg consider that teaching young people about organ transplantation is not particularly difficult. He considered that helping young people understand the problem of transplant increases the possibility that they clearly understand its importance. Probably young people in response to this teaching will discuss this issue with their families or with their peers, thus multiplying the educational effect. Intense and persistent educational efforts focused specifically on young people are relatively rare. Consequently, this leading educator not related to medicine, suggested that "the transplant community has to offer strong stimuli that induce professors in various places to assume such a task" [54].

Undoubtedly, the insufficient results of people's education, without significant changes over the decades, indicate the need to review the current methodology of teaching society about this severe crisis, consequently of their current behavior toward organ donation.

The introduction to the permanent curricula of study, in the different levels of education; structured by a commission of experts, that will analyze the insufficient achievements obtained with the current education methodology.

Introducing a new conceptual line of teaching that will provides new approaches and even new slogans to be transmitted to society, could be a necessary test in a search for a progress in the results so far obtained.

Clarify fundamental concepts previously mentioned and up-to-date information on organ donation and transplantation will help to understand fears and prejudices generated by ignorance.

Previous information to the teachers by experts in social communication and specialists in transplants will be fundamental as an initial step for the realization of a new program of education of the youth with transmission to the whole society.

Recent experiences in Argentina and Canada, inspired by the previously described conceptual suggestions, have produced positive results. In their responses to a questionnaire completed after the class, students (aged 10–16 years, from households of different socio-economic levels) showed a clear understanding of the concepts taught, and a coherent and logical interpretation of the problem.

The pilot trial consisted in a 45 min course followed by discussion and questions, with the following purposes:

1. Evaluate the possibility of application of the project.
2. Assess in countries with socio-economic differences, the understanding and acceptance of basic concepts concerning organ donation.

Materials and methods

- Mixed school in Argentina.
- Average age 12.9 years [11–14].
- Girls school in Canada.
- Secondary school: 45 students.
- Average age 14 years [13–16].

The following where the main topics included in the lecture:

1. History of transplantation.
2. Brain death.
3. Cadaveric and living donor.
4. Mortality on the waiting list.
5. Religious attitudes toward transplantation and organ donation.

This pilot test highlights the usefulness of a stable and universal introduction transplantation subjects in the curriculum of youth education [50].

2.2. The role of international organizations responsible for health and education

Considering the stagnant rates of organ donation, it is important to mention that in the search for possible solutions the potential role of a different educational strategy has not yet been significantly promoted by the World Health Organization (WHO) and international transplantation societies.

In contrast, it is interesting to note that the WHO and The Transplantation Society have developed an intense and positive legal and ethical interest into the serious problem of organ commerce and transplant tourism. It would be of great utility to promote joint activity by the WHO, UNESCO and Transplantation Societies, and religious authorities, in order to generate international consensus meetings, looking for efficient educational policies and search for other possible solutions to this global social emergency.

3. Conclusions

Organ and tissue transplants provide the possibility of new life and improved health and well being. However, the number of patients who died due to lack of donated organs increases daily. The main cause of this paradox is inadequate social behavior regarding organ donation, both in life and after death. Education at all levels of society may offer the possibility of improving this critical situation. In this chapter, we suggest a change of methodology based primarily on a modification of the message. We emphasize the need to focus education at an early age, starting with primary school and intensifying it at the university level, especially in medical sciences.

In the USA from 1988 to 2010, donation-related policies on organ donation and transplantation increased in number from 7 to 50. That is great progress with intentions to improve a crisis. Nevertheless as remarked by Chatterjee et al. strategies to encourage organ donation have had no observable effect [55]. Millions of dollars have been unsuccessfully spent on the education of society seeking to change feelings toward organ donation [27]. Consequently, international figures have suggested the controversial need to institute legal and economic incentives to living and deceased donors [37].

The current contradiction is that the global success of organ transplantation is growing as fast as the waiting list and the mortality of its members.

Fundamental measures looking to improve the shortage of donated organs have been scientific and technical; however, there has not been a significant increase in the number of organs and tissues obtained for transplantation. Almost inexplicably, society's communication and education methodology has remained practically unchanged over time. It is clear that human behavior regarding organ donation should be critically analyzed to identify the most effective

solutions for the shortage of donated organs. The virtual absence of positive attempts to modify human behavior concerning organ donation suggests a scientific stalemate for crisis resolution by the main protagonists. A change in the current philosophy of social education policies regarding organ donation and transplants is clearly necessary, as recognition and support by international health and education organizations will undoubtedly confirm this need.

As we have previously discussed, should be of critical importance to consider. In the development of new educational plans, the complex barriers to donation, deeply established in social behavior: fear of death and respect for the integrity of the body.

These psychological inhibitions have not been primarily considered in the educational programs. In particular, should be of interest that people can realize some crucial concepts such as that today the dead body represents a unique irreplaceable source of health.

An educational program developed by experts in sociology, psychology and theologians should be essential to carefully planned potential solutions of these real barriers to donation A change in transplant and organ donation education programs, efficiently reviewed, may be a challenge to change the inadequate people's behavior and the tragic consequences of organ failure. The persistence of current reality becomes an unanswered uncertainty.

We consider it of interest at the end of this article to hypothesize why the positive results obtained at an educational level in schools at Argentina and Canada, which have been internationally reported in specialized journals, have not been repeated, particularly in the analysis of the structural changes that have been suggested to carry out concerning education and the message to Society.

Models of social education whose qualities can generate important changes in individual behaviors can be resisted. All potential interested that has experienced long time traditional establishment educational programs usually develop resistance to a change.

Certainly, this resistance to educational changes, especially increases when it modifies ideas set up in the consciousness of people through the time.

The introduction of these educational changes with the aforementioned characteristics also seeks the learner's autonomy and the maximum development of their ability to change concepts firmly established in their knowledge.

In fact, these innovations cannot be taken in isolation or generated at individual levels. Undoubtedly they require the support of official or private institutions responsible for international education policies.

Dealing with ideas that will modify long-held concepts typically produces anxiety and worry.

Obviously, we can think, following concepts mentioned by Schoenberg in 1991 expressed "the transplant community has to offer strong stimuli that induces professors in various places to assume such a task" [54], that even for the main protagonists of the dilemma of organ shortage, the professionals responsible for the practice of transplantation, the influence of traditional educational establishment have not been totally overcome.

In practice, every action to modify this crucial problem should be supported by International Transplant Societies through discussions and forums, which must include, in particular, leading international organizations in education and preventive health policies such as WHO and UNESCO.

Alternatives to improve people's knowledge should be carefully planned so as to avoid the possibility that organ donation becomes equivalent to Shakespeare's disturbing reality of the dramatic symbol of "to be or not to be".

Conflict of interest

The author certifies that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Chapter 5

The Process of Organ Donation from Non-Living Donors: A Case-Based Journey from Potential Donor Identification to Organ Procurement

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Additional information is available at the end of the chapter

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Abstract

Each year, thousands of people worldwide succumb to end-organ failure while awaiting life-saving transplantation procedures. The shortage of organs continues with no signs of easing in the foreseeable future. The availability of organs from living donors continues to be constrained. At the same time, the cumulative knowledge of organ preservation is advancing steadily resulting in an enhanced ability to utilize a growing number of previously unsuitable tissue and organ gifts. Our ability to procure and preserve more organs is accompanied by the increasing use of so-called "expanded criteria" donors, or those whose organs may not have been suitable without modern advances in organ preservation science. Within the overall context of organ donation from non-living donors, the importance of physiologic and end-organ optimization cannot be understated. This chapter discusses our current state of understanding of optimized organ procurement approaches derived from practical experiences and "lessons learned" at a high-performing, community-based tertiary referral hospital.

Keywords: clinical optimization, ethical considerations, organ donation, organ procurement, organ procurement organization, transplantation

1. Introduction

The process of organ procurement from non-living donors (OPNLD) is multi-factorial and complex [1]. In the United States, significant progress has been made toward wider availability

of organs for transplantation [2, 3]. This includes increased use of organs from donors after cardiac death (DCD) as well as the inclusion of "expanded criteria" donors (ECD) and the introduction of information technology-based tools for better organ allocation [3]. Despite tremendous progress, major challenges remain including the growing number of patients entering transplant waiting lists [4].

It has long been known that more organs may be available than are being currently captured within the existing organ procurement organization (OPO) network [5]. In addition, some organs are lost through suboptimal organ donor resuscitation and/or lack of timely OPO notification [6, 7]. In this chapter, the authors will discuss the modern process of OPNLD, beginning with potential donor identification, then proceeding with physiological optimization, and finally ending with the procurement procedure. To illustrate key points more effectively, a realistic hypothetical case-based scenario will be presented.

2. Case vignette

A 65-year-old male presents via aeromedical flight after a head-on, two-car collision in which he was the restrained passenger. The crash occurred at 7:45 pm–approximately 45 min prior to his arrival at the regional Trauma Center. The patient's wife, the driver, was pronounced dead at the scene. According to the Emergency Medical Services (EMS) report, the patient had Glasgow Coma Scale (GCS) of 9 shortly after the incident and was complaining of left-sided chest pain. It took 30 min to extract the patient from the car.

The patient arrived at the regional Trauma Center at 8:32 pm. On initial assessment, blood pressure was 90/65 mmHg, pulse was 120 beats/min, respiratory rate was 28 breaths/min and the patient had a GCS of 4. Intravenous fluid administration was started and the patient was immediately tracheally intubated. The FAST ultrasound exam was negative. Chest radiograph showed multiple rib fractures and a pneumothorax on the left, for which a chest tube was placed. The secondary trauma survey showed an obvious deformity of the left parietal skull, a left chest wall injury, and a visibly displaced left-sided hip fracture.

Hypertonic saline infusion was begun for treatment of a presumed severe traumatic brain injury (TBI). Detailed timeline of subsequent events now follows:

- 9:00 pm: Once the patient was hemodynamically stabilized, he was taken for computed tomography (CT) of the head, cervical spine, chest, abdomen and pelvis. Immediate review of imaging confirmed left hip fracture-dislocation, multiple left-sided rib fractures and a pneumothorax on the left. Of special concern was the presence of large left epidural and subdural hematomas with evidence of diffuse axonal injury, as well as extensive subarachnoid hemorrhage. The patient was found to have 1.5 cm midline shift with uncal herniation.
- 9:15 pm: Emergent neurosurgery consultation was placed, with immediate arrival of the on-call neurosurgeon. The patient experienced a brief period of hemodynamic instability featuring both bradycardia and tachycardia, followed by the appearance of severe hypertension (systolic blood pressures >200 mmHg), and finally the appearance of bilaterally dilated, unresponsive pupillary exam. The injury was deemed non-survivable.

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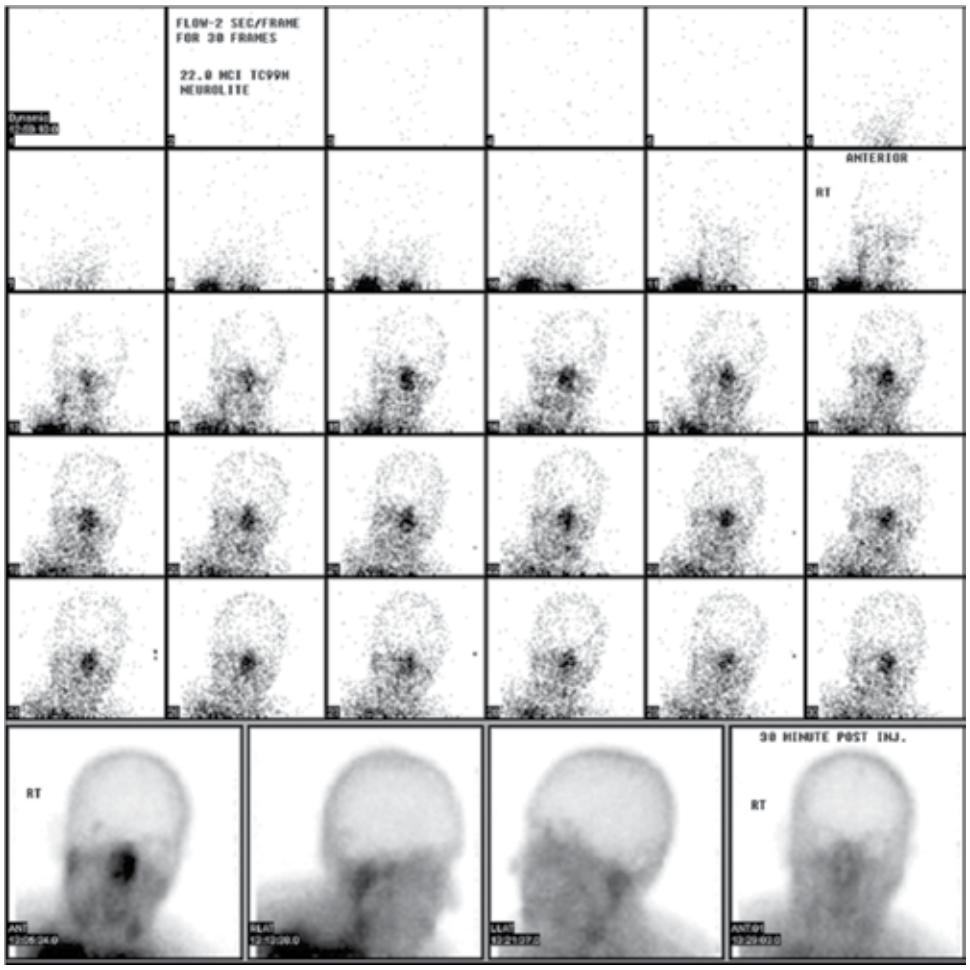

Figure 1. An example of a Tc-99 m radionuclide cerebral blood flow study showing typical appearance of "no flow" within the cranial vault (e.g., the white appearance). At the same time, the facial region is richly perfused with blood (e.g., the "hot-nose" sign), providing a stark comparison to the lack of intracranial flow. **Credit**: Jason Robert Young, M.D. Image used under Creative Commons Attribution-Share Alike 4.0 International license.

- 9:25 pm: The patient's neurological exam was consistent with brain death, as confirmed by two independent physicians credentialed in this clinical area of expertise. Confirmatory testing in the form of cerebral perfusion study was ordered and the patient was taken to the intensive care unit (ICU) in the interim for ongoing medical management. In accordance with applicable State Laws, The Local Organ Procurement Organization (OPO) was contacted for possible organ donation.
- 10:30 pm: The patient's family arrived at the hospital and discussed prognosis and goals of care with the clinical team. At that time, his family members indicated understanding of the diagnosis, and the gravity and irreversibility of his condition. They informed staff that the patient was an organ donor and that they would like to honor his wishes in the event of brain death. Medical management and stabilization continued in anticipation of the cerebral perfusion study.

- 8:30 am: After stabilizing the patient sufficiently for transfer to the radiology department for confirmatory brain flow study, determination was made to proceed with such testing (**Figure 1**). Following the confirmatory study to determine brain death, the patient continued to receive maximum medical management to ensure adequate organ perfusion and maintain tissue oxygenation. Representatives of the local OPO were introduced to the family and the formal process of organ donation was initiated.

- 9:00 am: All required procedures for determining blood type and tissue match were progressing as planned. Organ placement discussions between the local OPO and potential receiving institutions were ongoing. The patient underwent trans-esophageal echocardiography and bronchoscopy to determine his suitability as a heart and lung donor, respectively. Throughout this process, medical optimization of end-organ perfusion continued, including the use of advanced cardiac monitoring, as well as intermittent use of vasopressors and inotropes.
- 11:00 am: After determining that the patient will be able to donate his kidneys, pancreas, and liver. However, due to severe chest trauma his lungs and heart were not suitable for transplantation. At this time, final placement decisions were made.
- 12:00 pm: The patient's family members were allowed to see him prior to the organ procurement operation. The process was to occur within the next several hours, and was predicated on finding a suitable recipient for one of the kidneys. Medical optimization therapy continued.
- 15:30 pm: The patient was taken to the Operating Room for the procurement of bilateral kidneys, pancreas and liver. Three different institutions received kidney, liver and combined kidney-pancreas for transplantation, respectively. Recipients of the above organs underwent uneventful immediate post-transplantation recovery and were able to resume normal lives.

3. Historical background

Organ transplantation and procurement has a rich history, with early accounts of tissue removal and re-implantation involving skin, bone and teeth [8]. During the past several decades, significant progress was made in the area of human transplantation. The evolution of both surgical techniques and immunosuppression resulted in organ transplantation becoming commonplace [9, 10]. Notable medical pioneers of modern transplantation include Dr Christiaan Barnard, Dr Alexis Carrel, Dr Joseph Murray, Dr Thomas Starzl and many others who helped advance the basic scientific and medical understanding required to achieve today's state of knowledge and clinical reliability [11, 12].

From a clinical perspective, the first successful transplants of the modern era involved skin and corneal tissues, and took place in the early 1900s [8]. These experiences, especially involving skin grafting, were plagued by failures well before the concept of tissue compatibility and rejection was fully elucidated [11]. Solid organ transplantation beginning with the kidney was even more challenging. Russian surgeon, Dr Yuri Voronov is credited with the first recorded

report of a kidney transplant from a recently deceased donor in the mid-1930s [8, 13, 14]. Although unsuccessful, this procedure foreshadowed the various technical and ethical challenges modern transplantation would face well into the future.

The subsequent years and decades were characterized by a mixture of "trial and error" until the first successful living donor kidney transplant was performed in the mid-1950s by Nobel prize winner, Dr Joseph Murray [8, 11, 13]. The procedure was performed between identical twin brothers, both of whom survived for some time after [15]. Although the understanding of the organ rejection process was still very poor, Murray's successful transplantation strongly implied the need of genetic congruity between donor and recipient. Shortly thereafter, Main and Prehn discovered that chimerism could be induced by using radiation to weaken the immune system of mice, leading to improved acceptance of donor tissue [16, 17]. Several years later, Dr Murray attempted this method in his next successful kidney transplantation, but this was unfortunately preceded by significant mortality among patients who underwent total body irradiation prior to receiving new organs [11, 18]. Of note, this successful non-twin-twin transplant recipient was the first well-documented case to recover from rejection [11, 19]. Subsequent failures associated with total body irradiation, including significant morbidity and mortality, led to increased interest in other potential methods of immunosuppression [13].

As a result of intensive research efforts, immunosuppressive medications were soon introduced to help address the problem of graft rejection [20, 21]. Initially the use of monotherapy was attempted with limited effectiveness. It was Dr Thomas Starzl (whose success rates exceeded most in the field at the time) who proposed a cocktail of immunosuppressive agents capable of reversing rejection [11]. This was yet another critical discovery that over time facilitated the expansion of efforts into transplantation of other solid organs, including the first liver transplant in 1963 by Dr Starzl, the pancreas in 1966 by Dr Lillehei and the heart in 1967 by Dr Barnard [8, 22–24]. Although long-term survival of early transplants and their recipients varied, the 1960s ushered in a new era with transplant centers appearing across the world [8, 11, 24]. Organ preservation science developed out of the necessity to ensure organ viability during transport from donor to recipient [25].

Beginning in the early 1900s, Charles Guthrie proposed that cooling of organs may offer a way to preserve them during transport [11]. It was not until the mid-1960s that the use of cooling agents became standard practice with the introduction of the now widely accepted University of Wisconsin solution [11, 26, 27]. With progress being made in multiple aspects of transplantation, new hope arose for patients suffering from various forms of end-stage organ failure. As organ preservation and technical aspects of transplantation advanced, attention shifted to ensuring adequate organ availability [7]. Along with this challenge came the ethical and legal considerations surrounding death and organ donation, which will be addressed in greater detail later on in this chapter.

4. Ethical considerations

It is the responsibility of physicians to "above all, do no harm" [28]. This concept should permeate each clinical decision made. In theory, this ethical principle is paramount to an equitable

and just system of medicine, but oftentimes physicians find themselves in situations where they must weigh the risks and benefits of treatment, and answers are far from apparent [29–32]. The field of transplantation is among the most complex medical settings to navigate from the ethics standpoint. This is even more evident with the emergence of extremity and face transplants [33–35].

As Dr Murray embarked on the first living donor transplant, he faced an ethical dilemma. The concept of retrieving an organ from a perfectly healthy individual for implantation in another patient was, and still is, a gray area considering that by removing an organ from a perfectly healthy donor, you are exposing them to a number of risks. [36]. At the same time, certain organs (e.g., heart and pancreas) can only be procured from deceased donors, which raises a completely different set of ethical issues. These include questions of donor and recipient eligibility, fairness, procurement procedures, the legal definition of death, donor designation versus family permission and compensation [7, 36]. There is also a major concern regarding the potential of inequitable allocation of organs [36, 37]. This dilemma gained international attention with the first successful cardiac transplant in 1967 by Dr Barnard [38]. The concept of taking a still beating heart from someone considered "dead" created a significant conceptual and ethical problem in the eyes of many, with calls for a more concrete definition or list of objective criteria including non-responsiveness and other neurological signs that defined irreversible coma [8, 36]. It would be another 10 years before the Presidential Commission of the Study of the Ethics in Medicine proposed the current legal definition of death which included "irreversible cessation of circulatory and respiratory function" or "irreversible cessation of brain function including brainstem function" [36, 39]. The concepts of brain death and circulatory death will be discussed in greater detail later in this chapter.

Over 33,000 organ transplants were performed in the US in 2016, representing a 20% increase in donations over the past 5 years [40]. Yet about 115,000 individuals are currently on the waitlist for organ donation and 7000 waitlist candidates died in 2016, while awaiting a life-saving transplantation [40]. Although significant strides were made with regard to increasing donations, there continues to be an organ shortage, which has led to some ethically questionable practices [35, 41]. The National Organ Transplant Act of 1984 brought together top content experts and outlined key issues related to the different aspects of the organ procurement process [42–44]. This group established key ethical principles, including the requirement that there would be no payment in exchange for organs and that organs must be voluntarily given [36, 43, 44]. This act also established our current U.S. system of organ allocation [45]. As one can see, there are numerous ethical concerns to take into account from a purely systematic viewpoint. This does not even account for the sensitivity of broaching the topic of organ donation to a grieving family coming to grips with the loss of a loved one. Even with efforts to encourage individuals to make these end-of-life decisions early on through donor registries, it is still common practice in many states to consult the family prior to proceeding with the organ procurement process [7]. Because the primary focus of this chapter is to describe the organ donation process in non-living donors, we will not be discussing numerous other ethical issues that arise when taking into account living donors. The subsequent sections of this chapter will outline OPO's and their critical role in the donation process. We will then proceed to describe the organ donation process in the context of both the above ethical and historical considerations, as well as the vignette presented earlier in the text.

5. Organ procurement organizations

The organ procurement process begins with the identification of a potential organ donor, then proceeds through the stages of notification of next of kin, the decision to donate, the process of physiologic donor optimization, the process of organ procurement and finally the transplantation of donated organs. Throughout this entire sequence of events, the OPO plays a central and an integral role [7]. The evolution of OPOs stems from the increased demand for organ donation, the need to organize and prioritize the process, and the necessity to ensure that organ allocation is performed in a fair and impartial fashion while at the same time efficiently and effectively providing organs to those in need [8, 46].

Prior to the inception of the modern network of OPOs, the organ procurement and allocation process was the responsibility of individual transplant centers [8]. They also shouldered the costs of the procurement and transportation process. As one can imagine this created a fragmented system in which each center would instinctively focus on providing resources to those in need of organs within their own hospital or locality [47, 48]. The evolution of OPO's provided a structured, equitable solution to streamline the process from organ donation to transplantation [7]. The current scope of functions of OPOs is vast and diverse, including interfacing with patient families; providing support to grieving relatives while helping them make critical decisions concerning organ donation; working in conjunction with hospitals and health care practitioners to physiologically optimize donors prior to organ procurement; coordinating with the United Network for Organ Sharing (UNOS) to find proper donor matches; and facilitating professional and public education as well as related research [7, 49].

6. The process of organ procurement

From the time of identification of potential donor to successful procurement and transplant, the process of organ procurement is a complex and intricate undertaking that we will discuss in greater detail in this section. For the purposes of our discussion, we will direct our attention to donation after death since the subject of live donors is outlined in other chapters. A simplified schematic of the overall process is shown in **Figure 2**.

The initial step in donation is centered on the potential organ donor. Although each clinical situation is uniquely different, the first step in the process is the recognition of the irreversible process of brain death, or the circumstances leading to non-heart beating donation [7, 50]. When examining the topic of donation after cardiac or circulatory death, we must go back to the corresponding legal and ethical definitions [23, 51, 52]. How and when does one determine brain death and circulatory death? In the current chapter's vignette, the circumstances of brain death were unequivocal as the patient had suffered a non-survivable injury. We realize that in clinical practice it may not be this straightforward, and repeated exams or confirmatory testing may provide the family with a greater degree of certainty regarding the finality of this devastating diagnosis. More specifically, confirmatory brain death determination with the brain scan showing "no blood flow" to the brain was helpful in the current scenario.

Figure 2. Simplified schematic of the organ donation process. Following the identification of potential organ donor, a cascade of events takes place that ultimately ends with successful organ transplantation. Further details regarding this complex process can be obtained from Wojda et al. [7].

Another critically important consideration is the emotional state of a family coming to grips with the untimely and unexpected loss of a loved one. The grief combined with the immense responsibility of determining what a loved one "may have wanted" can place a significant burden on his or her relatives. This can be especially difficult for families of patients with no advance directive, living will, power of attorney, or prior conversation concerning their organ donation wishes. When dealing with issues related to organ donation, health care providers must be extremely sensitive to family needs and ensure that their local OPO is involved early on in the process in order to prevent any potential conflict of interest [7]. The separation of responsibilities during these proceedings is critical in alleviating any concerns regarding the simultaneous provision of care for the patient along with facilitation of the organ donation process by the same individual and/or team [53, 54].

From the time a potential donor arrives to the hospital and is determined to have non-survivable injury, it is important that they are managed under the assumption that they may donate organs, and that care is both optimal and timely [6, 55, 56]. This includes early notification of the local OPO regarding the presence of a potential donor [7]. A great deal of attention must be paid to prevent hypoxia and systemic hypo-perfusion, both of which could compromise the

viability of the donor's organs [56, 57]. As was the case in our hypothetical vignette, the patient should be stable before undergoing any confirmatory testing. In the current example, such stabilization required approximately 10–12 h of continuous effort by the critical care team. Once the decision is made to donate by the family in the case of brain death or circulatory death, the OPO helps coordinate the remainder of the care process, including the distribution of the organs and the provision of highly trained staff to prepare for the actual organ procurement and preservation, as well as highly efficient transport of preserved organs to each recipient's institution [7]. At this time, we will discuss key components of the process of organ donation, including the determination of death and physiologic optimization of the donor.

7. Determination of death

As discussed earlier in the chapter, declaration of death—whether that be circulatory death or brain death—has been a controversial topic over the years. In 2014, an international forum was held in Montreal, Canada with the objective to provide a functional definition of death that encompassed the concepts of brain and circulatory death. They reported that "Death is the permanent loss of capacity for consciousness and all brainstem functions. This may result from permanent cessation of circulation or catastrophic brain injury. In the context of death determination, 'permanent' refers to loss of function that cannot resume spontaneously and will not be restored through intervention" [58]. It is important for health care practitioners of all levels working with potential donor patients to have a good understanding of the definition of death in order to be able to explain to grieving families the reality of the situation and its finality [59]. It is well established that adequate explanation of brain death is one of the critical components of the donation process [60]. The optimal timing of the consent process is also of great importance [61]. Checklists are helpful in maintaining the thoroughness of brain death determination, but do not replace the expertise, knowledge and compassion of physicians in end-of-life discussions with families of the potential donor.

8. Brain death determination

When a patient presents to the hospital with concern for altered consciousness, it is imperative to rule out all reversible causes of coma, first excluding the presence of any substances of abuse, medication side effects, electrolyte, metabolic or acid-base derangements [62]. Once these are ruled out, imaging can often shed some light on potential causes of neurological compromise. In the current case vignette, the CT was utilized in order to give the treating physician an indication of the magnitude of injury and likelihood of recovery. With that being said, official declaration of BD is actually a clinical one [63]. From definitional standpoint, BD is considered to be present when there is irreversible damage to the brain and brainstem [36]. In order to assess brain function, several key components are required, with the most important one being a thorough neurological examination including assessment of brainstem reflexes [62]. Various ancillary tests can also be performed to assess cerebral blood flow and brain electrical activity in cases with equivocal exam findings. The final declaration of BD (including the official time of death) rests with the treating physician.

Determination of brain death (BD) can be a complex issue in the evaluation of catastrophic neurological injury. Clinical diagnosis of BD is relatively uncommon in the acute care setting. Usually acute injury does not progress to the degree of absent brainstem reflexes and apnea. "The small percentage of…cases may be related to many factors including early aggressive care like decompressive craniectomies, change in referral patterns, and early withdrawal of care or decision to proceed with a donation after cardiac death protocol" [64]. There are predefined criteria for the clinical determination of BD which may vary slightly from country to country. The neurological assessment of suspected BD typically requires at least 25 tests and verifications. "The overriding principle is simple: establish cause, exclude confounders, determine futility of interventions, examine brainstem reflexes and test for apnea" [64].

Due to frequent inconsistencies related to the determination of BD, the Quality Standards Subcommittee of the American Academy of Neurology (AAN) met in the 1990s to establish clear definitions of clinical terms and associated testing. The group also determined the validity of ancillary testing versus the clinical exam and its applicability to the organ donation process. Clinical criteria for BD require a formal assessment and are only undertaken once all other potentially reversible cause are excluded. The initial evaluation needs to ensure there are "no lingering effects of prior sedation, or prior use of illegal drugs or alcohol. A reasonable guideline is to calculate 5–7 times the drug's elimination half-life in hours and allow that time to pass before clinical exam is performed" [64]. A core temperature of 36°C is also recommended which can be aided by use of warming blankets if necessary. As in the current chapter's vignette, neuroimaging such as a CT scan of the head should be performed to help determine cause of mental status deterioration. Clinical examination must include a thorough neurological examination including assessment of patient's level consciousness, as well as evaluation for verbal and motor deficits. The above exam must also include the interrogation of brainstem reflexes including pupillary, corneal, pharyngeal, and tracheal responses, as well as oculocephalic reflexes with doll's eye and cold caloric assessments. Apnea testing requires documentation of absence of a respiratory drive after a CO_2 challenge. This methodology also has strict criteria that must be followed to ensure accurate determination of absent respiratory drive [65, 66].

Although ancillary testing, such as electroencephalography (EEG), cerebral angiography, nuclear flow scan, transcranial Doppler, CT angiography and magnetic resonance (MR) angiography, can be utilized in the process of determining BD—due to variability in the interpretation of these studies—it is not a substitute for the clinical examination [67–70]. In aggregate, the above tests can provide additional data on electrical brain function and cerebral blood flow and "…can be used when uncertainty exist about the reliability of parts of the neurological examination or when the apnea test cannot be performed" [71, 72]. Expertise in determining brain death can be inadequate due to multitude of factors, including lack of clinical experience. This is likely one of the reasons why 6 US states require confirmation by a second examiner and some specifically require at least one of these examiners to be either a neurologist, neurosurgeon or intensivist [73].

As one can see, the determination of brain death can be quite complex in and of itself and can be even further complicated when the question of organ donation is raised. This is why we stress the importance of early involvement of a local OPO [73]. After the declaration of BD, assuming the presence of consent for organ and tissue donation, the care of the donor shifts to optimizing organ perfusion and viability [7]. The preservation of organs after determination

of BD also requires excellent coordination between the OPO and the medical management team [7, 64]. The key concerns for the medical team are to ensure hemodynamic stability and avoid the development of hyper/hypo-glycemia, acid-base or electrolyte derangements and pulmonary edema [7]. Many diagnostic tests and interventions occur during this phase specifically to ensure viability of key body systems and organs. Accumulated clinical evidence suggests "that a delay in declaration of brain death not only prolongs the time to organ recovery but also may increase the risks to transplantable organs, resulting in more complicated post-operative phases for the recipient" [64]. Finally, there is also evidence suggesting that second BD examination may negatively affect organ donor physiology due to inherent time delays [74], thus lending indirect support for ancillary/confirmatory BD testing.

From historical perspective, transplantation of organs was premised on the so-called "dead donor rule", where donors must be declared dead according to established medical and legal criteria prior to donation [75]. According to Chaten [76], "the dead donor rule (DDR) maintains that it is illicit to procure vital organs from donors until after they have been declared dead". This rule also required adherence to strict BD criteria, directly referencing that the "dead donor rule mandates simultaneous life and death within the same body for organ donation, a biological status that is inherently contradictory" [76]. The best way to decrease variability in BD determination is for all hospitals to implement the established set of AAN brain death guidelines [71, 77]. This would lead to less confusion and fewer inconsistencies among institutions. Due to the many complexities of end-of-life discussions, it is imperative that BD determination protocols become increasingly uniform in both content and application. Wahlster et al. [78] looked at practices and perceptions regarding BD declaration in 91 countries, noting that "countries with an organized transplant network were more likely to have a brain death provision compared with countries without" [78]. Barriers to consensus on universal BD standardization include social, religious and economic factors specific to each country and/or culture. Wahlster et al. [78] further note that "future efforts for uniform policies will need to include physicians with neurologic and critical care expertise, representatives of national and international major medical organizations such as the World Health Organization or World Federation of Neurology, and scientific and medical advisors of government agencies" [76].

9. Circulatory death determination

The shortage of organ donors has prompted resurgence in the utilization of donation after circulatory or cardiac death (DCD) [23, 51, 79]. While the concept of BD has been extensively discussed and there is a reasonable consensus as far as applicable criteria and assessments are concerned, definitive guidelines with respect to DCD continue to pose a challenge. In 1993, the Pittsburg non-beating heart organ donation protocol was proposed in order to provide criterion for organ procurement in the case of circulatory death. This protocol has come under criticism due to its questionable ethical application [80]. Although a definitive consensus is yet to be made from a legal and ethical standpoint, various OPOs are performing organ procurements with their own sets of standards and protocols [80]. Of interest, DCD historically constituted the largest proportion of organ donations prior to the advent of donation after BD. Subsequently, its utilization decreased substantially due to superior graft survival outcomes following donation after BD [81, 82].

10. Physiological optimization of the organ donor

Throughout the process of organ procurement, it is critical to achieve physiologic normalization and maintain systemic homeostasis in order to optimize the number of organs procured from each donor [6, 7, 83]. With this in mind, it is essential that potential donors be attentively managed in order to prevent systemic hypoxia and hypoperfusion to vital organs [7]. As in the current chapter's vignette, the ICU is the ideal location for the resuscitation of organ donors and the associated complex physiological and management needs [56, 84]. Cessation of brain function sets off a myriad of multi-system manifestations (e.g. cardiac arrhythmia, hypotension, profound acid-base and electrolyte imbalances) many of which can result in subsequent end-organ insufficiency and/or failure [56]. Consequently, ICU teams must be cognizant of these considerations and proactively work to counteract deleterious effects of BD through a number of intensive interventions. Adherence to strict protocols for management of the organ donor is of utmost importance to ensure optimal conversion rates and graft survival among recipients [7, 84]. Because such protocols may differ from center to center, it is essential for ICU teams to work with local OPOs to ensure standardization in the approach taken to optimize the organ donor prior to the procurement procedure [7, 84]. This alliance can help standardize the care received by each patient, regardless of the institution, and also provide important feedback on what is working and what may not be working. This then helps facilitate performance improvement from an OPO system standpoint [7].

As previously mentioned, there are multiple adverse physiologic events that may occur when the brain ceases to function (**Figure 3**). These include reflexive hypertension and subsequent hypotension; systemic "endocrine failure" leading to significant hormonal derangements including diabetes insipidus; and finally secondary phenomena such as acute lung injury and neurogenic pulmonary edema [85–88]. When managing patients, who succumbed to BD following TBI and multi-system trauma, special care must be dedicated to preventing any secondary insults

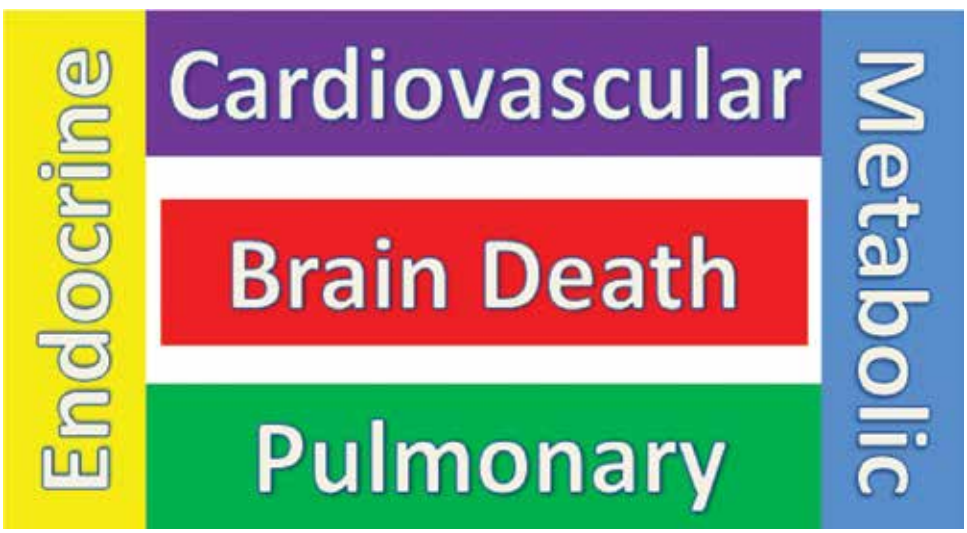

Figure 3. A diagram showing the most important and most commonly reported systemic manifestations following brain death. In the management of an organ donor consideration must be made to all of the above changes, including proactive approaches to normalize any deleterious physiologic disturbances.

from any injury that may pose a potential threat to end-organ viability [87, 89–91]. In order to properly monitor the various changes that may be rapidly occurring within the donor, frequent laboratory assessments and advanced cardiopulmonary monitoring are recommended [7, 57, 62, 92]. Correcting electrolyte and metabolic abnormalities can increase viability of donated organs according to an observational study conducted by UNOS [92]. Another important UNOS report stated that by setting certain parameters or goals for managing donors during the period leading up to organ procurement, care teams were able to augment the number of viable organs from each donor [83]. Among endpoints that increased likelihood of organ viability were maintenance of central venous pressure as well as PaO_2:FiO_2 ratio, optimization of cardiac ejection fraction and normalization of serum sodium and creatinine [83]. Thyroid hormone levels are another important element to closely monitor and correct during organ optimization. Exogenous thyroid hormone is routinely administered along with methylprednisolone and insulin to maximize organ donor viability [62, 83, 92]. Among more progressive developments, the use of extracorporeal membrane oxygenation has been proposed as a method for expanding the donor pool after cardiac death [93]. However, this approach may be prohibitive from a financial standpoint.

11. Conclusion

In summary, the field of transplantation has made significant strides throughout the years. Following its humble beginnings as an experimental science, it evolved into the primary therapeutic option for patients suffering from end-stage-organ failure. Modern transplantation offers hope to those who even a few decades ago would have none. Going hand-in-hand with that hope are the ethical and legal ramifications related to the donor and their families. With the demand for organs far exceeding the current availability, a better framework is needed for both maximizing the procurement of organs from eligible donors and better allocation of these gifts-of-life across patients on transplant waiting lists. Closer examination of all available organ donation avenues is warranted, including the assessment of opportunities offered by the use of expanded criteria donors and greater utilization of donation after cardiac death.

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Chapter 6

Family-Centered Care to Improve Family Consent for Organ Donation

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Abstract

The need for organ donation has increased over time, but the shortage of available donors is the major limiting factor in transplantation. Organ donation refusal from relatives of potential donors with brain death significantly reduces organ availability. We report a brief analysis about family conflicts in decision-making and causes for refusing donation; moreover, we describe new family-centered strategies in the intensive care unit (ICU) and our systematic communication approach between medical staff and patients' relatives. In 2016 we conducted a single-center, non-randomized, controlled and before and after study in our ICU, an 18-bed intensive care unit (ICU) of a university hospital. We compared the rate of consent for organ donation before and after the introduction of the new communication approach. The application of a new communication approach between medical staff and relatives of brain-dead patients was associated with a significant increase in the rate of consent to donation. The positive results of the 3-year period 2013–2015 have been confirmed in the 2-year period 2016–2017. Our results highlight the importance of empathy and counselor support of relatives in the ICU.

Keywords: organ donation, patient-centered care, intensive care, family

1. Introduction

The number of donors is inadequate although the need of organ transplant has increased over recent decades [1–4]. Most of the organs available for transplantation come from deceased

rather than living donors. Then, patients who have been declared brain death are the largest source of transplantable organs. The consensus rate improvement of solid organ donation from deceased donors is considered one of the main strategies to increase the availability of organs for transplantation. Unfortunately, a low percentage of people register their donation wishes in life. Furthermore the laws concerning individual consent expressed previously in life are dissimilar in different countries; above all, the population's adhesion to the law may not be comparable so that family members are often the only ones that can express consent to organ donation. Although the main factor limiting the number of donations from brain-dead potential donors is the low rate of consent from their families. Furthermore, in the clinical practice, even when the patient has registered their will on the organ donors' registry and there is no legal obligation to obtain consent from the relatives, if a relative denies the consent, organ donation may not proceed [5, 6]. The consensus rate improvement of solid organ donation from deceased donors is considered one of the main strategies to increase the availability of organs for transplantation. They get the bad news about the possibility and then of their loved one's death in a short time. The settings where family members receive this information are intensive care or emergency areas, unfamiliar, unknown and often confusing places. Several studies examined the reason why some potential donors' families refuse consent, while the others analyzed a series of "modifiable" factors related to meeting with the family(s) especially. Kerri Barber et al. in 2006 reported the results of an interesting audit of all deaths in intensive care units (ICUs) from 1 April 2003 to 31 March 2005 regarding 341 intensive care units in 284 hospitals in the United Kingdom [7].

Among the relatives of 2320 potential heart-beating donors who were approached for donation consent, 41% refused. The main reason for refusal is the knowledge of the desire not to donate expressed by the deceased person in life (16%). In the last 20 years different studies have emphasized how privacy and request timing, the involvement in the patient care team that gives information to the family of at least one member of the staff of organ procurement and a care to brain death significance explanation are key factors to improve relationships [8–10]. Furthermore several authors pointed out that religious, cultural and social beliefs play an important role in the family's decision-making process. Besides, concerns on exact time of death and body integrity after death and emotional vulnerability are equally crucial. The process is also influenced by education, income, sex and age of the family members [6, 7, 9, 10]. There are many relationship elements and emotions involved in the donation process. Ignoring family's emotions without taking care of the relational aspects can hamper fully aware choices. De Groot and colleagues in 2015 reported the results of a qualitative research in a group of donors' relatives regarding the decision-making donation process. We reported the main results of this research in this context. The authors confirm how the stressful sudden event, the interaction with unknown people, the difficulty of mourning and making a decision for the loved one whose loss is being wept over are determinant factors in the decision-making process of the family. The occurrence rapidity does not allow us to be aware of the reality we are experiencing and of any decisions that must, in any case, be taken. The potential donors' relatives describe the decision-making process as complex mainly because they had to make a decision on behalf of the deceased (surrogate decision). The conditions that might contribute to this complexity are the feeling of having limited time and

a sense of urgency, the feeling of not being competent to decide and a sense of despair and crisis and the need for an agreement between all relatives. The ethical considerations regarding the possibility of helping others, the integrity of the body and life after death reveal the emotions and personal motivations coming into play going beyond the event itself and the immediate and concrete decisions that family members are called to take [11]. Vincent A et al. also reported the common reasons for family refusal: relatives not wishing surgery to the body (concerns regarding disfigurement), feelings that the patient has suffered enough, feeling incompetent regarding the patient's wishes, disagreements among the family group, religious/cultural reasons, dissatisfaction with the health-care staff and process, concerns over delay to the funeral/burial process, inability to accept death, lack of understanding of brain death, concerns regarding integrity of process and the fact that they were emotionally exhausted themselves.

The same authors pointed out that several studies come to the conclusion that following elements could be useful [12]:

1. Guaranteeing the right timing of a request
2. Guaranteeing an appropriate setting
3. Providing emotional support
4. Imparting specific information (e.g. regarding the nature of brain death)
5. Guaranteeing adequate staff training
6. Guaranteeing staff involvement in a planned process of the organ donation request

Italy as a whole is undoubtedly the country that has developed a model similar to the Spanish one. Spain has become a reference point for European and global solid organs donation and transplantation with the highest donation rates. Italy has a cultural and health structure similar to Spain and it is needed to create an organizational structure since the 1999 law [13, 14]. In 1988, the Council of Europe Committee of Experts on Transplantation (SP-CTO) was established. The Committee included more than 30 countries with observatories from Canada, Japan and Israel and was incorporated by the Eastern countries for which it represented the only contact with the great Western countries' transplant referents for many years. The Organización Nacional de Trasplantes (ONT) held the presidency of this Committee for 7 years (1995–2000 and 2003–2005). The majority of the documents you need as a basis for the preparation of the Commission and the European Parliament actions on transplants have been processed in Spain. Following the approval of Article 157 of the Amsterdam Treaty, the European Union has developed the European directives on transplantation, guaranteeing the quality and safety of the tissue and cell organs. In May 2003, the Executive Council of the World Health Organization (WHO) accepted to set up an international group of experts to examine the issues related to transplants, including xenotransplantation. Spain is the promoter of a punctual organization in the field of transplants that Italy has been sharing. In particular, Italy shares with Spain the following key points regarding transplantation

- The health status of a country or region is also affected by a good functioning of the donation transplantation system. This is conditioned and conditions a good quality health system
- A training program is needed for intensive care and operating theaters' doctors who work with potential and receiving donors

Spain has collaborated very actively organizing different types of courses some of which specifically concern family members' interviews. The proximity between Italy and Spain and the similarity of the language facilitate the collaboration process between the two countries. Italy currently has a three-level (national, regional and hospital) transplant system organization and a training system that follows the Spanish model. Initially, the percentage of refusal to donate was quite high; currently, the percentage of waste is about 30% and it has been stable for some years. We believe we can still do a lot to reduce this waste amount. The WHO provides technical support for the correct development in the field of transplants, promotes international cooperation and continues the examination and collection of global data on allogeneic transplantation safety, quality, efficacy and ethics.

The work strategy adopted to implement donations is based on a process called global base of knowledge about transplant (GKT) defined in resolution WHA57.18. The GKT consists of four lines of work that require progressive development and includes the following aspects:

- GKT1: It includes activities and practices in allogeneic transplants.
- GKT2: It includes allogeneic transplants legislation and organizational systems. The main objective of the registry is to gather information on organs, tissues and cell donation and transplantation activities, as well as information on the legislative and organizational aspects of transplantation all over the world and to make professionals and the general public know them.
- GKT3: It includes response to transplants, risks, survival and surveillance systems, safety aspects and ethical aspects. Creating systems is considered a surveillance priority that guarantees transplants safety, so that any effects and adverse reactions, both in receiving and in living donors, can be communicated to take any necessary measures. From an ethical point of view, it is intended to obtain information on the measures taken from member states to protect the poorest and most vulnerable groups, for example, from organ and tissue trade.
- GKT4: It includes xenotransplantation. Xenotransplantation could be the alternative to the lack of human origin organs and tissues. However, experimental preclinical tests haven't justified human clinical trials yet. Transparency is a fundamental and mandatory requirement for the WHO in donation and transplantation-related practices, as well as in information collected around the world.

To find out more about the world situation regarding organ transplants, we recommend the website [2].

No religious beliefs preclude organ donation; people usually refer to personal conscience. The national frame guarantees quality and safety for the donor and the receiver [15].

With regard to all that was mentioned above, we believe that optimizing the relationship with the potential donors' relatives might represent the main organ procurement strategy. The intensive care setting is the place where this relationship can be created.

The past two decades witnessed an increasing interest in the importance of health-care humanization. The recent guidelines suggest organized interventions and approaches aimed at supporting the families of critical patients. The objectives are twofold: to reduce the impact of serious illness and to prepare family members for decision-making and assistance needs. An international consensus recognized a new definition: "family" and "family-centered care" to identify this approach. The term "family" intends to identify a group of individuals who support the patient and with whom the patient has a significant relationship. "Family-centered care" is a respectful and responsive approach to health-care that meets the needs and values of individual families and is mainly characterized by: family presence in the ICU, family support and communication with family members. Family members will not only be present within the ICU but also actively participate in the care process. It is recommended that validated tools exist to optimize communication quality, medical understanding and reduce family decision-making conflicts, in setting the ICU up. Care practitioners must apply standardized and agreed communication approaches with family members of deceased patients and above all for those who died with brain-death criteria, especially [16]. Seaman J.B. and colleagues in Annals ATS (2017) suggest that the goals of clinician-family communication should be diversified and concern different aspects [17]. We very much share the elements discussed by these authors and we comment on some of them that seem relevant and in line with our choices. First of all, it is about establishing trust. The most important element of the quality of care for seriously ill patients' family members is the condition of trust in the care team. Sharing the decision-making process requires trust in the care team and at the same time allows to achieve a more stable relationship with the family members. Because family members can decide (when they are called on to do so) they must understand what has happened (and what the clinical consequences are), the effect of the choices on the beloved and the risks and benefits of the shared pathway. The second one is providing emotional support. ICU patients' family members have high levels of emotional stress and experience intense negative emotions such as fear and anxiety. These feelings are exacerbated by the communication of the bad news (death or threat of death) and by the decision-making process itself. Research in the neuropsychological field suggests that strong negative emotions such as fear and anxiety do not allow processing information detaching the subject from the reality (as already mentioned above). Therefore, attention to the relationship also allows us to devote a time to recognize emotions and reflect on them. Third element is conveying clinical information. Clinicians should take into account that family members should be informed in a clear, simple and precise manner regarding the diagnosis, prognosis and possibilities of patient treatment. Families need to be involved in the decision-making process. Lack of correct information can lead family members not to take the right decisions for the patient as well as being a source of stress and frustration. The authors confirm the importance of the interdisciplinary team role (involving psychologists, social workers, volunteers, care coordinators and communication facilitator) to improve family satisfaction and decrease psychological symptoms.

2. Our experience and discussion

2.1. New communication approach

Since 2013, as we have recently published, we developed a new communication approach addressed to relatives of patients admitted to the intensive care unit (ICU) ([18], with permission]). It consists of a patient/relative-centered approach, in which doctors, nurses, psychologists and volunteers support relatives throughout the care process. First, they try to acquire information on the family's social and cultural background and adjust the communication accordingly and second, they aim to understand the patient's will, a task that can be challenging in the intensive care context. When the patient first enters the ICU, the physician must give priority to treatment and can only speak briefly to the relatives. He reassures them that there will soon be time to acquire information and ask questions. As soon as the patient's conditions allow an interview with the relatives is performed so as to establish a relationship between the physician and the family. The physician who followed the patient's acute phase, the nurse who is in charge of him or her and a psychotherapist or a psychologist conducts it in a dedicated room. The staff also takes note of the relatives' phone numbers. The following interviews take place in the patient's room. During the first interview, the medical staff harmonizes on the needs and feelings of the family and retraces the patient's history and the recent acute event. This interview also aims to identify the main caregivers and establish the timetable and program for the following days. We applied a well-defined model, which can be divided into several steps:

1. Giving a warm welcome to the patient and his family unit;
2. Identification of the caregiver among the family members;
3. Taking care of the patient and relatives in a multidisciplinary way;
4. Early involvement of a psychotherapist;
5. Communication to the family in the patient's room;
6. Giving information on the patients' clinical conditions by the physician, the nurse and in presence of a psychotherapist and a volunteer;
7. Communication between the family and the psychotherapist and volunteer, with special regard to the family members' emotions and feelings.

2.2. Relationship with donors' families

The changes in the interaction modalities with family members and with the patient, when possible, have been consolidated since 2013 in our ICU; the changes represented a structured intervention. We believe that the new relationship's modalities with the patients' relatives, so far exposed and described in their reliability, have favorably influenced the reduction of opposition to organ donation by the family members of the deceased patient. Our ICU is an 18-bed, multidisciplinary ICU. It is a referral center for acute respiratory failure as well as a

trauma center. Relatives of brain-dead patients were approached according to an internal protocol, inspired by NICE guidelines, which temporarily distinguishes two phases: communicating brain death and proposing organ donation. These guidelines deal with delivering the end-of-life communication and developing a supportive relationship with potential beating-heart donors' families [19]. Often, patients who develop brain death did not express their opinion on organ donation during their lifetime. In our ICU all patients and relatives including relatives of brain-dead patients have been approached by the medical staff to establish a relationship since 2013, aiming at making them feel better and understood. The number of acceptances to organ donation in our intensive care was observed before and after the implementation of two major interventions: the opening of the intensive care (project called "OpenICU") to relatives and the introduction of the innovative communication approach mentioned above. Opening ICUs should come about not so much in answer to pressure generated by a growing social awareness, or in simple recognition of a right, but because this policy addresses more comprehensively the issue of respect for the patient, as well as providing more appropriate responses to many needs of both patients and families. It is a decision which requires doctors and nurses to rethink their relationships with patients and their families, which calls for original solutions for each individual situation and which should be subject to periodic checks. Psychotherapists support the relatives in finding a meaning to their experience and to understanding their own reaction and attitude. Further elements could have positively influenced the decline in organ donation, such as:

1. Increased attention to the initial welcome to the patient and his family;
2. The creation of a multidisciplinary team, giving a new value to non-medical figures, such as psychologists and volunteers;
3. The enhancement of giving information in the patient's room.

The Open ICU is realized when the whole team aims to abolish all of the unnecessary limitations at a temporal, physical and relational level. Opening the ward to family members allows patients and their relatives to be actively involved, fueling the healing process through affection and contact with their beloved. Besides, it helps patients to better tolerate hospitalization. When the Open ICU first opened, an innovative concept was introduced: interview with relatives no longer took place in a separate and impersonal "medical staff room." It was moved into the patients' room. This gave the opportunity for relatives to be physically close to their beloved while receiving bad news. This physical nearness soothes the relatives' grief. Being in the patients' room means sharing the environment with him or her: they hear the same sounds, feel the same temperature and see the same colors. The patient, his family and the physician now share the same scene. The relationship is still asymmetrical as the physician decides what to do and is trusted. However, the patient and his family are now considered as central elements of the scene. In fact, during the interview, there is an exchange of information between the physician and the family; the former is open to questions and doubts expressed by relatives, reducing errors related to a subjective interpretation of reality. Rather than speaking to the patient's family, the physician speaks with the patient's family. Conducting the interview in the patient's room also facilitates questions on machines and therapies with

which relatives are not familiar. Apart from verbal expressions, body language (which comprehends movements toward or from the patient, facial expressions, position of the relatives in the room) is part of the relationship between physician/nurse and the patient's family. In this regard, the interview taking place in the patient's room recalls the historical role of the doctor, who visited patients at home. Just as in the past, the doctor moves toward the patient. This movement is symbolically meaningful in the relationship. We think it is also important to recognize the emotions of both the family and the physician, who are all involved by seeing the patient while the interview takes place. The physician, the patient and the relatives recreate a family unit, giving more humanity to a very difficult moment that involves communication of the ICU patient's condition.

Besides, there are a few gimmicks that help improve the family's comprehension and memorization of information:

1. Communicate one piece of information at a time, in a specific, accurate and coherent way, "need to be honest, but should aim to mitigate the stress rather than exacerbating the fear and uncertainty";
2. Explain the patient's priorities in that moment;
3. Invite the family member to ask questions;
4. Verify that family members have understood what is explained to them.

The interview with relatives has several functions: it is informative, it is clarifying and it contains the family's emotional reactions. This last one is paramount in an ICU, where patients' deaths or losses of functional capacities can take place unpredictably. Historically, trust in doctors has been an unconditional feeling. During emergencies and critical events, families have no choice but to trust physicians, who are in charge of their relative's lives. Nevertheless, this trust must be respected and preserved because it is no longer unconditional. Nowadays, it is based on the physician's empathy with the relatives' emotions and on giving explanations to their worries and questions. By taking the family's emotions in charge, the physician creates a trustworthy relationship with them and can then make realistic predictions of survival and prognosis with them, also facing the topic of terminal illness. Many patients experience anxiety because of hospitalization and the impending threat to their lives. Similarly, psychotherapists help relatives to decrease their level of anxiety, allowing them to experience the ward as a more "human place," where there is space for relationships with the caregivers, who respond to help requests and throughout which emotions can be shared. The physician respectfully listens to the patient's or relatives' worries, allowing them to elaborate their emotions. At the same time, the physician conveys clear information authoritatively, though in a sensitive and truthful way. This is identified by Castagna as a counseling relationship, through which individuals develop awareness of their experiences and needs. Thanks to such relationship, patients manage to handle a challenging moment of their life by expanding their inner strength, even when reduced by critical illness. By communicating, we improve our shared knowledge, the so-called "common sense," the essential precondition to the existence of a community. Among the multidisciplinary team, a special

mention must be addressed to nurses. Nurses are probably the health-care professionals who spend more time with patients. Because of the Open ICU, they often work while relatives are in the room and most of the time not in the presence of physicians. For these reasons, they are in charge of explaining to the relatives what physicians told them during the interview. Their relationship with the patient and with relatives is unique and contributes meaningfully to the care-taking process. Even though they are not professionals, volunteers act as a connection between the world outside the hospital and the ICU. When nurses and physicians are occupied in emergencies or in routine clinical activity, relatives find an important referral in volunteers.

Potential donor patients' family members receive a favorable impact from the host, support and relationship strategies described so far. When brain death is declared, the family is entrusted by the care team to a dedicated team of the organ procurement. Relatives of brain-dead patients were approached according to an internal protocol, inspired by NICE guidelines, which temporarily distinguishes two phases: communicating brain death and proposing organ donation. These guidelines deal with delivering the end-of-life communication and developing a supportive relationship with potential heart-beating donor families.

The end-of-life communication recognized the following seven details:

Suggested locations: doctor's office, conference room, relatives meeting room, no hallways and common areas.

Meeting participants: intensive care specialist in charge of the decedent's care, nurse appointed to provide specific support, physician and nurse in charge of the transplant coordination system, family members wishing to be informed and psychotherapist. It is crucial that both staffs are present during this phase: the medical staff in charge of the patient, which will introduce the transplant coordination system staff.

Environment arrangements: sitting in a circle, if possible, access to phone calls, paper handkerchiefs, glasses and water. Avoid placing writing desks between the speakers and the relatives. Do not behave/act with detachment or indifference: avoid folded arms, fisting and fiddling; do not look away from the interlocutor; do not speak in a formal or distant way.

Delivering the communication (how and when): only after the first observation to assess the patient's death according to the Italian legislation. The assessment declaration is clearly and simply formulated by the intensive care specialist: "The EEG tracing we've just performed reveals the absence of brain electrical activity, there are no reactions to external stimulation, and the patient is not able to breathe autonomously. These circumstances unfortunately describe a death diagnosis. The legal-medical procedure to assess brain death has just started, and it will go on for 6 hours. At the end of the 6 hours, we will stop the artificial respiration procedure that is now keeping the heartbeating." A summary of the patient's clinical and therapeutic history can be added. Verify that relatives understand the meaning of brain death.

Developing a supportive relationship: give the family the appropriate time to react to the communication. Do not try to control or limit their reaction. Let them express rejection, denial, incredulity, anger, violent anger, desperation and so forth.

Medical personnel cope with reactions caused by the end-of-life communication: keep a silent and empathic behavior. Take care of actual necessities such as drinking, making telephone calls and handkerchiefs. Listen empathically to the relatives' memories on the patient's life and on the history of the illness that caused his death (accident dynamics, health-care delays, diagnosis mistakes, disappointed expectations on surgery and treatments, family problems, and so forth). Do not make obvious or inappropriate statements such as: "I'm so sorry for you," "I can understand your pain," "I know you're angry and I understand this," and so forth.

Visiting the bed: the nurse was appointed to support the family or the transplant nurse coordinator introduced themselves and their job. They take the relatives wishing to visit the patient to the patient's bed. Group visits are allowed (according to the size of the patient's room), preferably after the first reactions to the communication of death. The nurse answers to every question about heartbeat after death in a simple way: ".heartbeats and blood pressure are still being monitored because the heart is beating. We are sending oxygen to the heart artificially with a ventilator that is pumping air into the lungs but, unfortunately, there is no brain activity. The patient is not able to breathe autonomously and his chest is still moving just because of the ventilator." The nurse can offer the assistance of a religious person to administer the last rites and pray with the family.

A donation proposal recognized a following four details:

Suggested location: doctor's office, conference room, relatives' meeting room, no hallways and common areas.

Participants: intensive care specialist in charge of the patient's care, nurse appointed to provide specific support, physician and nurse in charge of the transplant coordination system, family members wishing to be informed and psychotherapist.

Environment arrangements: sitting in a circle, if possible, access to phone calls, paper handkerchiefs, glasses and water. Avoid placing writing desks between the speakers and the relatives.

Delivering the communication (how and when): The organ and/or tissue donation proposal follows in all cases the death communication, the bed visit and the last farewell. Before the donation proposal, it is advisable to give a brief summary of the patient's clinical conditions, focusing on the seriousness of initial conditions and prognosis. The donation proposal is communicated in a direct and simple way: "we propose to you an act of solidarity toward people who are in critical conditions. We propose to donate organs of your relative." Provide detailed information on the organ donation process and on its potential benefits. The proposal can be followed by a moment of privacy for the family to discuss and decide.

The Italian Transplant Coordination System monitors, audits and oversees organ donation, harvesting and transplant in our country. The Italian Transplant System controls organ donation, allocation and transplant; it is organized into three levels: National (National Transplant Center), Regional (Regional Transplant Center) and Hospital (Hospital Transplant Center). The Fondazione Agostino Gemelli Hospital Catholic University is one of the major regional

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hospitals in Italy. As one of the five level-1 regional hospitals in Lazio, it has approximately 1600 beds and 95,000 accesses per year. It is one of the leading centers for identification of patients who have been declared dead with neurological criteria. Our intervention could have a beneficial effect on the rate of consent to organ donation (COD) by the relatives of brain-dead patients. To test this hypothesis, we compared the rate of COD before and after the implementation of the protocol into our ICU [13]. In our work we analyzed the family consent rate (potential and real donors' ratio) before and after the introduction of the new communication protocol. We observed that a consent rate increased from 71% in the pre-intervention period (2007–2012) to 78.4% in the post-intervention period (2013–2015) with a specific increase of 82.75% from 2014 to 2015. In 2017, we observed a consent rate of 78.6% with a steady rise in the number of identified potential donors. At the same time we registered the regional consent rate of 68.1 and 73.1% in 2016 and 2017, respectively. During these periods, no significant variation of organ donation consent has been recorded at a national level. Our center has kept a constant commitment in increasing the observation rates, keeping the opposition to COD rate unvaried at first and then contributing to reduce it significantly. On the whole, the center's opposition rate compared with both national and regional average is significantly lower: 2017: national, 28%; regional: Lazio, 27%; our ICU, 21% (**Figures 1** and **2**).

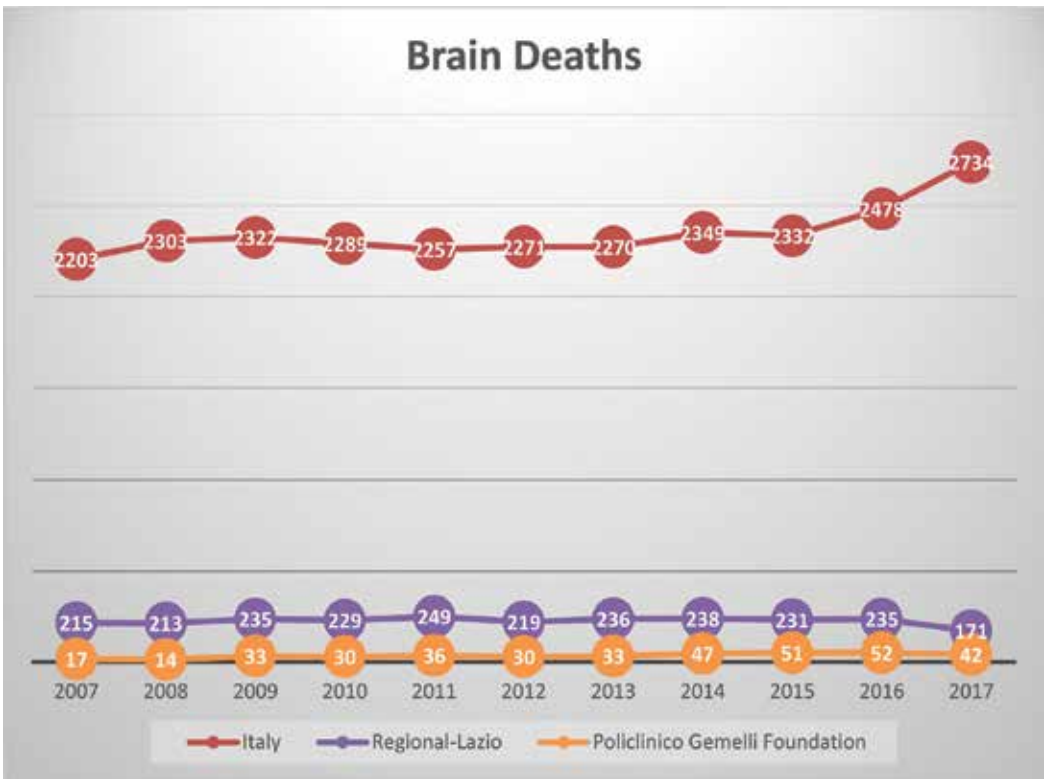

Figure 1. Brain deaths.

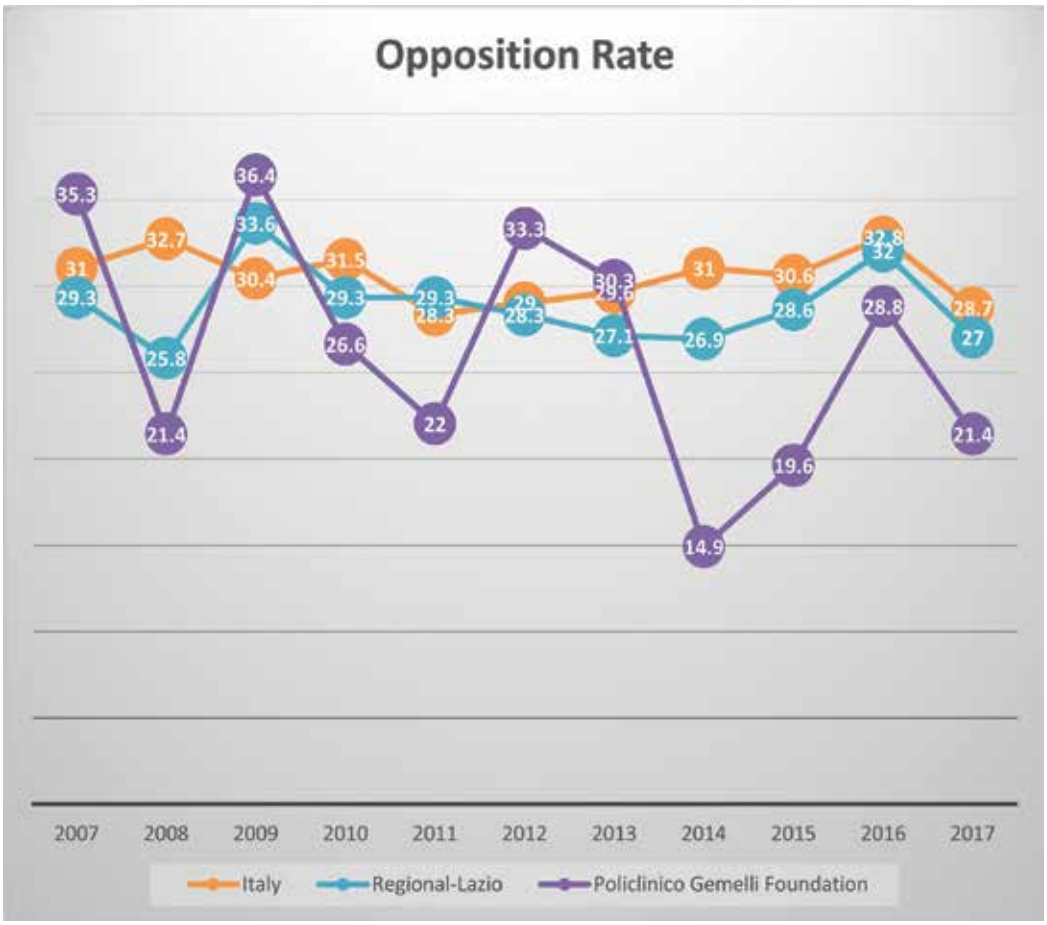

Figure 2. Opposition rates.

In our opinion, in order to suggest behavioral strategies to the care team involved in the assistance of potential donors and their family members, the following factors must be taken into account: family members are facing an acute event and high stress conditions; they may find different nursing teams and different people giving information they first learn about the life-threatening conditions and then about the death of the beloved. In this sudden critical situation, they are asked to take the place of the dear deceased, to make decisions about his body on his behalf. Family members have an extreme difficulty in contacting a condition of understandable reality. This is worsened by the fact of being in an unknown place, with unknown faces managing with emotions and personal convictions. Clinicians in the ICU should use structured approaches to communication including active listening, expression of empathy and considering the importance of explanation care. Communication is the process of sending or receiving messages through verbal and non-verbal means; therefore, an information field may consist of one or more subfields of information items such as thoughts, emotions and ideas. When, among individuals or among an individual and a group, there is a collaborative

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and ongoing message exchange, aimed to understand each other, the communication is integrated in our social realities and we can define this process as "transactional." In the transactional process the people involved in the act of communicating are actively and simultaneously sending information as well as receiving them. Participants perceive their communication as intentional. The information transfer between them takes place in a particular situation affected by relationship and culture. The speaker and the audience are co-communicators in the process with equal responsibility and power to create, as well as understand, a message. People encode their messages based on their own unique perceptions. Our past experiences, values, attitudes, knowledge, culture and feelings all influence our messages and also the way we interpret the messages of others. These influences are our unique perceptions or the way we see things around us. Before messages can be transmitted to another person or group, we must encode these messages. When the message is encoded it's ready to be transmitted or sent to another person or group. The receiver must then interpret the message, by filtering the new information through his past experiences, culture, attitudes, values, knowledge and feelings. This interpretation is called decoding the message. The receiver decodes messages based on his perceptions, which are different from the sender. The sender needs to make sure that the receiver understood the message; therefore, it is the receiver's job to convey a message back. The receiver's reply to the sender is called feedback. The feedback allows the sender to ensure that the original message was interpreted correctly by the receiver. Feedback helps the communicators make sure that the message has been decoded correctly. Once the cycle has gone full circle, it will repeat itself for as long as the conversation continues. We could even say that sender and receiver change roles throughout the process depending on who is sending the message and who is responding to feedback. The location and the time (the situation) in which communication takes place are relevant and they influence the encoding and decoding process. The latter is paramount in an ICU, where deaths or losses of functional capacities of patients can take place unpredictably. Historically, trust in doctors has been an unconditional feeling. The clinician should know all the elements described that are particularly relevant in the relationship with the potential donors' families. Family members often have difficulty understanding the condition of brain death. Their loved one still has a beating heart and a present breath (although assisted by the machine). The elaboration of death in this condition is not always simple. The doctor also needs to share the consent to the donation as soon as possible with the relatives. A narrative approach can be very useful to create a relationship of trust and support. The critical event causing the current situation is reported as telling a child a story already known. The narrative will describe the growing role of health-care workers who enter the history of the relatives and of the beloved. The care practitioners make supportive statements around non-abandonment and decision-making. We advise to remember the continuous need of feedback and of narrative approach. We also believe it is essential that ICU clinicians receive family-centered communication training as an element of critical care training to improve clinician self-efficacy and family satisfaction. These explanations promote a relationship in the here and now when both health-care practitioners and family members experience a state of stress and intense emotions. Knowing and seeing what is happening to the loved one, feeling part of and understanding the healing process is preferable to the anxiety generated by what is "unknown." Family members are forced by their relative's illness into an unwanted role, which provokes discomfort, dependence and anxiety. The relative is

vulnerable and asking for help; the doctor and the nurse, who are experts in medical care, take him or her in charge. Developing a good therapeutic and empathic relationship with the family, taking care of their emotional issues during this process, appears to lead families to opt for donation. The relationship with the care practitioners and family members can allow the creation of a sense of reality of the place and the moment. Then, it can make the relative aware of himself, of the event, of recognizing roles and responsibilities, to give permission to emotions and decision-making power to what is recognized as right and achievable. When considering patients in critical conditions admitted in the ICU, the care-taking process of the medical staff is addressed toward relatives more than toward patients, who continue to receive high-quality care. Clinically speaking, the communication-based relationship has a central role and a positive action on health improvement in the care process [18].

3. Conclusion

Further studies are needed to evaluate the effectiveness of an open intensive care unit and of a new systematic approach to communication between relatives and medical staff to decrease the rate of donation refusals. In our ICU, we applied this new approach based on the introduction of a multidisciplinary team and an increased attention for the patient and his family. In our single-center study, the higher increase in organ donations was registered in the 2014–2015 period, 1 year after the introduction of the new communication approach. This increase was also confirmed in the years following 2015. These results highlight the importance of effective communication with patient's relatives and the need to dedicate attention and time to them, especially in the ICU.

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Chapter 7

Current Situation of Organ Donation in China

Yingzi Ming, Baoren Tu and Quan Zhuang

Additional information is available at the end of the chapter

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Abstract

The shortage of organs is a worldwide challenge for transplantation. To alleviate such organ shortage and keep pace with the world's development and experience on organ transplantation, the pilot program of organ donation after citizen death (DCD) has been carried out in China, with support and attention from the Chinese government. From 2010, with the joint efforts of the government and medical workers for these years, a series of laws, regulations and related process management have been formulated, and great achievements have been made in DCD work. Currently, the main source of organs in China has come from DCD. However, some difficulties still restrict organ donation rates. Firstly, resistance to organ donation in China is often due to the conventional view among citizens. Secondly, some medical workers do not fully understand the definition and diagnosis criteria of brain death and therefore do not uphold and promote DCD work. Thirdly, the existing laws and institutions for organ transplantation fail to implement and remain defective. Nevertheless, China has made a firm and strong stride in DCD work. In order to carry out DCD work better, Chinese government, people and medical workers have to do much more.

Keywords: organ donation, transplantation, China, DCD, OPO

1. Introduction

Organ transplantation in China began in the 1960s and has gone through semicentennial of hard work and development [1]. With the continuous development of the technology in transplantation, we have made great progress in organ transplantation and have gradually improved and completed in aspects of operation technology, long-term patient maintenance, transplantation-related scientific research and transplant-related management. At the same time, more and more patients with end-stage organ failure get prompt treatment

and long-term survival. The curative effect has been recognized by the public. At present, it has become a routine treatment in our country.

However, from the 1960s to 2006, organ transplantation in China lacks supporting and supervision of specialized regulatory and law [2]. Due to the specificity of organ transplantation, which involves various of parts and links, it requires strict and orderly management of the identification, assessment, procurement, preservation, function maintenance, registration, distribution of organ resources and selection, evaluation, postoperative maintenance of recipient and other aspects. All these need to establish a scientific donation system at the national level to ensure the legal operation of organ transplantation, to guarantee the legal rights and benefits of the donor and the recipient, to ensure the quality of transplanted organs and the fair distribution and rational use of organs. The selection of recipient should also be based on the severity of illness rather than the other requirements. Meanwhile, strict control indications to ensure the quality of medical care and safety of recipients.

In 2007, our country promulgated "the Regulations on Human Organ Transplantation" (hereinafter referred to as "the Regulations") [3], which marks that our organ transplantation has embarked on a legal track [4]. After the relevant departments took a series of positive measures, initial results were obtained. The objective of implementing the "the Regulations" is to establish an ethical and sustainable organ transplant system. In the past, there were some serious problems in organ transplantation in our country, such as organ trading, tourism transplanting, opaque distribution, and so on. In the meantime, due to the lack of voluntary organ donation, there had been a history of reliance on organs of deceased prisoners [5]. These are all serious mismatches with the development of our country's modernization and seriously restrict the sustainable development of organ transplantation [6]. It is not only difficult to guarantee the quality and safety of transplantation but also undermine the image of our civilized power. With the rapid and steady development of all walks of life in our country, the firm establishment of legal system, the increasing emphasis on health of people and the increasing demands on the quality of life, the medical industry in our country has become the industry which needs more improvement. The development of organ transplantation in our country has entered a crucial stage.

Since the 1980s, China began to try organ donation-related work in the light of international advanced experience. In June 1986, experts from various disciplines in China drafted the first draft "Brain death criteria," and in 1995 and 2003, they repeatedly modified and improved the draft. In July 1997, the first brain-dead organ was donated in Shanghai, and two cases of kidney transplantation were successfully performed. In February 2001, the second case of brain-dead organ donation and the first DBD liver transplantation were performed in Nanjing, China. In July 2005, the first case of donation after cardiac death (DCD) was successfully performed in Guangzhou, China. The first case of DBD heart transplantation in China was successfully performed in July 2006. The first DBD lung transplantation in China was obtained in March 2007. The successes of these attempts have also inspired our continued search [7].

In view of the declining source of cadaver donation in our country, living donors are in violation of the international principle of "no injury" to healthy people. In order to avoid the trade of organs, our country strictly investigates and limits the donor's status and relatives' scope [8]. Living donors cannot be the major source of organ transplantation. China urgently

needs to establish a sustainable organ donation and transplantation system in line with social ethics and China's national conditions. In August 2009, the Red Cross of China and the former Ministry of Health held a conference for human organ donation work in Shanghai to jointly announce the establishment of a human organ donation system and promote DCD across the country [9].

2. The gradual processing of DCD work in China

After a long period of deliberation and discussion by health authorities and scholars of transplantation and law, China started pilot work of organ donation and procurement in 2010, that is, donation of cardiac death (DCD). Chinese Red Cross, entrusted by the ministry of health, hosted the pilot work. Since then, China's organ transplantation has entered a new stage [10].

In March 2010, the Ministry of Health (now the China Health and Family Planning Commission) and the Red Cross Society issued a document to carry out organ donation pilot work in 10 provinces and cities in China. The 10 provinces and cities are Tianjin, Liaoning, Shanghai, Jiangsu, Zhejiang, Fujian, Jiangxi, Shandong, Hubei and Guangdong [11]. Since Hunan Province had begun organ donation work and made some achievements before that, in June 2010, the Ministry of Health and the Red Cross General Union jointly approved Hunan Province to join the pilot provinces for organ donation [12]. At the same time, a document was issued on the "Organ Donation Pilot Work Program." Several requirements were put forward in the work of the 11 provinces and cities: (1) to establish the organization structure and team of organ donation work; (2) do a good job of personnel training; (3) to ensure the start of this work and ensure the work funding; (4) attach great importance to propaganda work; (5) explore the establishment of compensation and relief mechanism and incentive mechanism to ensure the sustainable and healthy development of donations; and (6) on the basis of common goals and principles, explore the formulation of implementation rules in line with the actual conditions in the region. In July 2010, the "Guidance to Chinese Organ Donation after Cardiac Death pilot work," which was written by the former Ministry of Health and the Red Cross of China together with related experts, was published in "Chinese Journal of Organ Transplantation" and initiated the DCD pilot work in China [13]. At the same time, the "Organ Donation Pilot Work Program" has also been formulated, which specified the duties and institutional framework of organ donation system and preliminary scheme of donation work process (**Figures 1** and **2**).

2.1. The determination and classification of DCD

International classification of cardiac death organ donation is mainly based on the Maastricht standard established in 1995. In view of the immature conditions of legislation on brain death in our country, our country has formulated the DCD classification standard of China according to China's national conditions so that the combination of "brain death" and "cardiac death" is well applied, in which way can also avoid the misunderstanding in our country's cultural identity and make the donation procedure comply with the law. In 2011, the Ministry of Health issued

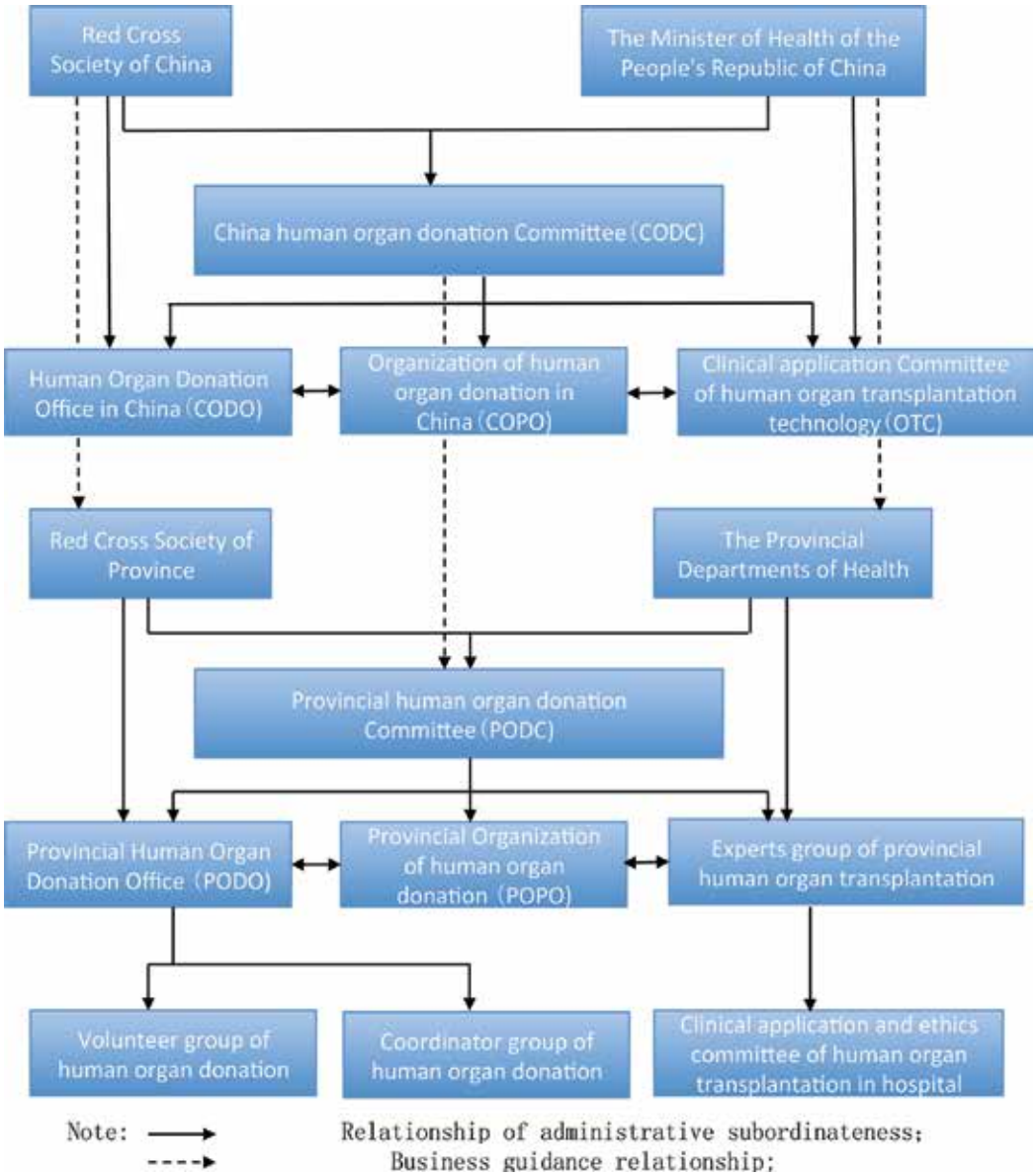

Figure 1. The structural chart of Chinese human organ donation system [11].

"Notice of the General Office of the Ministry of Health on Initiating Cardiac Death Donor Organ Transplantation" and proposed "China Classification Criteria" and related definitions:

1. Definition of DCD: DCD refers to the organ donation after the citizen's death. In the past, it was also called non-heart beating donation (NHBD)
2. DCD classification: Currently, the DCD classification standard as defined by the 1995 Maastricht International Conference in Holland is adopted internationally. According to China's national conditions combined with internationally accepted standards, in February 2011, the

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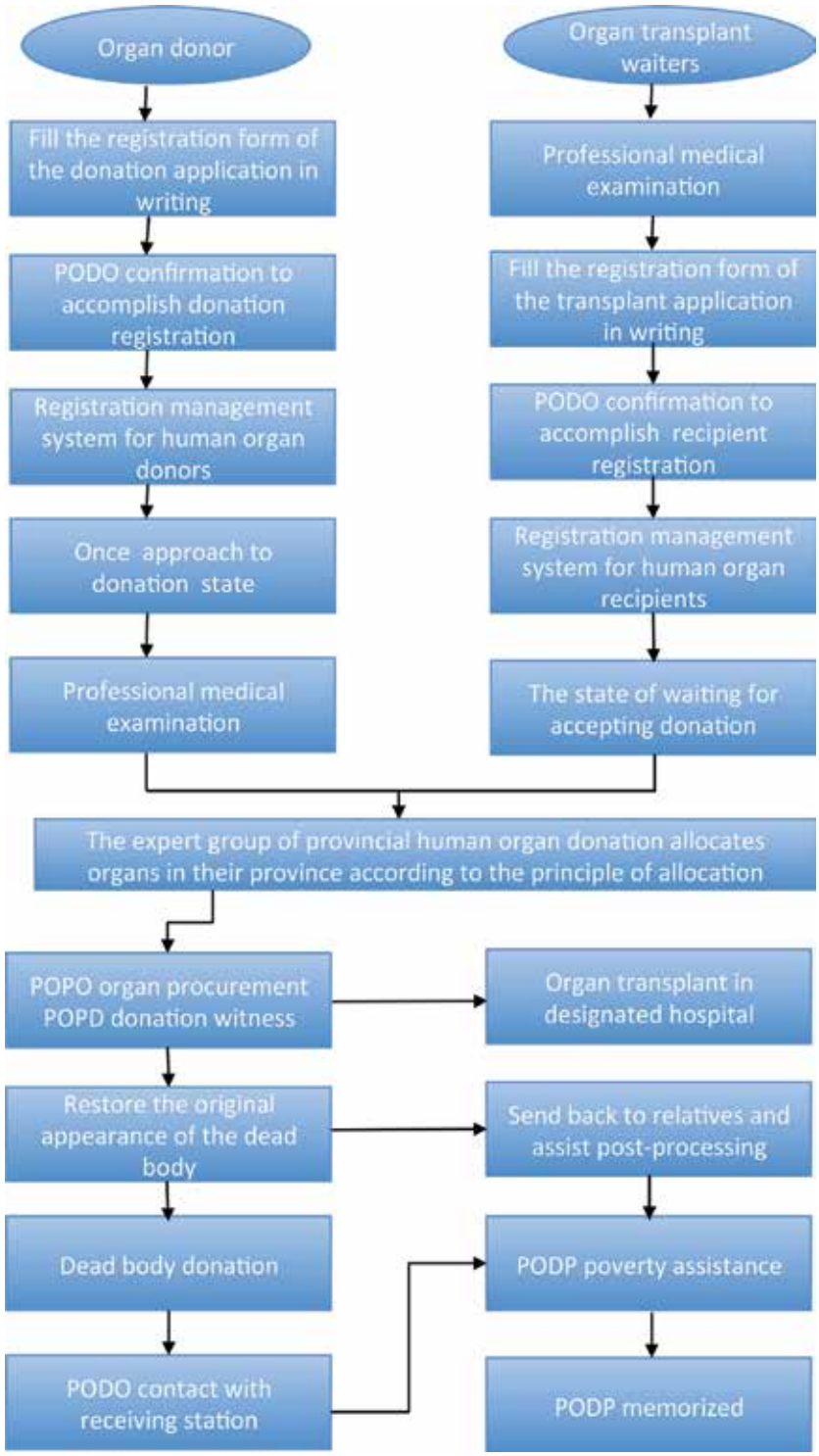

Figure 2. The process chart of Chinese human organ donation work [11].

China Human Organ Transplantation Technology Clinical Application Committee passed and announced the Chinese human organ donation classification standers, that is, the following three categories:

China Category I (C-I): This includes internationally standardized donation after brain death (DBD), that is, brain death cases. After rigorous medical examination, the indicators of potential donors are in line with the latest international and current domestic brain death standards (China Journal of Cerebrovascular Diseases, 2009 Volume 6, Issue 4), by the Ministry of Health commissioned by the agency-certified brain death experts who clearly identified it as brain death. Family members also come under this category, who fully understand the situation and choose to stop the treatment and donate organs, at the same time, having obtained the approval and support from the hospital and relevant leading departments.

China Category II (C-II): Internationally standardized cardiac death organ donation (DCD) includes the I–III case of the Maastricht standard classification.

China Category III (C-III): This includes donation after brain death awaiting cardiac death (DBCD). Similar to Maastricht's standard class IV, it is a controlled type and meets the diagnostic criteria for brain death. Since the brain death law has not yet been established, and family members cannot accept donations of organs under cardiac beating, donations should be made according to the DCD procedure, that is, life support should be removed, and donations should be made after cardiac arrest. The C-III is in line with China National conditions [14].

2.2. The pilot project of DCD has been carried out smoothly

In March 2012, the National Health and Family Planning Commission (formerly the Ministry of Health) and the Red Cross Society jointly held a wrap-up meeting on the organ donation pilot work throughout the country in Hangzhou, Zhejiang Province, and summarized their experience in organ donation work [15]. After 2 years of pilot work, under the joint promotion of the Red Cross and the National Health and Family Planning Commission, the Pilot Regions had to do a lot of work and achieved initial success in setting up sound organizations, applying for specialized agencies, revising of laws, exploring relief mechanisms, standardizing work processes and coordinating training, guiding the public in a scientific way and carrying out scientific assessments. As of March 15, 2012, the pilot completed a total of 207 donations, contributing 546 organs and saving more than 500 lives [16]. In August 2012, the National Health and Family Planning Commission and the Red Cross jointly issued the "Opinions on Further Promoting human organ donation work" (Zhonghong Zi, No. 39) in order to continue this steady progress. It specified the guiding ideology, basic principles, work objectives and specific measures for human organ donation. It also announced the organizational structure and responsibilities of the organ donation system and the organ donation process after the death of Chinese citizens, including eight important aspects, which are registration, donation evaluation, donation confirmation, organ acquisition, organ distribution, body processing, memory and humanitarian aid [17]. On March 25, 2013, the National Health and Family Planning Commission and the Red Cross jointly held a video conference on organ donation work throughout the country,

demanding to carry our organ donation work in the whole country this year [18]. As of August 14, 2014, 169 units completed 2107 human donations and 5787 organs were donated, saving the lives of more than 5000 patients by transplants [19].

3. Organ donation work procedure after Chinese citizens' death

On August 1, 2012, the Red Cross Society of China and the Ministry of Health published the "Opinion on Further Promoting human organ donation work (Zhonghong Zi, No. 39)," officially announcing the organ donation process after the death of Chinese citizens. The main contents include enrollment registration, donation evaluation, donation confirmation, organ acquisition, organ distribution, body processing, memory, humanitarian assistance, and so on (**Figure 3**).

DCD organ procurement process (**Figures 4–7**).

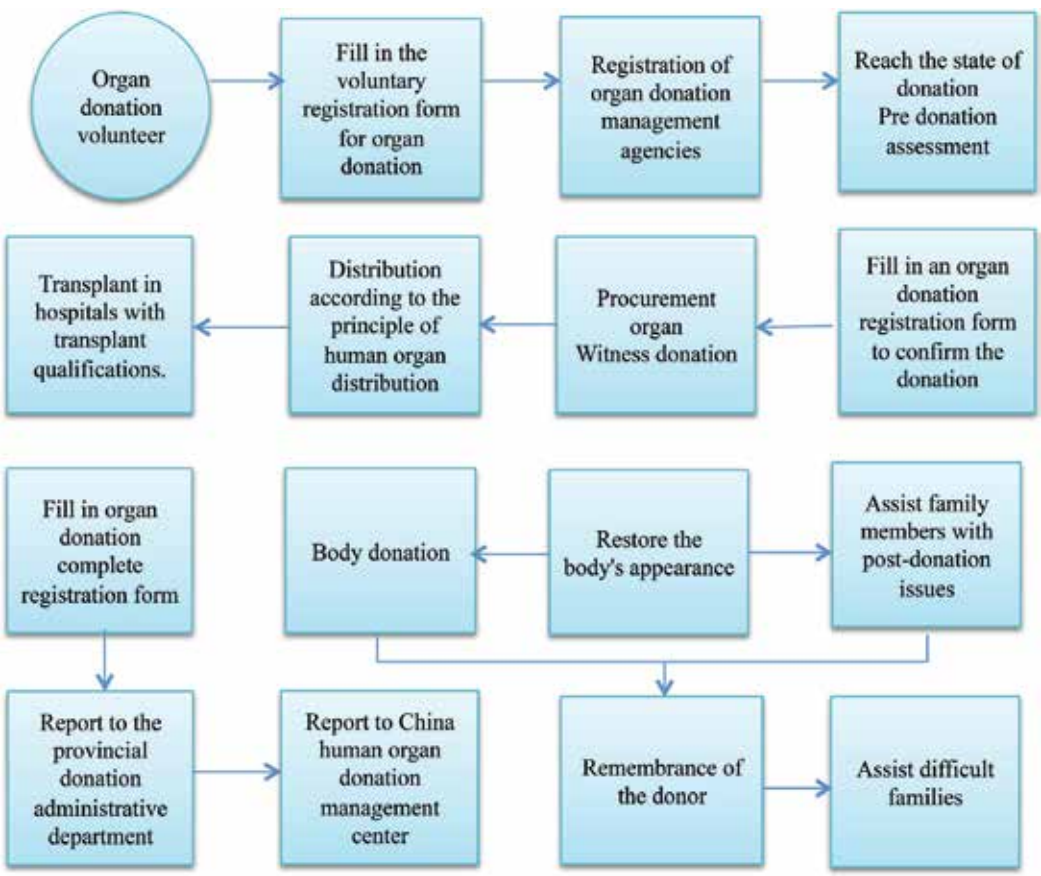

Figure 3. The process chart of Chinese DCD work [11].

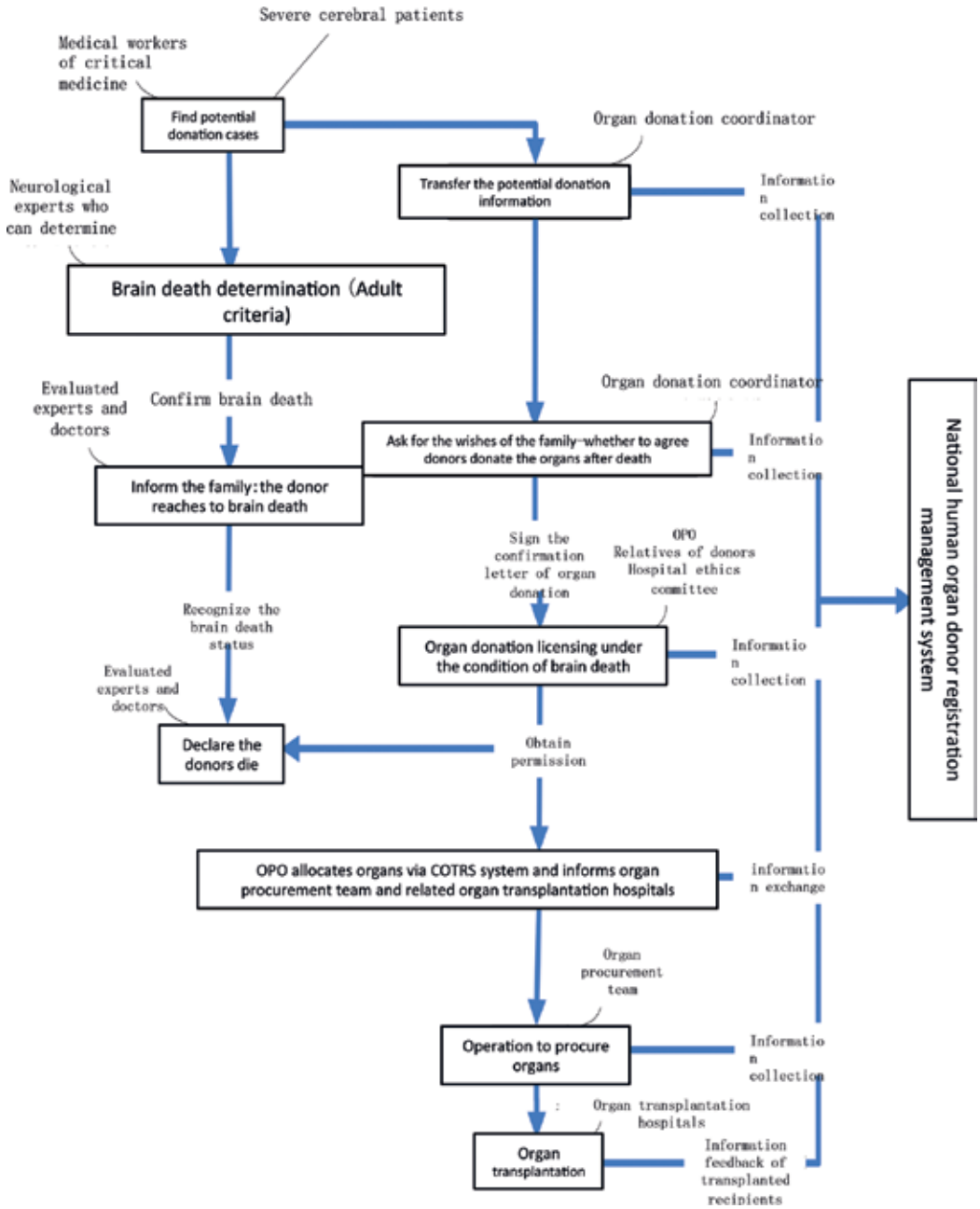

Figure 4. Flow chart of organ donation and procurement (C-I) [20].

The distribution of organs is based on the following seven basic principles of "Basic Principles for the Allocation and Sharing of Human Organs in China (2010 edition)" [21]:

1. The distribution and sharing of human organs should meet the medical needs.

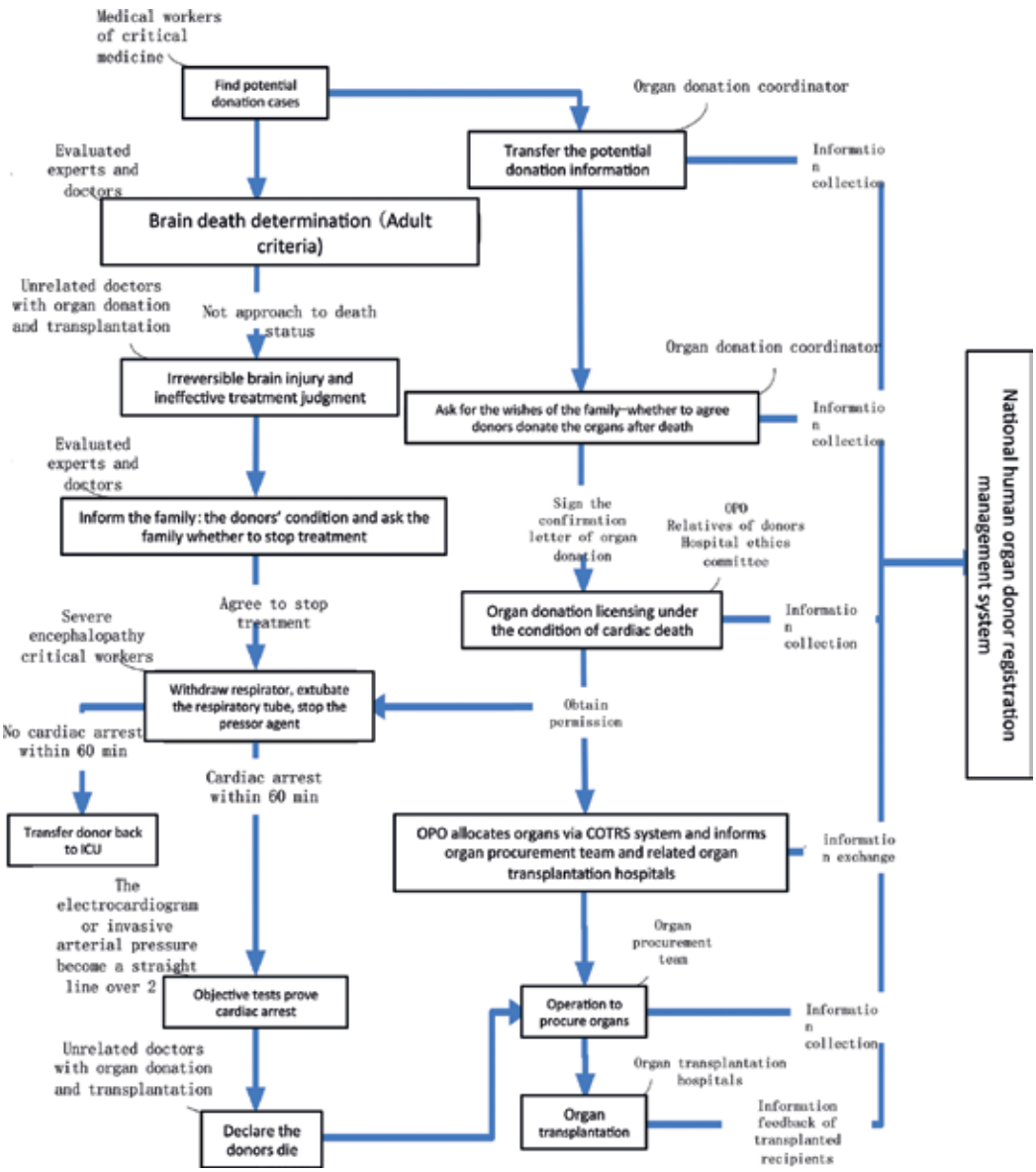

Figure 5. Flow chart of organ donation and procurement (C-II) [20].

2. The transplant hospital has the right, based on sound medical judgment, to refuse to accept unsuitable organs for transplant wait-ins.

3. Human organ distribution and sharing must take place according to the transplantation of hospitals, provinces (municipalities and autonomous regions), three levels of organ distribution and sharing level by level

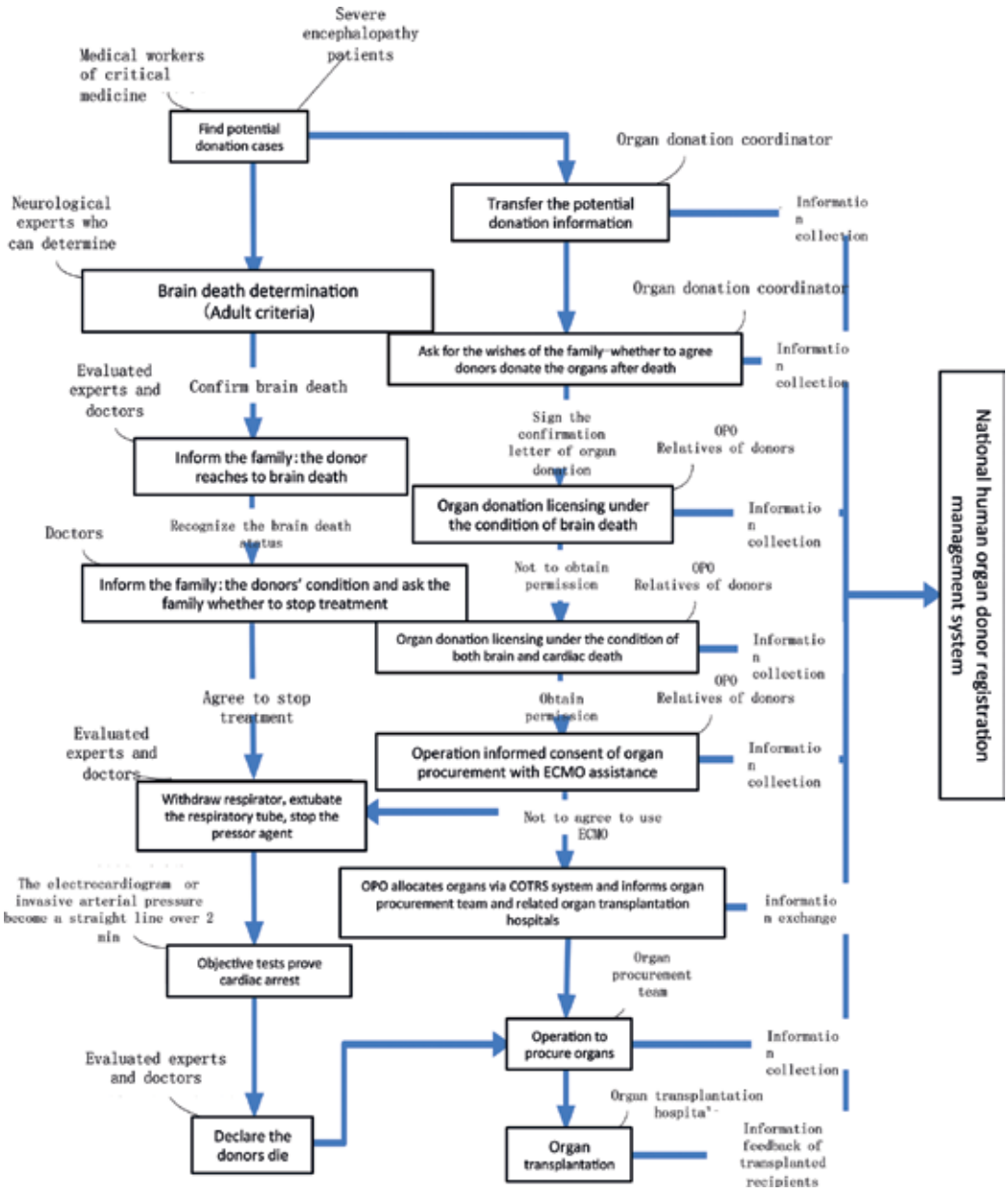

Figure 6. Flow chart of organ donation and procurement (C-III) without ECMO assistance [20].

4. Human organ distribution and sharing process should avoid the waste of organs to maximize the patient's chance of receiving a transplant and improve the efficiency of organ distribution.

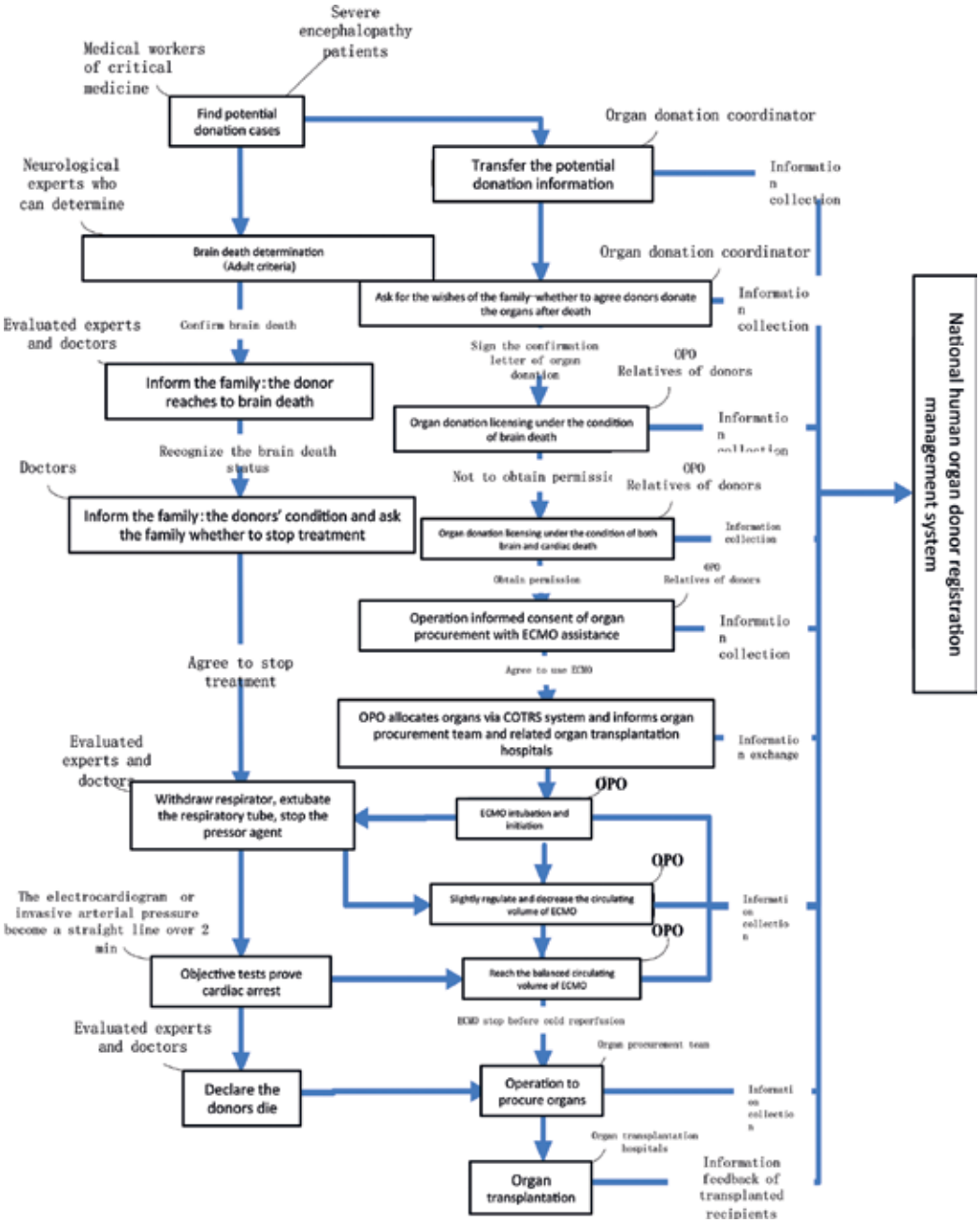

Figure 7. Flow chart of organ donation and procurement (C-III) with ECMO assistance [20].

5. To optimize the matching quality of organs and recipients and improve the postoperative survival rate and quality of life of transplant recipients, on the premise of ensuring the lowest possible death rate in the transplant waiting list.

6. To ensure the fairness of organ distribution and reduce the physical, pathological and geographical differences caused by uneven distribution of organs.

7. Regularly review and amend human organ distribution and sharing policies.

With the progress of the human organ donation pilot work in our country, in order to further standardize organ procurement, distribution and transplantation, the National Health and Family Planning Commission issued the "Regulation on the Administration and Accession of Human Donated Organs (Trial Implementation)" in 2013. The regulation further develops the organizational structure and normative process of organ procurement and distribution system. It also has consolidated the legal basis for the establishment of the organ procurement and distribution system in our country. Not only does it promote the birth of organ procurement organization (OPO) but it also empowers the China Organ Transplant Response Systems (CORS) (www.cot.org.cn) to exercise the power of organ distribution. The regulation compulsively demands that donated organ must be distributed through the organ distribution system, and organizations or individuals cannot allocate donated organs outside the organ distribution system to ensure the origin and fairness of organ donation. At the same time, it requires OPOs to obtain the organ according to the china organ donation after cardiac death classification standard [22].

In order to guarantee the implementation of laws, regulations and policies, the medical database associated with it has also been gradually improved in recent years. Following the establishment of the four major scientific systems of kidney, liver, heart and lung transplantation, the China Organ Transplant Response Systems (CORS), China Organ Transplant Surgeon Registration System and Human Organ Donation Coordinator Registration System have also been constructed and put into use one after another. Since the "Regulations" were implemented through April 30, 2015, the organ distribution system has performed a total of 6170 organ matches, including 1738 liver and 4432 kidneys. Among them, 83.2% (5136 cases) were assigned to wait in hospital and 16.8% (1034 cases) were shared with other transplant hospitals in and outside the province. According to the statistics, the organ distribution system calculates the matching list for no more than 8 s and takes an average of 1.2 h for organ sharing [23]. The organ distribution system operates efficiently without human intervention. Through the assistance and data exchange between the various systems, invisible monitoring network of Chinese organ procurement and transplant is formed, which means that China's organ transplant, based on the legal management, further deepens scientific information management.

4. The composition and responsibilities of the DCD working group

The DCD team includes the donor's responsible doctor, organ donation coordinator, member of human organ procurement organization (OPO), surgical group, anesthesiologist, organ donation management committee, organ transplantation ethics committee, and so on. The above members constitute the DCD working group to participate in the DCD implementation process, each of which does its job, divides the labor and cooperate and jointly decides the key procedures.

4.1. DCD working group

1. The donor's responsible doctor: the doctor participates in the entire donation process except for the removal of the organ. Major responsibilities: find potential donors, have a preliminary assessment of potential donors with donation conditions; responsible to inform the families of patients, after the family members express the intention to terminate treatment; contact the Provincial organ Donation Committee (PODC); submit the basic information of potential donors; assist in organ donation coordination and discuss organ donation matters with family members; consult with family members to decide to remove cardiopulmonary support treatment; responsible for the implementation of the removal; confirm and announce the death of the donor; carry out necessary medical intervention of donors before donation; fill DCD records; review the case and report to the hospital organ donation committee and hospital organ transplant ethics committee.
2. Organ donation coordinator: the main responsibility is to discuss the organ donation with the family members and obtain the legal documents such as the donation of informed consent and so on. The organ donation coordinator is trained and qualified by the Red Cross.
3. Organ donation evaluation expert: the team is composed of high-quantified chief physicians or above-ICU physicians, neurologists and surgeons and is mainly responsible for confirming whether the patient meets organ donation conditions.
4. OPO group: the team is mainly responsible for organ removal and not participating in the removal of cardiopulmonary support.
5. The Hospital Organ Donation Committee/The Hospital Organ Transplant Ethics Committee: the team checks whether the relevant legal documents for supervision and donation are perfect, whether the donation process accords with the informed consent principle and supervises the DCD reporting medical records and record management.
6. Other related members, including the anesthesiologist, operation staff, and so on, mainly assist the OPO team to complete the organ procurement [24].

4.2. Organ procurement organization

1. Constituent conditions: The Organ Organization (OPO) is established by provincial health administration department and comprises one or several human organs' organ transplant surgeons, neurologists, critical care physicians and nurses under the unified leadership of the Health and Family Planning Commission. The OPO must establish a team of human organ donation coordinators with specialized skills and qualifications. OPO also needs to formulate medical standards of identifying and screening potential donors, to establish standard human donation organs' procurement, technical specifications and provide specialized personnel and equipment to ensure the quality of organ procurement.

2. Responsibilities: (1) appropriate medical assessment of potential donors in their service area; (2) in accordance with the "Regulations," sign the human organ donation informed consent or other human organ donation legitimacy document with the donors or their spouse, adult children, parents (or other legal guardians), and so on. (3) maintenance of organ donation; (4) entry of clinical data and legal documents of potential donors and donated organs into CORT; (5) use of organ distribution system to start donation of organs; (6) acquire, save and deliver donated organs and confirm the transfer of donated organs according to the distribution result of the organ distribution system and the hospital with human organ transplantation qualification where the organ transplant recipients who obtained the organ are located; (7) carry out medical treatment on the remains of donors according to ethical principles and participate in the memory and condolences work; (8) protect the personal information of donors, recipients and waiters and protect their legal rights and interests; (9) organize relevant medical staff in medical institutions within the scope of their services to participate in professional training and assist the executive on the regular OPO coordinator training and assessment, conduct scientific research and academic exchanges; and (10) publicity and education on knowledge of human organ donation to the general public [25].

4.3. Division principle of OPO service areas

In order to encourage and promote effective DCD work in all the provinces and avoid unnecessary competition and looting, under the coordination of the health administrative departments, various provinces and cities established a number of independent OPO organizations and divided the service scope of OPO organizations at the same time. The OPO group must comply with the following principles [26]:

1. Provincial OPOs cannot procure organs across provinces.
2. Each OPO cannot procure organs across their own service area.
3. All organs must be assigned to the China Organ Transplant Response System for uniform distribution.
4. Each OPO has a priority in the allocation of its organs but should ensure that organs will be used effectively.

The number of OPOs varies from province to province. Most provinces adhere to the principle of free combination among transplant hospitals. For example, hospitals that are more advanced in DCD work and have a higher transplantation professional level and can carry out multiple organ transplants can form independent OPOs. A strong transplant center can lead a weaker transplant center to form an OPO. An experienced transplant center can lead an inexperienced transplant center to form an OPO. To avoid organ waste, centers that only can carry out one kind of transplantation can form an OPO with centers that can carry out multiple kinds of transplantation.

Taking Hunan as an example: With a population of 67.83 million (in 2015), Hunan Province has a total area of 211,800 km^2 and a total of 14 cities and 122 counties. There are eight

hospitals in the province that can carry out organ transplants, of which three hospitals can carry out a variety of organ transplants and the remaining five can only carry out kidney transplantation; there are a total of four existing OPOs in Hunan Province and under the joint consultation divided into four OPO service areas [27].

In order to carry out the work of DCD better, each transplantation hospital has set up some working groups: the OPO leadership group, the DCD ethics committee, the DCD work hospital coordination group, the full-time coordinator team, the OPO procurement group, the DCD work post-processing group, and so on. The composition and responsibilities of each group are as follows [28]:

1. OPO leadership group

   Composition: includes the hospital main party and government leaders, functional departments and potential DCD clinical department leaders and transplant center responsible person.

   Responsibilities:

   1. to protect the development of DCD work and provide a good platform for collaboration;
   2. to carry out publicity work inside and outside the DCD and regularly report to the higher authorities;
   3. regular inspection, to grasp the legality of the DCD process;
   4. to give DCD work in financial support and financial supervision; and
   5. to protect, such as ambulances, equipment, care, medical, legal and other aspects of support to ensure the smooth flow of DCD work.

2. DCD Working Coordination Group: Composed of the Medical Services Department, the Department of Health, Propaganda Department, the vehicle team, the hospital legal adviser, potential DCD medical department director, nurse and coordinators.

3. DCD Ethics Committee

   Composition: Includes the management, medical, psychological, nursing, pharmacy, law, ethics, community representatives and other aspects of the composition of the residents.

   Responsibilities:

   1. confirming and identifying that medical institutions have the relevant qualifications and conditions;
   2. discuss and approve DCD organ transplantation and confirm that the donor source is legitimate and matching type is reasonable;
   3. to confirm that the donor and the recipient of the documents meet the requirements of legal documents, that there is no sale of human organs;

4. to confirm whether necessary inspections have been given to the donors and recipients to ensure clinical efficacy.

4. DCD determination group

Composition: Includes the brain death or DCD identification of neurological and surgical experts. Requirements:

1. experts in determination group at least are associated experts and
2. experts must undergo a specific brain death and DCD training.

5. OPO procurement group

Composition: Includes professional transplantation medical staff and related staff.

Responsibilities:

1. receive DCD-related information and DCD authentication written information;
2. evaluation of donor quality;
3. assess the recipient indications, contraindications, general condition and timing of surgery; and
4. being responsible for the acquisition, preservation and transshipment of organs.

6. DCD post-processing group

Composition: Formed by the Medical Department, the vehicle team, the Propaganda Department, logistics department, coordinators and Red Cross staff, and so on.

Responsibilities:

1. body maintenance;
2. coordinate with the relevant departments and arrange the cremation;
3. communicate and coordinate with relevant departments and arrange the implementation of the cemetery and the memory activities;
4. cooperate with the Red Cross in publicity; and
5. deal with other left-over issues after DCD work.

DCD work in Hunan Province has always been in a leading position in the country. The success of DCD work in Hunan Province is mainly attributed to the strong support and cooperation of the Provincial Health and Family Planning Commission, the Red Cross Society and the transplant hospitals. **Figure 1** shows DCD in eight hospitals in Hunan Province for 2010–2016 and **Figure 2** shows implementation of DCD in China. The work of organ transplantation in China is also known as the "Hospital president project" and all the transplant centers that have better development are fully supported by their presidents [29].

5. Chinese organ donation coordinator

5.1. Basic requirements of coordinator

"There is no organ transplant without organ donation, no organ donation without coordinator" [30]. This fully demonstrates the importance of coordinators in organ donation and transplantation. At present, there are two main types of coordinators in China: Red Cross Coordinator and OPO Coordinator.

Based on relevant regulations "Management of human organ donation coordinator" promulgated on June 1, 2013, the coordinator shall meet the following conditions [31]:

1. has good conduct and loves organ donation career
2. has medical and other related disciplines' specialist qualifications
3. has at least 2 years of work experience
4. are formal or hired personnel of local regulatory agencies or persons recommended by medical institutions

According to the "Regulation on Procurement and Distribution of Human Donated Organs (Provisional)" issued by the National Health and Family Planning Commission, the OPO coordinator should meet one of the following conditions:

1. bachelor's degree or above in medical college, holds a valid "People's Republic of China physician practicing certificate", with more than 2 years of clinical work experience and is a licensed doctor engaged in medical work in medical institutions.
2. has a college degree on nursing or above and has "People's Republic of China nurse practicing certificate." The person must have more than 2 years of clinical nursing experience and is a registered nurse engaged in clinical nursing activities in a medical institution.

In addition, the coordinator should also have a serious working attitude, a certain degree of communication skills and language skills as well as good psychological quality and psychological endurance. Only in this way can we properly handle the relationship with donors and their families, do a good job in organ donation coordination and push forward the donation of human organs [32].

5.2. Coordinator training in China at the current stage

From 2010 to 2012, the Chinese Red Cross is mainly responsible for the training of coordinators. After the establishment of China Organs Donation Administration Center after 2013, it specially undertook the training of coordinators and organized 4–5 training courses each year. It invited experts in medicine, law, ethics and psychology from all over the country to give lectures and organized domestic relevant experts who compiled the "Training Materials on Human Organ Donation Coordinator in China" (the first and second volumes) [33]. The coordinators were required to attend relevant studies and pass the qualification examination.

At present, a management system of coordinators with rigorous management, impartial examination and scientific training has been formed, which has delivered a large number of high-quality coordinators for the DCD work in China and, at this stage, and has made great contributions to the successful development and promotion of DCD work in China.

5.3. Organ donation coordinator working procedures in current China

1. discover potential DCD donors [31]
2. report the information and wait for evaluation
3. after the assessment team finds that it meets the conditions of donation, communicate with potential DCD family members
4. after donors' family members agree to donate, witness the completion of China organ donation registration form and family's informed consent
5. witness the work of the OPO team to ensure that all work is conducted in a reasonable and lawful manner
6. assist in the burial, memory and other post-processing work
7. assist the aftermath of the donors' family members
8. assist in completing legal instruments related to organ donation

6. China's current DCD work archives establishment and management

The organ donation archive is a strong evidence of DCD's legal compliance but also important information for late summing up experience and conducting scientific research. With the increasing number of DCDs, the management of donors' archives is becoming increasingly important. Establishing a set of scientific and standardized donor archive management standards is of great significance to promote the sustainable development of DCD [34].

Since the launch of the pilot DCD program in China in 2010, great attention has been paid to the collection of archives and data. All provinces and transplant hospitals have done a great deal of work and summed up some experience. For each case of donation, corresponding archives were set up and collected the important information. In the early stage, it is mainly the collection and preservation of paper materials; the project basically includes the following [35]:

1. China organ donation completion form
2. China organ donation registration form
3. donor and their relatives' ID cards
4. donor's basic information table

5. donor comprehensive evaluation table
6. potential organ donor's family informed consent
7. brain death determination family member's application form
8. brain death determination family's informed consent
9. brain death determination form
10. the donor electroencephalogram inspection report
11. DCD organ transplant ethics form
12. donor management form
13. organ procurement record form
14. the donor discharge diagnosis/patient history information
15. the HLA matching report
16. the recipient information
17. recording video or photo material throughout the process of organ donation
18. donor medical death certificate
19. cremation certificate
20. organ donation process record form

However, there are also some problems. For example, the standard norms are not uniform, and the paper materials are not conducive to long-term preservation; inspection and data statistics are inefficient. Through continuous discussion and exploration and the cooperation of various field experts, we have made use of information technology and Internet technology to develop an organ transplant file management system that is suitable for China. The

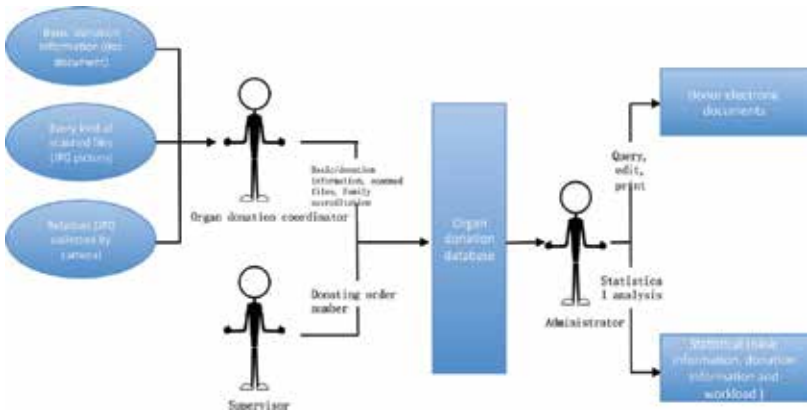

Figure 8. Flow chart of organ transplant file management system [33].

registration of this system basically covers all the information and data above. The process is as follows (**Figure 8**):

Real-time reporting, monitoring of implementation, data analysis and follow-up were conducted in every case of this organ donation database to ensure the regulation of DCD work and also accumulated a large amount of data and laid the foundation for the long-term development of DCD.

7. China's DCD organ donation problems

7.1. Organ donation and transplantation supply and demand situation is still grim

At present, great progress has been made in organ donation and transplantation in our country. With the continued development of our society and economy, especially the improvement of the levels of medical service, it is still a huge challenge to solve the contradiction between the rapid growth of people's health needs and the overall shortage of service provision. About 300,000 end-stage organ failure patients in our country need organ transplantation each year, yet the number of organ transplants is less than 20,000 each year. From a global perspective, the number of organ donations in our country is at the forefront, but the donation rate per million population needs to be improved. How to improve the organ donation rate so that more patients receive prompt treatment is still the long-term goal of our work.

7.2. Citizens lack the correct understanding of organ donation

First of all, under the influence of "maintaining the integrity of the body" and the wrong "death concept," Chinese citizens' willingness to organ donation is not strong, and due to the fact that the law relating to brain death is still lacking in our country, it also hinders the practice of "brain death" standard to a certain extent in our country. Secondly, the lack of social propaganda makes citizens lack the recognition of the donation process and give up donation. Coupled with the wrong public opinion, such as some exaggerated organ buying and selling speech, etc., also make citizens concern whether the use of donated organ is properly or not, which mislead citizens' cognition of organ donation, affecting their willingness to donate. At the same time, most medical workers have not been involved in organ donation and are not familiar with the criteria for determining brain death, the classification and conditions of organ donation and their reluctance to ask their patients' families' attitudes and wishes toward organ donation, which also hinder the discovery of potential organ donors.

7.3. Organ donation and transplantation-related systems still need to be sound

China's organ donation worked from scratch, achieved by leaps and bounds. At present, we have initially established a donation and transplantation system for human organs in line with China's national conditions, culture and social ethics. However, all aspects of system design and legal norms still need to be explored and developed in practice. To establish a sound organ donation and transplantation system that is compatible with the level of social-economic

development level, improve a more equitable and efficient organ distribution system and to scientifically plan the regional distribution of transplanted hospitals so as to ensure the equalization of organ transplant services for all citizens, we also need to keep it up.

For example, various provinces in China have formed an independent organ procurement organization (OPO) and delineated their corresponding service scope. Unlike other countries, most of the OPOs in China are composed of transplant medical teams in organ transplant hospitals. This form is advantageous in the initial stage of DCD work, but with the development of this work, some disadvantages are also exposed, such as the limitation of organ distribution and the irrational division of service areas, and so on, which all need to be constantly adjusted and improved in following-up work.

At present, there are two organ donation registration websites in China: organ donation administration center website by the Chinese Red Cross and "Shi and Suo" organ donation registration website administered by the National Health and Family Planning Commission. Current organ donation and transplantation network systems include the donor system, China organ transplant response system (CORTS). The use of websites is yet to be promoted, just as CORTS, a site that plays a major role in organ allocation, the number of patients who have registered on CORTS for transplantation has a large discrepancy from the actual number of 300,000 patients who are waiting for transplant. Compared with the UNOS website in the United States, the COTRS website in our country did not give full play to its functions, and lacked the legal effect and administrative system. Its authority and compulsion needed to be improved [36, 37].

8. The achievements and prospective of China's DCD work

After several years of hard work, China has made gratifying achievements in the work of DCD. Some of the figures are shown in **Figures 9–19**:

China has successfully resumed the transition from relying on the judicial channels to procuring organs through the voluntary donation of citizens. The reform is the result of the concerted efforts of the top leadership of the country, relevant departments, social organizations, the vast majority of transplant medical personnel and Red Cross workers, who obtained the understanding of society and the recognition of the international community. Based on the guiding principles set by the relevant international organizations and based on the actual social and economic development and cultural traditions in China, we adopted methods suited to China's national conditions and promoted the continuous progress of organ donation and transplantation in China. After several years of pilot research, the influence of organ donation after citizen death to our society has surpassed that of the transplant medical service itself. Such love charity is gradually becoming a common practice in China. With the joint efforts of the health administrative departments, the Red Cross Society and the transplant medical institutions, organ donation after citizen death has taken a solid first step. However, China has a large population, huge demand for organ transplants and some traditional concepts are still affecting us. In particular, we are still in the process of improving laws and regulations, strengthening government supervision and departmental coordination, clarifying responsibilities of all parties, refining work processes and strengthening international exchanges and cooperation. There is still a lot of work to do [38].

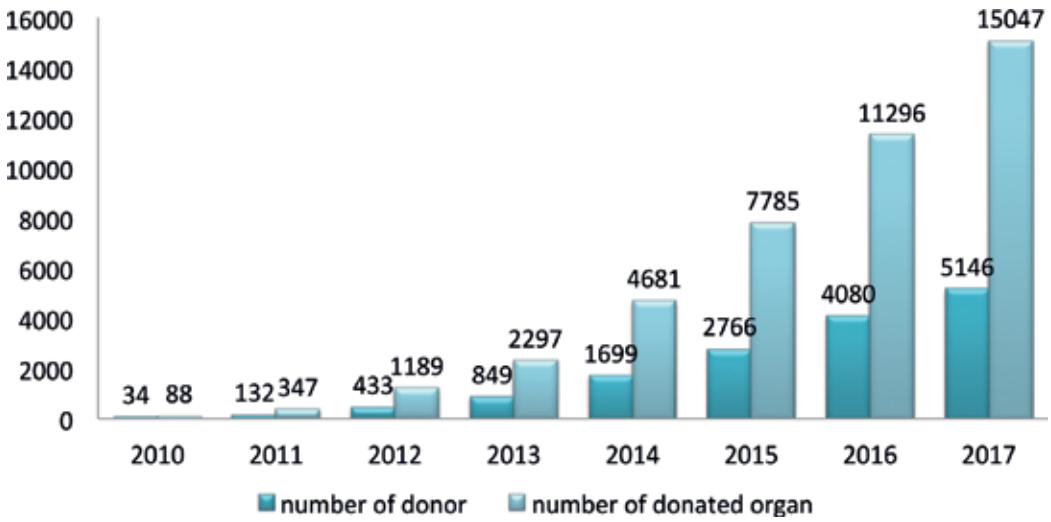

Figure 9. Overview of organ donation according to the data of China organ donation administrate center, by the end of 2017 [data from China organ donation administrate center].

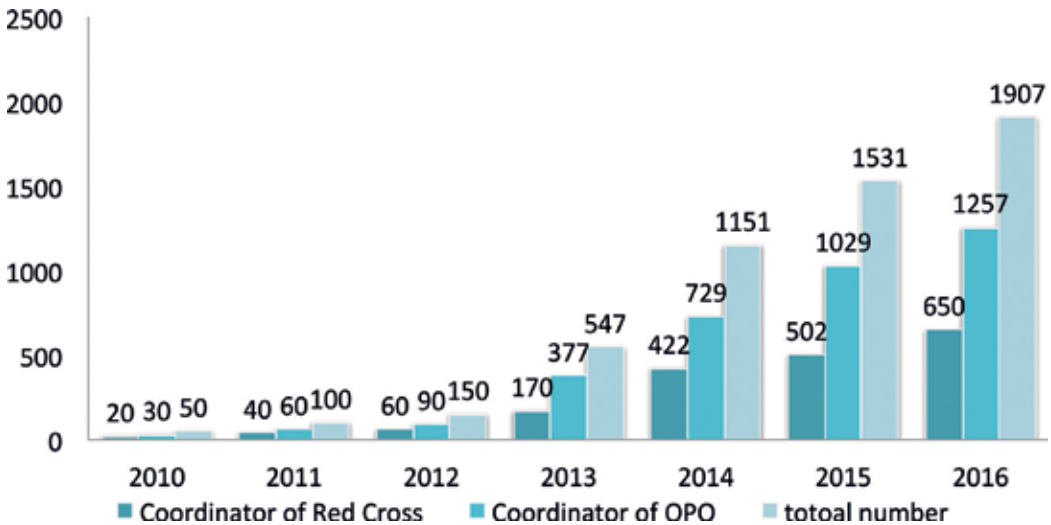

Figure 10. The change of the number of coordinators according to China organ donation administrative center [data from China organ donation administrate center].

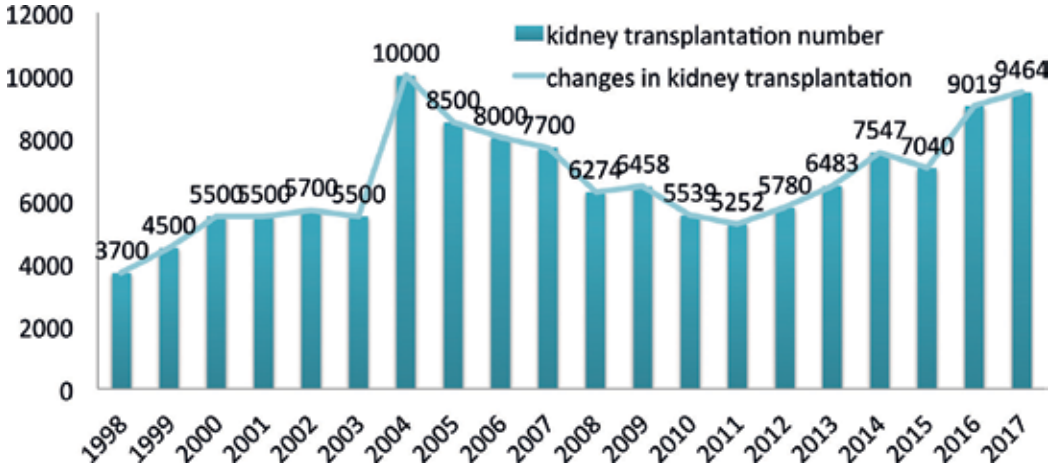

Figure 11. Annual data on kidney transplantation (20 years) [data from Chinese scientific registration of kidney transplantation (CSRKT)].

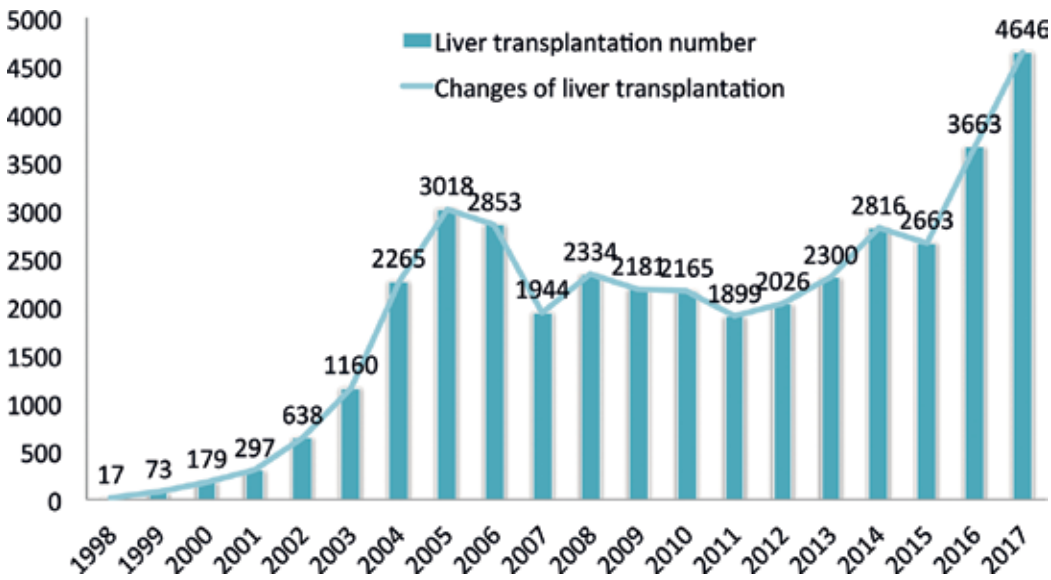

Figure 12. Annual data on liver transplantation (20 years) [data from Chinese liver transplantation registration].

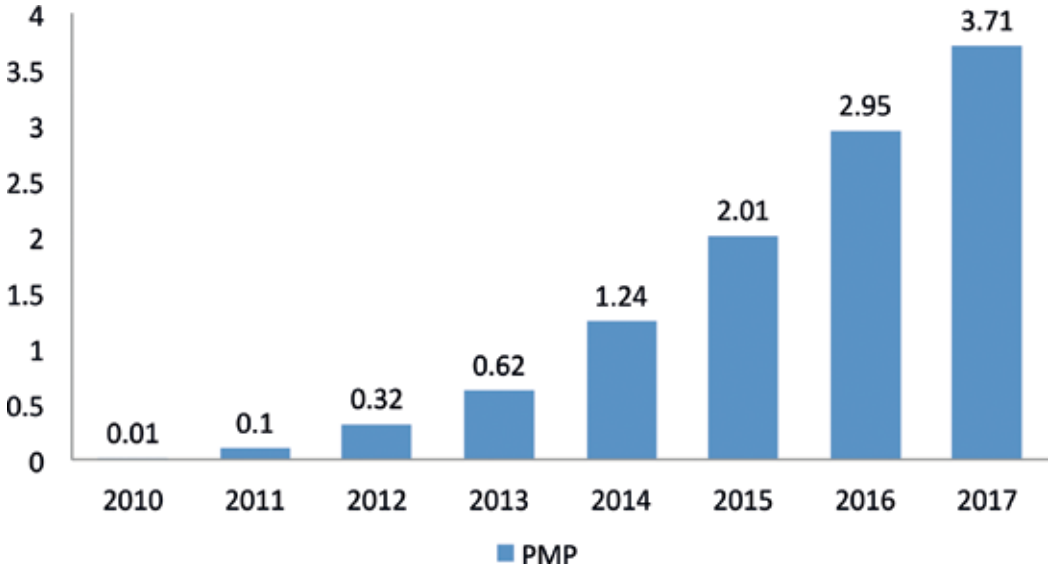

Figure 13. Annual data on organ donation PMP [data from China organ donation administrate center].

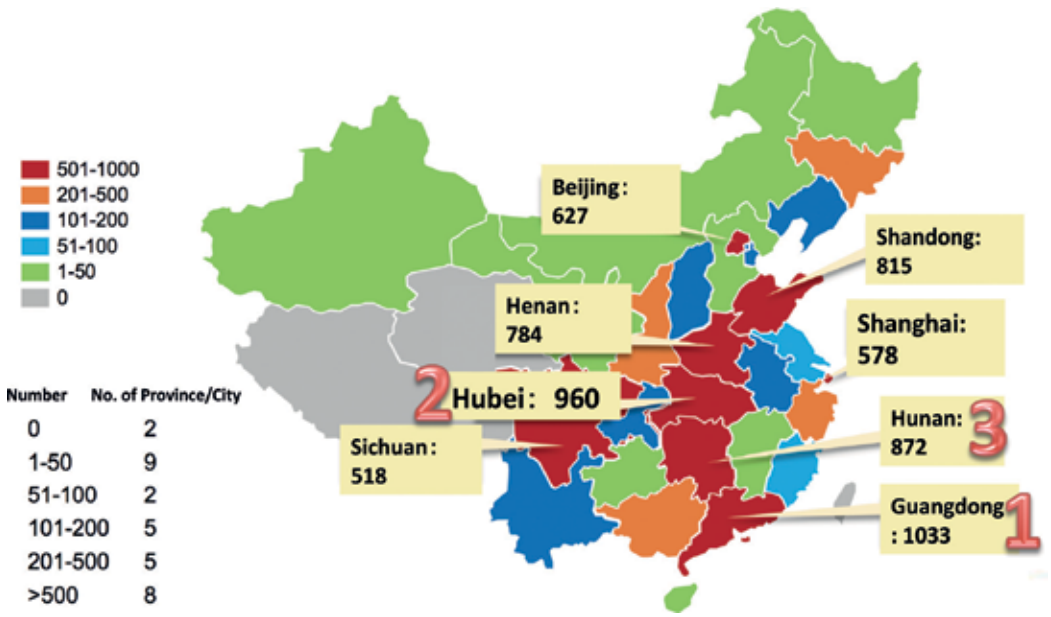

Figure 14. The overview of kidney transplant in 2016 [data from Chinese scientific registration of kidney transplantation (CSRKT)].

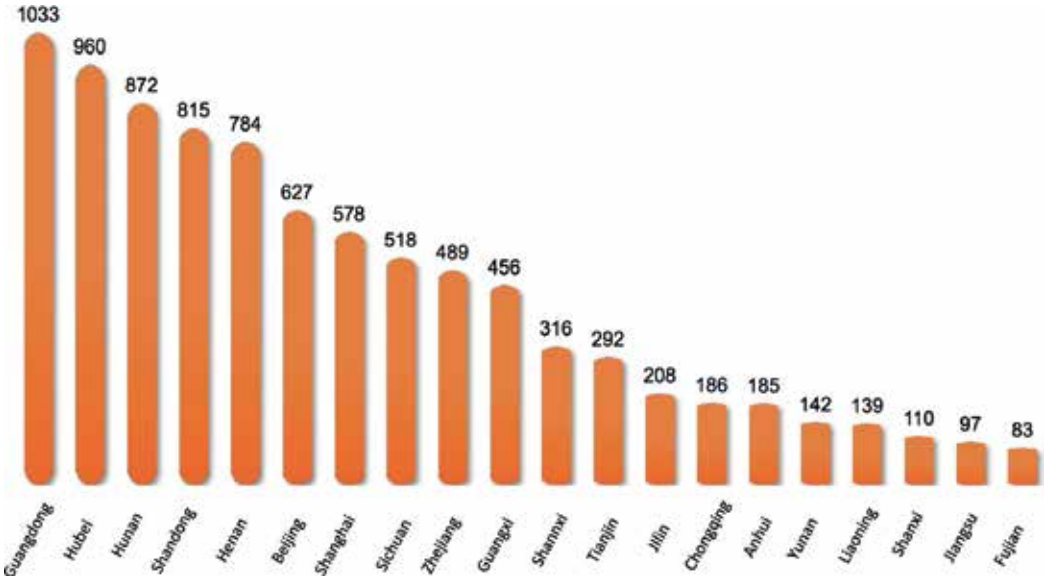

Figure 15. Number of kidney transplantations in each province in 2016 top 20 [data from Chinese scientific registration of kidney transplantation (CSRKT)].

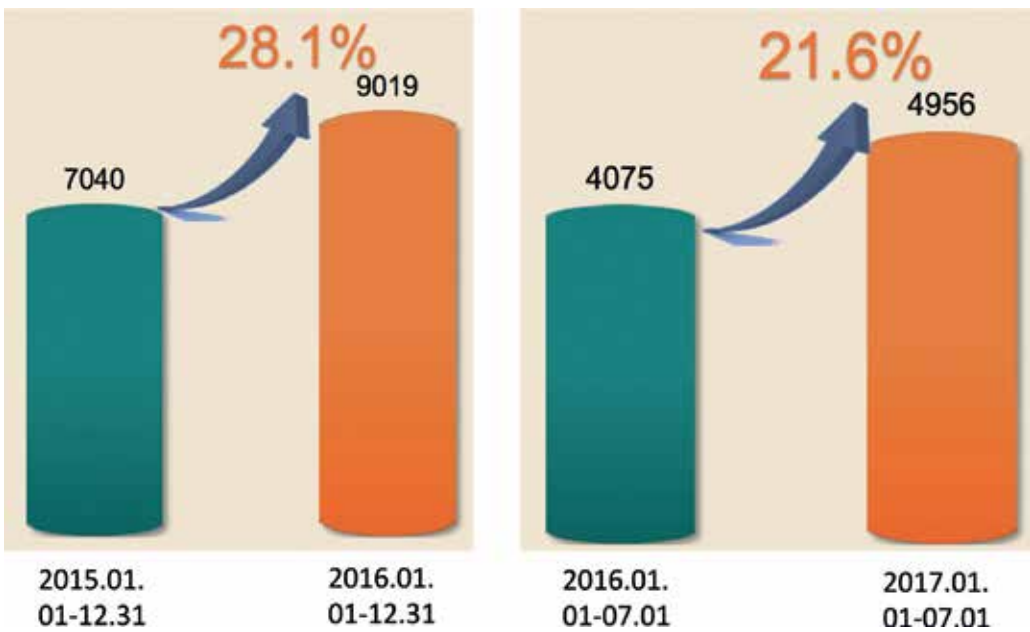

Figure 16. During the same time period, the number of kidney transplantations has been continously increasing, that is, more than 20% [data from Chinese scientific registration of kidney transplantation (CSRKT)].

Figure 17. The increasing of donation rate [data from Chinese scientific registration of kidney transplantation (CSRKT)].

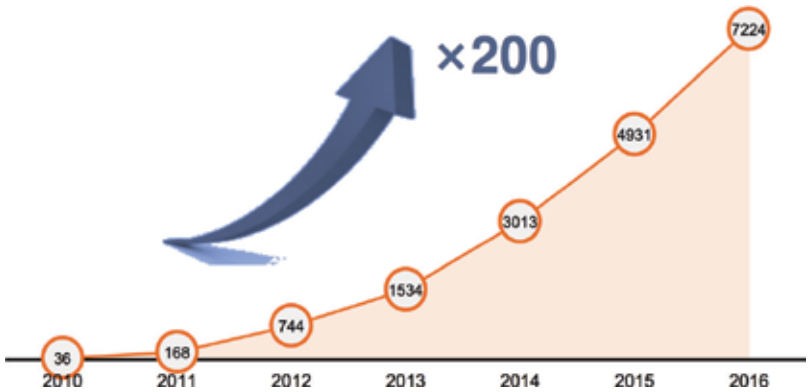

Figure 18. The increasing of DCD cases [data from Chinese scientific registration of kidney transplantation (CSRKT)].

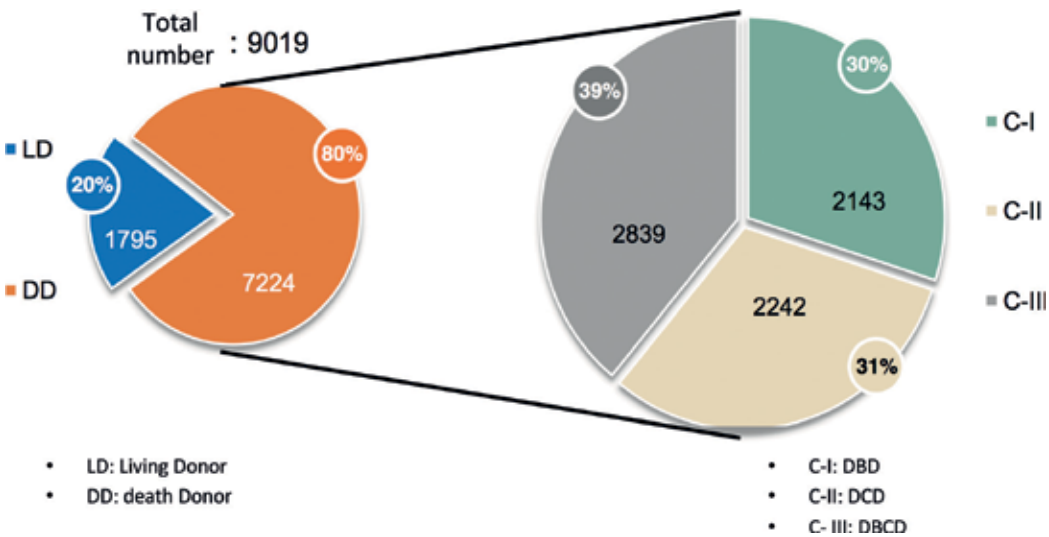

Figure 19. Organ donation in our country is based on death donors at current stage.

9. Conclusion

The shortage of organs was the bottleneck of transplantation. According to Chinese own features and the international development situation, China has carried out DCD work, which is fruitful, innovative and achievable. This has guaranteed the organ donation issue of China to develop healthily and sustainably. Although there is still some imperfection, Chinese DCD work will be better and better with our continuous promotion and international help.

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Conflict of interest

No conflict of interest.

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Chapter 8

Experimental Brain Death Models in Liver Transplantation

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Additional information is available at the end of the chapter

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Abstract

Most organs for transplantation are currently procured from brain-dead donors; however, brain death is an important risk factor in liver transplantation. In addition, to counteract the shortage of liver grafts, transplant centers accept the use of sub-optimal livers, which may show higher risk of primary non-function or initial poor function. Very few literatures exist regarding liver transplantation using brain-dead donors, or about brain death and its effects on sub-optimal grafts in such surgical situation. This chapter aims to describe the pathophysiological changes occurring in liver grafts during brain death and focuses on the strengths and limitations of experimental models used to study the effect of brain death on optimal and sub-optimal (specially steatotic) liver grafts. Depending on the use of experimental models that simulate as much as possible the surgical conditions present in clinical practice, therapeutic strategies designed in animal models could be more successfully translated to the bedside.

Keywords: liver transplantation, ischemia-reperfusion injury, brain death, experimental models

1. Introduction

Liver transplantation (LT) has evolved to become a standard therapy for certain end-stage liver diseases [1]. Nowadays, 80% of organs come from donors who have suffered brain trauma (brain-dead donors) [1–4]. Brain death (BD) has been defined as the irreversible loss

of brain and brain stem function, usually caused by major hemorrhage, hypoxia or metabolic dysregulation [5, 6]. Hemodynamic events, hormonal changes and inflammation and immune activation occur consequently to BD [6–9]. It has been described that BD markedly reduces the tolerance of liver grafts to preservation and reperfusion injury and reduces graft survival [2–4, 10]. The detrimental consequences of BD have been clinically described; however the underlying mechanisms and their relevance in LT remain poorly understood. Indeed, few studies have evaluated the effect of BD on LT, and most of the experimental studies focused in establishing surgical or pharmacological strategies to reduce liver graft damage associated with transplantation have been performed in the absence of BD [6].

Shortage of donor organs remains a major obstacle to the widespread application of LT in patients with end-stage liver disease [11–14]. To overcome this problem, transplant centers developed strategies to expand the organ donor pool [15]. This shortage could be alleviated by routine use of sub-optimal donor livers including elderly donors, steatotic donors, split livers and donor with viral infections or with malignancy. These sub-optimal livers exhibit a greater risk of organ dysfunction and primary non-function compared with optimal livers, when used as grafts in transplantation [1, 4, 9]. Multiple methods are currently being investigated to minimize the effects of ischemia-reperfusion (I/R) injury to allow the use of sub-optimal liver grafts, including anti-inflammatory approaches to attenuate cytokines, blockade of adhesion molecules, antiapoptotic strategies, among others. However, these studies are performed in non-BD surgical conditions. Only a recent study describes, for the first time, a potential treatment in steatotic liver grafts undergoing LT from cadaveric donors [1].

Since consequences of BD are linked to an inferior graft function and a more potent immune response, studies in animal models could be critical in uncovering its basic mechanisms and to provide a rationale for the development of novel targets that may prevent the deteriorating consequences of BD in clinical practice [16]. Virtually, all experimental organ transplantation studies generally utilize young, healthy living animals as donors; in clinical practice; however, a relatively low percentage of organs are acquired from living sources. Among other variables, the difference between living and brain-dead donors includes the effect of profound physiological and structural derangements [17]. Although a wide variety of animal BD models have been used to study the effect of BD on donor organ quality (without transplantation) [18–26], the lack of standardization of BD models makes difficult to compare results from different research groups. Undoubtedly, there is a need for a more controlled and standardized BD model allowing studies of organs in a situation closely resembling the reality in the intensive care unit [27].

In the first part of this chapter, we highlight the actual knowledge and new insights into the pathophysiology in BD focusing on liver graft undergoing transplantation. Following, the different experimental models reported in literature used to study the effect of BD on quality of optimal and sub-optimal liver grafts are presented, focusing on the strengths and limitations. Since recognize the underlying mechanisms of detrimental effects of BD is a critical question to design therapeutic strategies to protect liver grafts undergoing transplantation, we discuss if the existing BD animal models mimic the clinical findings in transplants with BD donors, and highlight which BD model could be more appropriated.

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2. Effects of brain death on liver graft undergoing transplantation

Liver I/R injury is a local proinflammatory response mediated by the immune system. The central nervous system plays a fundamental role in the regulation of molecular markers triggering inflammation and tissue damage, and BD results in a breakdown of these mechanisms, hence initiating the cascade of I/R injury liver transplants and exacerbating the hepatic damage caused by I/R [9]. However, although the detrimental consequences of BD have been clinically described, the underlying mechanisms and their relevance in LT remain poorly understood [6]. Following, systemic events that happen after BD and the specific effects of BD on hepatic graft undergoing transplantation are presented.

BD is followed immediately by an acute and transient rise in blood pressure (Cushing reflex), that is immediately followed by a transient bradycardia communicated through parasympathetic activation [16, 24, 28–31]. Later after BD, deteriorating hemodynamics and a compromised perfusion of abdominal organs is becoming evident. Accordingly, a shift from aerobic to anaerobic metabolism and acidosis is registered, clinically reflected by elevated serum levels of lactate and free fatty acids and promoted by decreasing insulin secretion and hyperglycemia [21, 32, 33]. Alterations of specific mitochondrial functions may lead to an impaired production of ATP and a reduced uptake of substrates for mitochondrial metabolism and eventually limit resistance and survival of cells and organs to damaging insults [34]. It is well known that ATP degradation during hepatic ischemia leads to an acceleration of glycolysis [35]. Although glycolysis is essential for cell survival, it may also be detrimental because of the accumulation of glycolytic products such as lactate [35]. In the liver, the increase in cAMP levels due to ischemia triggers the activation of glycolysis. This causes the accumulation of hexose 6-phosphates, which proceed down the glycolytic pathway to form lactate [35–38]. Some authors [39] have shown that normotensive BD had no influence on the general viability of the liver, as measured by ATP content. In this research work, no differences were found in ATP content in livers from BD-induced rats and control rats, implicating that the phase of BD up to 6 h does not reduce general liver viability. However, it has also been described that the ATP content after 3 h of reoxygenation after graft harvesting was significantly increased in liver biopsies from brain-dead rats, may be because of a stress response, caused by increased catecholamine levels induced by BD and exaggerated reoxygenation times of liver tissues, exacerbating the I/R injury [39].

Hemodynamic instability, hormonal alterations, blood coagulation factor consumption, lung tissue changes, hypothermia, and electrolyte disturbance generated by BD invariably influence hepatic functions. Increased AST and ALT concentrations indicated liver dysfunction in brain-dead pigs and hepatocyte edema, hepatic sinusoid compression, and other microscopic observations in such animals demonstrated damage to liver cells [40, 41]. Also, BD causes time-dependent general dysfunction in rats, as indicated by elevated LDH and creatinine, AST and α-GST levels [42]. The disruption of liver function seems to be due to circulatory collapse and hemodynamic instability [41]. Morphological changes in the liver following BD are even less well defined. Recent experimental findings show that hepatocytes in livers from brain-dead donors show an altered cell membrane permeability and integrity [17, 43]. It has also been reported increased caspase-3 activity indicating that apoptosis occurs in liver tissue of brain-dead donors [3].

A systemic inflammatory response is present in most patients at the diagnosis of BD [44]. The intense cellular and molecular activation that quickly follows the acute onset of BD involves acute transcriptional upregulation of inflammatory cytokines, both on a systemic and intra-organ level [16]. In liver, aside from microcirculatory changes, early effects of I/R injury and BD are mediated via Kupffer cells. When I/R injury occurs, Kupffer cells and neutrophils are activated, together with an increased expression of adhesion molecules and infiltration of monocytes and lymphocytes [9, 45]. Cell death by necrosis and apoptosis is initiated. In addition to mitochondrial damage, synthesis of oxygen-free radicals is boosted and accompanied by a decreasing activity of antioxidant enzymes. All of this results in immediate organ damage, contributing to hepatic failure after transplantation. In BD models many of these events have been also documented, exacerbating the hepatic damage that already occurs by I/R. In this sense, expression of adhesion molecules in endothelial and epithelial cells, initiating the release of proinflammatory cytokines and the infiltration of immune competent cells is induced by BD [16, 46–50]. VCAM-1 expression was found after 6 hours of BD in both hypotensive and normotensive donors. The pattern of VCAM-1 expression was similar to that described by others during periods of inflammation or rejection of the liver. Also, the ICAM-1 expression observed in all brain-dead rats was similar to expression patterns during episodes of inflammation [42, 51–55]. Leukocyte recruitment to the underlying parenchyma was facilitated, as expected, with upregulated VCAM-1 and ICAM-1 expression, with a significant increase in infiltrating leukocytes in the liver tissue of brain-dead rats. Also, naive as well as activated macrophages (i.e., ED1- and ED2-positive cells) were significantly increased in brain-dead donors versus controls [42]. The detrimental changes observed during experimental BD were confirmed in more limited clinical studies. Inflammatory changes were found in cadaveric donor livers that showed increased proportion of CD3+ lymphocytes, CD68+ monocytes, and macrophages relative to livers from living donors [33, 56, 57]. Clinical events after BD included more frequent acute rejection episodes and increased lymphocytic infiltrates [33, 56]. In human liver transplants, donor BD triggered upregulation of inflammatory cytokines IL-6, IL-10, TNF-α, TGFβ, IFN-γ, and MIP-1α. Those findings correlated with more pronounced cellular infiltrates, a higher incidence of primary graft dysfunction, and frequent acute rejection episodes [16, 58]. Findings in small animal models of BD parallel clinical observations [16], for instance upregulation of IL-10 and iNOS mRNA in liver has been demonstrated after 6 h of BD [39]. On the contrary, another study has not found iNOS induction in brain-dead animals. This contradictory result may be explained by the fact that the phase of BD in this later study was maintained for only 2–3 h [39].

3. Experimental models used to study the effect of brain death on liver graft quality

Future research in experimental models of LT using BD donors is required to understand the pathophysiology of BD and elucidate the consequences of BD on graft function and survival [6]. The knowledge of the underlying mechanisms on the detrimental effects of BD is a critical question to promote better preservation of the organ and to improve outcome after LT [1].

A wide variety of BD models have been described in the literature, and in most of them an increase in intracranial pressure is generated by an expanding intracranial balloon, finally leading to BD [26]. This section will discuss about experimental animal models used to evaluate the effects of BD on optimal and steatotic liver grafts.

3.1. Experimental BD in small animals

Irreversible pontine ischemia is the essential hallmark of experimental BD. As hemodynamic instabilities related to cardiovascular collapse may bias experimental results, invasive monitoring is important for accurate determination of volume and maintenance of physiological blood pressure. The initial spike in blood pressure during brain stem herniation, apnea, and transient spontaneous reflexes with subsequent absence of spinal reflexes are characteristic criteria of the central catastrophe in experimental models of BD [16, 24, 28, 29, 31, 59]. To produce BD in small laboratory animals, a Fogarty balloon catheter (2–4 French) is inserted through a parietal bore hole into the subdural space and inflated. Reported inflation volumes for induction of the condition range from 200 to 500 μL in rats and 80–103 μL in mice. The inflated balloon catheter is left in place during the entire period of observation to avoid intracerebral hemorrhage and hemodynamic collapse. Computerized axial tomography or magnetic resonance imaging in brain-dead animals can document that the catheter inflation causes the hindbrain to herniate through the foramen magnum [16, 21, 28, 29, 60, 61]. Two types have been described: the so-called 'sudden onset' BD model and the 'gradual onset' model. In the gradual onset model described by [26], induction of BD was started by gradually increasing the intracranial pressure by inflating the balloon with 16 μL saline per minute. During balloon inflation, a hypotensive period occurred followed by a short peak and a subsequent drop in blood pressure. When the blood pressure returned to its basal level during an increasing peak, inflation of the balloon was stopped and anesthesia was withdrawn. The state of BD was confirmed 30 min after the onset of BD, by the absence of corneal reflexes and a positive apnea test, and such condition was maintained during 1 h or for 4 h [26]. In contrast to the sudden onset model, the gradual onset model simulates cerebral hemorrhage by slowly increasing the intracranial pressure, resulting in less hemodynamic instability and maintenance of normotension during BD for several hours. So far, only a few studies in abdominal organs have described BD induction using a gradual expansion of an intracranial balloon [24, 26].

Authors using a sudden onset model of BD [60] have demonstrated upregulations of proinflammatory markers in different organs, including the liver. BD was produced by rapid balloon inflation of a Fogarty arterial embolectomy catheter introduced into the subdural space through an occipital burr hole; this maneuver suddenly increases intracranial pressure and causes herniation of the brain stem within 20 min in all animals. The rats were tracheostomized and mechanically respirated for periods up to 6 h. [60]. Authors conclude that the experimental system described provides a potentially clinically relevant model in which to study the systemic effects of BD in detail and suggests means to prevent changes in peripheral organs [60]. Thus, it has been postulated that sudden onset BD reflects the situation of the hemodynamically instable donors [23]. In a sudden onset' BD model described by [23], through a

frontolateral trepanation a balloon catheter was introduced in the subdural space with the tip pointing caudally. Inflating the balloon for 1 min increased the intracranial pressure thereby inducing rapid progressive brain injury leading to BD, which was based on the sharp rise and subsequent drop of blood pressure and heart rate. The state of BD was confirmed 30 min after induction by the absence of corneal reflexes and an apnea test and the effect of BD was studied at 1 h and 6 h after BD induction [23]. Hemodynamic changes in this model, mimicking acute isolated cerebral trauma, included first a sudden increase and subsequently a gradual drop of both heart rate and mean arterial pressure [23]. This BD model appears to be a reliable tool to mimic the type of fast occurring brain injury due to isolated cerebral trauma in man. In this BD model that reflects the situation of the hemodynamically instable donor, expression of immediate early genes was found in tissues of liver and kidney coinciding with progressive dysfunction of these organs and suggest a progressive detrimental effect of BD on donor organ quality, especially in livers from hemodynamically instable donors [23].

In these experimental models described above, only the effect of induction of BD on liver tissue was evaluated. Unfortunately, the other conditions (ischemia and reperfusion) that are present in the clinical practice of transplantation were not included. It is important to evaluate such conditions, since they negatively affect liver grafts already damaged by BD when these grafts, especially steatotic ones, are obtained from cadaveric donors.

Given the prevalence of hepatic steatosis in the population, this represents a large potential pool of donors. However, the clinical problem is still unresolved as steatotic livers are more susceptible to I/R injury and, when used, have poorer outcome than non-steatotic livers [6]. To date, only one experimental study has evaluated the effect of BD on optimal and steatotic liver grafts, which were subsequently subjected to cold ischemia and transplantation. In such research [1], authors have tested the influence of hepatic steatosis and BD separately and in combination in an experimental model of LT [1]. A balloon catheter was introduced through the drill hole in the extradural space with the tip pointing caudally. The intracranial pressure was increased by inflating the balloon for 1 min. The increase in intracranial pressure induced rapid brain injury, leading to immediate BD, simulating a condition comparable to acute isolated cerebral trauma in humans. The state of BD was confirmed by the absence of corneal reflexes and an apnea test and liver grafts were extracted from donors at 6 h after the induction of BD [1]. Steatotic and non-steatotic liver grafts were preserved during 6 h and then were transplanted and submitted to reperfusion for 4 h. Authors report for the first time that the injurious effects of BD in LT are exacerbated in the presence of hepatic steatosis and occur before liver grafts are retrieved from donors [1]. In addition, the mechanisms responsible for the detrimental effects of BD were different depending of the type of the liver, which would interfere with protective pharmacological or surgical strategies applied in liver grafts, avoiding its potential benefits [6]. In such a study, BD-induced dysfunction in the cholinergic anti-inflammatory pathway and prevented the benefits induced by ischemic preconditioning, a surgical strategy that shows benefits when it is applied in non-BD clinical situations. In fact, the study indicated that the combination of acetylcholine and ischemic preconditioning could be considered as a feasible and easy-to-perform intervention to reduce the adverse effects of BD and improve the quality of liver grafts. So authors propose that the time frame between the declaration of BD and organ retrieval provides an important window for cytoprotective intervention, which may counteract the detrimental effects of BD [1].

Table 1 summarizes the experimental models used in small animals to study the effect of BD on liver graft quality.

3.2. Experimental BD in large animals

In the baboon, BD is produced by creating intracranial hypertension. Under full inhalation anesthesia, a Foley catheter was introduced into the subdural space through a frontal burr hole in the skull and filled with 20–30 mL of saline, then BD occurred within 20 min [62, 63]. BD has been also induced in pigs by ligation of both brachiocephalic arteries, from which arise the carotid and vertebral arteries. Both experimental models of BD led to a series of major pathophysiological changes that may be collectively referred to as the autonomic storm. Though there was a brief initial period of excessive parasympathetic activity, evidenced by a marked bradycardia, most of the effects of this autonomic activity were brought about by the sympathetic nervous system [63].

Porcine BD models have been widely used in transplant surgery studies. A research group [64] performed a study with a lengthening of the induction phase to 60 min with gradual epidural balloon inflation up to 15 mL. Furthermore, they suggested that prolonged BD process might give the necessary preconditions to trigger a systemic inflammatory response that might contribute to organ dysfunction. Compared to the clinical situation, other authors [27] considered that 60 min was still a relatively short time. They therefore wanted to prolong the BD induction phase up to 200 min by stepwise increase of intracranial volume, followed by a 30-min observation period. Analysis of the monitoring results showed a classic intracranial pressure-volume relationship. Furthermore, intracranial compliance decreased gradually when intracranial pressure increased, which reflects a decreasing ability to compensate for added intracranial volume [27]. The described so-called Cushing response with arterial blood pressure increase, bradycardia, and respiratory irregularities was also demonstrated [27]. Authors believe that their model has the advantage of applying gradual progressive changes in intracranial pressure, cerebral perfusion pressure, and brain tissue oxygenation, leading to cellular injury in parenchymatous organs. Therefore, the model provides the possibility

Small animals				
Article	**Type of BD**	**Time of sustained BD**	**Support during BD**	**Cold ischemia**
Ref. [1]	Sudden	6 h	Ventilation	6 h
Ref. [23]	Sudden	1 or 6 h	Ventilation	Not realized
Ref. [26]	Gradual	1 or 4 h	Ventilation Inotropic support	Not realized
Ref. [31]	Gradual and sudden	6 h	Ventilation	Not realized
Ref. [42]	Sudden	1 or 6 h	Ventilation Inotropic support	Not realized
Ref. [60]	Sudden	6 h	Ventilation	Not realized

Table 1. Experimental models in small animals used to study the effect of BD on liver graft quality.

of studying the effects of BD processes on organ quality and function as well as outcomes after transplantation. Furthermore, the model also may be used for explorative studies of brain injury mechanisms and for evaluating new neuromonitoring devices [27]. In another experimental model [65], BD has been induced by surgically placing an epidural balloon and gradually increasing the inflation to increase intracranial pressure to 15, 25, 35 and 45 mm Hg, maintaining each pressure level for 30 min, in piglets [65]. However, opposite results in comparison with the experimental model using a BD induction phase of 200 min [27] were observed, this means a decrease of the arterial blood pressure and tachycardia. The reason for this difference is unclear, but it is well-known from the clinical situation that a Cushing response is not always observed in patients with BD developing [27].

More recently, [66] have established a clinically relevant, reproducible, large animal model of BD in pigs, based on a controlled cerebral hemorrhage. To induce intracranial hemorrhage, a needle was inserted through a burr hole over the left hemisphere, and stereotaxically placed in the internal capsule at the level of the lateral ventricle. Blood was withdrawn from the central arterial catheter in a 10 ml unheparinized syringe and then was injected in the brain with a rate of 40 ml/h. The infusion of blood continued for 60 min to maintain an intracranial pressure sufficient to ensure BD. Pigs in the control group underwent surgical preparations similar to the BD group, including burr holes and tissue glue but without injection of blood. Computerized tomographic angiography was performed 120–180 min after BD [66]. Irreversible damage to the brain stem was validated by a negative atropine test, disappearance of corneal and pupillary light reflexes, and a negative cerebral perfusion pressure sustained for more than 60 min. The disappearance of intracranial pulse pressure waves supports the diagnosis, as this is a clinical parameter associated with BD [66].

Further investigations will be required to investigate whether all of these experimental models of BD above mentioned are adequate to study the effect of BD on a graft that also presents I/R injury and which one best simulates the conditions present in clinical practice.

Another experimental model of BD in large animals has been reported [67], which has been previously established and neuropathologically validated [19, 67]. In this model the effect of BD on liver grafts also undergoing cold preservation and transplantation was evaluated. BD was induced suddenly by the injection of 8–17 ml of normal saline solution into the catheter balloon over a period of 5–10 min using dogs as experimental animals. This produced a sudden rise in arterial blood pressure (systolic and diastolic) and heart rate, reflecting an explosive intracranial pressure increase and defined as the "Cushing reflex" [67]. Once the diagnosis of BD was established, the dog was monitored for 16 h before the liver was removed [67]. Livers were stored floating in 1000 ml of the UW preservation solution at 4°C for 24 h [67] and at the end of the preservation time, the livers were transplanted orthotopically using a modified technique with a sutured upper caval anastomosis and cuffed anastomoses for the infrahepatic vena cava and the portal vein [67]. Although not all the clinical scenario of BD could be replicated, this experimental model simulates the sudden rise in intracranial pressure caused by acute cerebrovascular lesions or traumatic brain injury [67]. As reflected by the hepatic enzyme release during the course of BD, no evidence of deterioration of function in livers retrieved from BD donors was found. These findings are different from rodent models. In addition, it should be borne in mind that in clinical practice it is well documented that BD negatively

Large animals				
Article	**Type of BD**	**Time of sustained BD**	**Support during BD**	**Cold ischemia**
Ref. [27]	Gradual	1 h	Ventilation	Not realized
Ref. [64]	Gradual	6 h	Ventilation	Not realized
Ref. [65]	Gradual	30 or 60 min	Not described	Not realized
Ref. [66]	Gradual	8 h	Not described	Not realized
Ref. [67]	Sudden	6, 12 and 16 h	Inotropic support	27 h

Table 2. Experimental models in large animals used to study the effect of BD on liver graft quality.

affects the function of liver grafts that are procured from a cadaveric donor and subsequently transplanted [68]. Therefore, to achieve a successful design of therapeutic strategies to protect liver grafts against harmful effects of BD, it is important to take into account only experimental models in which the negative effects of BD on the liver tissue have been demonstrated.

Table 2 summarizes the experimental models in large animals used to study the effect of BD on liver graft quality.

4. Conclusions

Most organs for transplantation originate from BD donors. Although the detrimental consequences of BD have been clinically described, the underlying mechanisms and their relevance in transplantation remain poorly understood. Indeed, few studies have evaluated the effect of BD on transplantation, and, as stated along this chapter, most of the experimental studies focused in the pathophysiological changes occurring during BD without transplantation. Moreover, these studies have been performed in the different sudden and gradual BD models, so dissimilar results on pathophysiological changes and treatments have been described.

Comparison of the results of animal studies and their extrapolation to human beings is feasible, but with limitations such as differences in BD and ischemia tolerance, anatomy of the liver of various species, surgical conditions used in clinical practice and those used in the experimental models. Importantly, studies performed in small animals are of limited applicability to human beings due to their different size and anatomy of the liver and their faster metabolism. Large animals exhibit greater similarity in their anatomy and physiology to humans; however, their use is restricted by serious logistical and financial difficulties, ethical concerns and limited availability of immunological tools for use in large animal species. In addition, in some cases, experimental models of BD in large animals did not mimic the clinical conditions in liver transplantation from BD. Despite the limitations of the experimental animal models, these are the best options to study hepatic I/R, especially considering that the progress of human studies is slow, the majority of human tissues are not routinely accessible for research, and there is very limited opportunity for interventional studies. The clinical application of strategies

Clinical effects	Sudden model		Gradual model	
	Small animals	Large animals	Small animals	Large animals
Release of catecholamines [7]	Observed [31]	Not described	Few [31]	Not described
Hemodinamic deterioring [16, 24, 28–31]	Acute [1, 18, 23, 42]	Observed [67]	Few [24, 26]	Observed [27] Not observed [65]
Hormonal alterations [33, 63]	Observed [1]	Not described	Not described	Not described
Inflammatory response [44]	Observed [60]	Observed [63]	Observed [26]	Few [64, 66]
Inotropic support [33]	Applied [1, 42]	Applied [67]	Not necessary [26, 31]	Not described

Table 3. Reproducible clinical effects of brain death in the different experimental models.

designed at benchside will depend on the use of experimental models of BD that resemble as much as possible the clinical conditions that happens in BD and LT [69]. **Table 3** summarizes the reproducible clinical effects of BD that happens in the different experimental models.

We recognize the complication, but future research in similar experimental models of transplantation using BD donors is required to understand the pathophysiology of BD and elucidate the consequences of BD, especially in sub-optimal liver grafts. Thus, multidisciplinary research groups should devote additional efforts to better understand the pathophysiology of BD to ultimately develop effectual therapeutic strategies aimed at improving graft viability, and at significantly increasing the organ donor pool.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 9

Pancreas Retrieval for Whole Organ and Islet Cell Transplantation

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Additional information is available at the end of the chapter

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Abstract

For more than five decades, we have been refining advances in pancreas whole organ and islet cell transplantation as clinical therapies to treat the ever-increasing number of patients suffering from type- 1- diabetes. Research and clinical practice have contributed to making both whole organ and cellular transplantation viable therapeutic options for a broader range of patients. Furthermore, both forms of clinical transplantation results have progressively improved, due to the ongoing refinement of organ donation and its various technical processes, combined with the evolution of immunosuppression and patient care now offering excellent long-term treatment for both type- 1- diabetes and concomitant renal failure. This chapter provides an overview on how this has been undertaken and achieved over decades to ultimately provide outstanding outcomes on par with other organ transplantation results. Briefly, we cover the history of pancreas retrieval procedures, the importance of donor selection, the intricate processes of the organ donor operation, preservation of the pancreas, and the ideal ways to best improve outcomes for transplantation. Improving and providing the optimal donor and preservation conditions underpinning the success of subsequent whole pancreas or islet transplantation as a safe, effective, and feasible therapeutic option for an increasing number of patients suffering from type- 1- diabetes.

Keywords: diabetes, insulin, islet, islet cell, islet cell allotransplantation, islet cell transplantation, islet cell isolation, organ perfusion, organ retrieval, renal failure, type 1 diabetes, whole organ transplantation

1. Introduction

Since Kelly and colleagues performed the first whole pancreas transplant in 1966, significant advancements in pancreas transplantation have been made. [1] There was a gap following the first series of whole pancreas transplants due to poor graft outcomes with significant impact from poor organ preservation of the pancreas playing a major role. It took almost 20 years for the development of newer surgical techniques including use of newly developed perfusion solutions, segmental grafts, advances in ductal drainage including bladder drainage, and effective immunosuppression regimens such that whole organ transplantation burgeoned, with great advances made by Sutherland and colleagues at the University of Minnesota [2]. However, it was not until much later following many years of experimental research that pancreata for islet cell isolation and transplantation became a reality. Over the past two decades in particular a great deal of effort has underpinned making islet cell transplantation a viable therapy for a broader range of patients with type 1 diabetes (T1D). Clinical results have progressively improved, now demonstrating outcomes on par with other organ transplants, specifically in terms of insulin independence, and graft and patient survival [3]. We are now at the point where islet cell transplantation, in the form of allotransplantation, like its forebear whole organ transplantation, has become widely accepted as a clinical therapy for patients affected by T1D.

Now more than five decades on and with many organ donor operations having been performed since the advent of organ donor procedures as we know them, we have refined and perfected the organ donor process since the first organ retrieval of a brain dead donor in 1963 [4] and the subsequent adoption of the "Acceptance of Brain Death for Organ Donation" issued by the Ad Hoc Committee of the Harvard Medical School [5]. We have seen an increasing emergence of specialized organ retrieval teams with focus on the overwhelming need to improve organ donor rates for the ever increasing recipient patient population [6]. Always a dedicated surgical pursuit, research into organ donation and the surgical retrieval process for the pancreas and most other organs has often been overlooked in favor of recipient-related research into the prevention of rejection, and improving immunosuppression and tissue matching. This is particularly problematic when it comes to whole pancreas and islet transplantation as the pancreas is a less retrieval tolerant organ than other solid organs, and requires extra attention both during and after retrieval to ensure that the organ's valuable islets, which are especially susceptible to hypoxia and the ischemic insult, are effectively preserved [7, 8].

In this chapter we provide a general overview of Pancreas Retrieval for both Whole Organ and Islet Cell Transplantation, but it should be noted that there are clear overlaps in this process for both whole organ and cellular transplantation. As such the way the processes are performed can be utilized for retrieval for either type of subsequent transplant. Overall, we have seen significant improvements to pancreas transplantation results, in particular in the islet cell arena, due to the significant research undertaken to improve graft outcomes by improving donor selection and organ procurement and preservation [9]. On the recipient side we have also further improved outcomes with changes to the transplant and to the

pharmacological treatment of recipients such as newer focused monoclonal immunosuppressive strategies that better control graft rejection [9].

This chapter focuses on the optimal process for deceased donor pancreas retrieval and its role in maximizing graft function and survival. However, with a great number of processes to outline, only the major ones will be covered in this chapter. In particular, we will emphasize major improvements in donor selection, surgical retrieval techniques, pancreas retrieval in the context of multi-organ donors, back-table preparation of the pancreas, perfusion fluid types, and future perspectives including the utilization of technologies such as machine perfusion and persufflation. These factors will be discussed in the context of improved outcomes to the engraftment, function and survival of the transplants. It is also acknowledged that there remains the ongoing need for further improvements to both whole organ and islet cell transplantation, however both techniques clearly offer safe and achievable therapeutic options for the ever-expanding number of patients suffering from T1D [10].

2. Historical timeline

The original retrieval processes of the modern era were initially developed for and used in kidney only retrieval surgery. As per **Figure 1** the procedure first introduced in 1963 utilized cold lactated Ringer's or low-molecular-weight dextran solutions infused directly into the renal artery of the retrieved kidneys, performed only after their removal from the donor [11]. These were the beginnings of modern donor retrieval but they were less than ideal techniques due to the time taken to perfuse the organs, and therefore a number of more active and by far more effective methods of perfusion and cooling of organs were subsequently developed in order to minimize ischemic insult and subsequent damage to organs. These techniques were based upon the concepts from cardiothoracic surgery, involving active patient cooling during procedures to prevent ischemic damage [12, 13]. The transplant fraternity quickly adopted these intravascular perfusion-related cooling techniques, which were standardly utilized as a first step in the preservation of all whole-organ grafts. The currently accepted modern cadaveric donor procedure is performed using some basal form of the *ex situ* techniques developed and performed in the mid to late 60's by Starzl and colleagues [14] for not only kidneys but also incorporating the pancreas and liver. Further refinements saw the perfusion and addition of heparin to the perfusate solutions and also the donor. Ensuring removal of blood by *ex situ* perfusion as described by Belzer et al. [15] resulted in improved but only satisfactory kidney preservation of several days. However, this technique was eventually abandoned in most kidney transplant centers when it was learned that the quality of 2-day preservation was no better than with the simpler *"iced slush"* methods [16].

The underpinning method of *iced slush* for shipping was based around experimental work on kidneys [17]. This research and practice focused on perfusion fluids of differing intracellular and extracellular fluids consisting of electrolytes with varying osmotic and oncotic effects that were infused into the allograft before placing it in a cold storage container. Collins

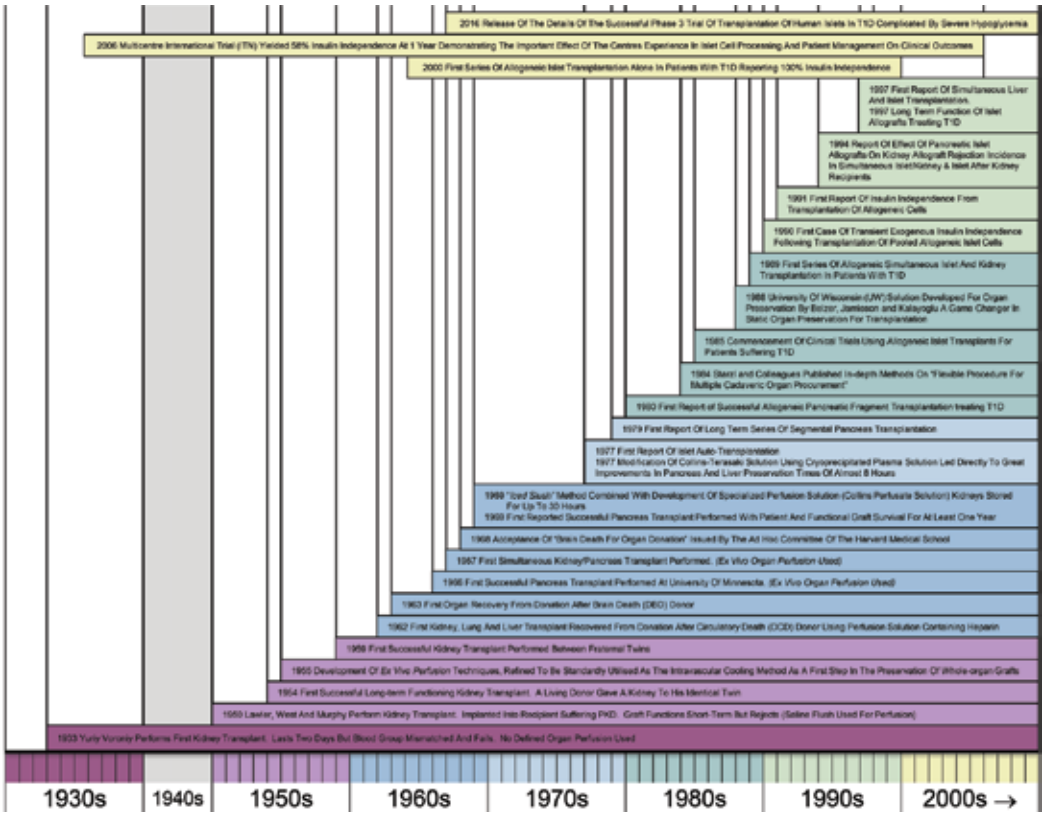

Figure 1. A time line in the significant development of transplantation over the years with focus on the techniques used for whole pancreas and islet cell transplantation.

and colleagues developed a relatively simple technique (infusion of mannitol, phenoxybenzamine, and their Collins perfusate) providing good preservation in kidneys stored for up to 30 hours [17]. Other perfusates, such as Ringer's lactate and 10% invert-sugar solutions, gave inferior results. The new perfusate solution and technique extended the time of simple ice storage from 12 hours to 30 hours. Continuous hypothermic perfusion saw further additions by Ackerman and Snell [18] and Merkel, Jonasson, and Bergan [19] who following many organ donor studies developed the widely accepted and much more simplified core cooling. These utilized cold perfusion solutions with the infusion of the fluids being performed directly to the vascular bed of all the organs via the distal aorta and demonstrated significant improvement for the pancreas but they were still less than ideal for this most sensitive organ. However, the development of these techniques used throughout the 70's meant that organs could generally be removed without causing issues when retrieving multiple organs, which included the liver and sometimes pancreas. Kidney preservation became more feasible along with the other abdominal organs seeing times of 1 to 2 days, long enough to allow tissue matching and sharing of organs between hospital units even interstate or in Europe between countries. However, these were purely focused still on the kidneys rather than the other abdominal organs and as such a number of groups undertook experiments focusing on other organs including the pancreas and liver; landmark papers included those by Benichou

et al. [20], using the Collins-Terasaki solution, and de Gruyl et al. [21] using cryoprecipitated plasma perfusion preservation of duct-ligated pancreatic allografts, along with Wall et al. [22] using similar plasma-like solution. This led directly to great improvements in pancreas and liver preservation and allowed organ sharing amongst transplant centers, although the preservation period was still limited to less than 8 hours.

The development of University of Wisconsin (UW) solution for organ preservation by Belzer, Jamieson, and Kalayoglu in the 1980s was a game changer in static organ preservation for transplantation [23]. This new flushout solution for preservation of the pancreas was tested in the dog model of segmental pancreas autotransplantation. The solution has an osmolality of 320 mOsm/L (K+ = 120 mM, Na + = 30 mM), and contains lactobionate, and raffinose as impermeants. The role of hydroxyethyl starch (HES), the colloid component of UW solution, was shown to be particularly important for pancreas preservation, in comparison to the liver and kidney [24]. UW perfusate solution preservation almost tripled the time of safe preservation of the various organs, including the pancreas, making national sharing of most organs a viable and practical process [25].

However, despite significant success the preservation or extended preservation of the pancreas still required further refinement, and significant research using animal models of static perfusion were pursued, in particular for use in islet cell transplantation. Along with perfusion fluids a number of standardly used retrieval techniques became more readily adopted [19]. However, until 1981 transplantation of the extra-renal organs was an unusual event such that the focus of perfusion only really focused on kidneys. By the mid-1980s, it became apparent that multiple organs would start to become transplanted in earnest, with liver, pancreas and thoracic organ transplant procedures becoming more widely accepted. A safe and effective method for multi-organ procurement and preservation was required by which the abdominal organs, kidneys, liver, and pancreas, could all be suitably retrieved using the same solution. At this stage Starzl and colleagues published an in-depth method on their "flexible procedure for multiple cadaveric organ procurement" [26], which was adopted by many centers worldwide.

However, even at this point the pancreas was often over-looked with the focus on the kidneys and liver as the principal organs to be retrieved. Starzl's publication stated "*If the whole pancreas is transplanted as we recommend, the combination of liver and pancreas removal is incompatible*" and it was often the case when surgical teams were procuring the liver and pancreas together that there were issues relating to the suitable separation of their vasculature [27]. At this time, our own surgical team also retrieved the pancreas with the liver, but always removed liver to the back-table before the pancreas and kidneys. The major perceived reason for this was the need for the life-saving liver to take priority. Furthermore, as the portal vein and the branches of the celiac trunk, drain or supply both organs, preference was given to sacrificing the pancreas' vessels instead of the liver. It was a number of years before this routine surgical practice would change.

Whole organ research utilized canine models as the dog pancreas is more anatomically similar to humans in comparison to the tri-lobed porcine pancreas. These models allowed replication of the clinical situation and further refinement of the retrieval and transplant procedures [28, 29]. From these came the widespread implementation of newer perfusion fluids such as UW solution, and the utilization of vascular extension grafts to the pancreatic vasculature helped resolve the situation of shortened pancreatic inflow and outflow conduits due to preference to

the liver in combined retrievals [30]. The other major change to the procedure was the adoption of an *en bloc* liver pancreas retrieval technique, where both organs were rapidly removed in a bloodless field post perfusion, then separated on the back-table. Furthermore, the sharing of organs from a common donor by recipient teams from different units became routine by the early-1990s, in particular due to the use of UW solution, which had clearly been shown to be a real advantage in pancreas retrieval both experimentally and clinically [31].

In the 1990's the focus on research and advances relating to the retrieval process started to shift, with attention once again shifting to the perfusate solutions, which were thought to be especially impactful for islet cell transplantation. A number of groups also investigated additives to the perfusate solutions such as the use of antibodies to reduce inflammation and further improve graft outcomes, although this was met with limited success [32]. In the 2000's it became generally accepted this was achieved via cannulation of the aorta alone, with or without additional access to the portal venous system with variations that have been seen specifically in relation to multiorgan retrieval where some groups chose to perform 'dual' perfusion technique which are all discussed in greater detail later in this chapter [33, 34].

3. Use of the pancreas for whole organ or cellular transplantation—donor selection

Underpinning the entire transplantation process, regardless of whether the donor is for whole pancreas or islet cell transplantation, is appropriate donor selection such that the donor organ is of a suitable size and quality to allow for use in either type of therapy. In order to be utilized in clinical transplantation, it is imperative that the donor be appropriately screened to ensure the organ to be retrieved is free from any disease that may subsequently manifest in the donor, including cancer, and infections with viruses, bacteria, fungi, or prions [9]. It is paramount that we avoid the more commonly occurring diseases when screening the donor before accepting the pancreas for organ donor retrieval and subsequent clinical transplantation. Infectious risk factors depend on the history of patient, any underlying disease of the organ donor, and the immunosuppressive treatment administered to the recipient [35]. Transmission of most pathogens is possible, but their frequency varies according to the endemic population from the transplanted organ, the selected immunosuppressive therapy and prophylaxis utilized in the recipient, and also at the donor procedure [36]. Obviously, there are many more variables with regards to organ donor selection criteria, and these will be discussed in more detail in the following sections.

4. Pancreas retrieval

4.1. Surgical techniques

Pancreas retrieval for both whole organ and cellular transplantation necessitates meticulous surgical technique. In comparison to the liver and kidneys, the pancreas is more commonly damaged at retrieval, which subsequently results in non-utilization of a significant proportion

of procured pancreata [37]. The organ must be procured without any parenchymal and/or capsular breach, and its arterial inflow and venous outflow vessels must be clearly identified (tagged) and maintained for subsequent back-table reconstruction when used for whole pancreas [38]. The extent of organ and vascular dissection depends upon whether the retrieval is from a brain-dead (DBD) or circulatory death (DCD) donor; a large proportion of pancreas dissection can be undertaken in the warm phase for DBD donors, whilst pursuit of the DCD pathway necessitates wholly cold-phase dissection, which is potentially more difficult as appropriate anatomy is harder to identify.

4.1.1. Anatomical considerations

The pancreas is situated in the retroperitoneum, nestled within the curvature of the duodenum. Important relations are both kidneys posteriorly, the spleen laterally and attached to the pancreas via its pedicle contained within the lienorenal ligament, the superior mesenteric vessels, bile duct, and portal vein in the region of the pancreatic head/neck, the inferior vena cava (IVC) deep to the head and portal vein, and the aorta, left suprarenal gland and left renal vein deep to the body. Pancreatic blood supply is primarily derived from the celiac artery in origin via the splenic and superior mesenteric arteries (via the inferior pancreaticoduodenal artery), and also the gastroduodenal artery (via the superior pancreaticoduodenal artery). The celiac trunk gives off the splenic artery, which emerges at the upper pancreatic border and runs along this border in a tortuous fashion until turning towards the splenic hilum within the lienorenal ligament. The superior mesenteric artery (SMA) emerges from the aorta inferior to the celiac trunk, and is directed inferiorly on the posterior aspect of the pancreatic neck, to then lie on the uncinate process and then the 3rd part of the duodenum prior to entering the root of the mesentery. Venous drainage occurs via the splenic vein for a large part of the pancreas, whilst the superior and inferior pancreaticoduodenal veins drain the head into the superior mesenteric vein (SMV) and portal vein confluence. It is the shared vasculature of the pancreas with the liver that often causes retrieval issues as the origin of the splenic artery is from the celiac, and the outflow of the splenic vein is through the portal vein, necessitating delicate surgical dissection and care in separation to ensure shared and usable vasculature for both organs [39].

4.1.2. DBD retrievals—pancreas-specific considerations

Pancreas retrieval in the DBD donor is a controlled process that allows significant preliminary organ and vascular pedicle dissection. The Cattell-Braasch maneuver is utilized to expose the aorta and IVC distally, with the proximal extent of dissection limited by the SMA overlying the left renal vein; this maneuver will incorporate mobilization of the small bowel mesentery and pancreatic head/duodenum [40]. Our approach to exposure and dissection of the remaining pancreas [41] is to access the lesser sac by mobilization of the greater curvature of the stomach; the greater omentum is detached at its origin using ultrasonic shears (Harmonic Scalpel) as per **Figure 2**. The short gastric vessels are also detached using this method at the upper portion of the greater curvature. The splenic flexure of the large bowel can thence be mobilized onto the lower pole of the spleen. Once the spleen is free of its surrounding attachments, it can be lifted and used as a handle to mobilize the tail and body of the pancreas without directly

handling the pancreas itself. The Harmonic Scalpel is also very useful in the dissection of the superior and inferior pancreatic borders, particularly the relatively vascular splenic flexure of the colon. The posterior surface of the pancreas can be mobilized with standard electrocautery in a relatively bloodless plane. The SMA/SMV pedicle inferior to the pancreas needs to be skeletonized such that it can be divided using a vascular stapler prior to pancreas removal in the cold phase. Superiorly, the bile duct is ligated and transected proximal to the point of ligation; residual bile is flushed out its open proximal end using saline instilled into the gallbladder. We will also free attachments around the gastroduodenal junction and duodenojejunal flexure, which are then identified with circumferential vessel loops for stapled division later in the cold phase. The inferior mesenteric vein is ligated *in situ* post perfusion as subsequent retraction of the divided vessel may make it difficult to identify on the back-table. Diluted povidone-iodine solution is instilled into the duodenum via a nasogastric tube as a decontamination step, and is subsequently removed through the same route. Some authors report concerns with subsequent duodenal mucosal toxicity related to instillation of povidone-iodine, and

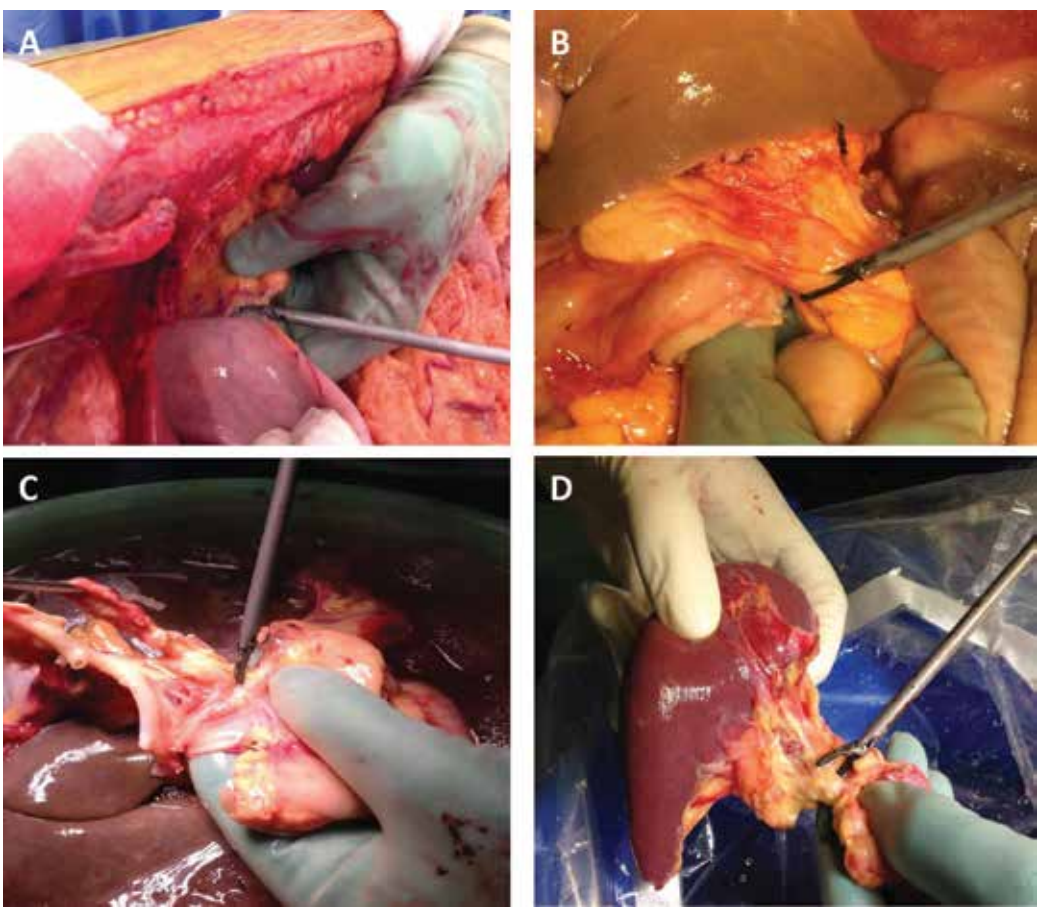

Figure 2. The harmonic scalpels utilization during pancreas procurement. (A) Mobilization of the greater curvature of the stomach, (B) creation of the superior mesenteric pedicle (cold phase), and back-table separation of (C) the liver-pancreas block, and (D) the pancreas and spleen.

suggest additional back-table flushing of the duodenum with an alternate solution [42, 43]. Alternatively, duodenal decontamination can be completed using an antibiotic solution, such as amphotericin [40]. However, the most important factor is to utilize a decontamination procedure to reduce the potential risk of cross infection to the recipient. Our own unit has utilized povidone-iodine solution instilled into the duodenum via a nasogastric tube as a decontamination step in more than 500 SPK transplants at our own center with no duodenal mucosal toxicity identified [44]. In the cold phase the duodenum is then divided above and below the pancreatic head with a linear cutting stapler, after carefully withdrawing the nasogastric tube from the duodenum into the body of the stomach. Any remaining superior mesenteric pedicle dissection is also completed, and a vascular (cutting) stapler is utilized to divide this pedicle. It is of paramount importance that the pancreas is not injured during this step as this will cause serious issues in both whole organ and islet cell transplantation. Furthermore, if the mesenteric pedicle is divided too close to the pancreas, or includes part of the uncinate process, there is a risk that blood supply to the pancreatic head via the inferior pancreaticoduodenal branch of the SMA will be compromised, creating a significant problem for whole organ transplantation [40, 45, 46]. Additionally, for the whole organ transplant an arterial and venous conduit should be retrieved for back-table pancreatic vascular reconstruction. This usually consists of a segment of the external iliac vein for use as a portal vein extension graft if required, and the common iliac artery bifurcation, including a length of the internal and external iliac arteries, to fashion a Y-graft connecting the native SMA and splenic artery. It is essential that the common iliac bifurcation is not damaged during this process [45]. Like a number of other major units our center preferentially retrieves the pancreas *en bloc* with the liver, with separation of both organs performed on the back-table (see below) [47].

4.1.3. DCD retrievals

DCD pancreas retrieval is technically feasible, and can achieve excellent outcomes in selected donors certainly in the whole organ arena (see Outcomes, below). In contrast to DBD procurement, the first step in all DCD retrievals after a rapid laparotomy is cannulation and cold perfusion via the aorta [48, 49]. Venous venting is conducted via the IVC. Alternatively, if local laws allow, an *in situ* flush can be achieved using femoral cannulae inserted prior to the withdrawal of life support [49, 50]. Ante-mortem interventions including heparinization have been shown to also provide significant improvements to pancreas retrieval outcomes in the DCD setting [51]. Standard pancreas retrieval can then be undertaken as described for DBD donors, although donor hemostasis is no longer a concern and therefore sharp dissection is commonly utilized. The use of energy devices such as the Harmonic Scalpel at this stage may help minimize recipient bleeding however, as described in the DBD setting.

4.1.4. Pancreas retrieval and multi-organ donors

Pancreas retrieval is almost never undertaken in isolation, but rather it is usually procured in the context of a multi-organ retrieval, often in the presence of multiple retrieval teams. Meticulous retrieval technique therefore needs to be maintained and balanced in the presence of these competing factors, especially in the presence of concomitant liver procurement, which is still given preference owing to the critical requirement of liver transplant recipients.

Pancreas-alone donors are uncommon in this day and age due to developments in procurement and preservation techniques. Some authors raised concerns that combined liver-pancreas retrieval, in contrast to pancreas retrieval alone, resulted in significant "flush" injury to the pancreas owing to a higher volume of perfusion solution and the utilization of dual aorto-portal cannulation in the combined donors [52]. However, other studies clearly demonstrated that multi-organ retrieval, including combined liver-pancreas retrieval, was not detrimental to pancreas transplantation outcomes [53–58]. Another factor that previously precluded combined liver-pancreas procurement was aberrant hepatic arterial anatomy, in particular the presence of an aberrant or accessory right hepatic artery originating from the superior mesenteric artery [58]. Abandoning retrieval of the pancreas due to this situation is now rare, as a preserved length of the right hepatic artery originating from the SMA stump can effectively be anastomosed to the GDA as part of a back-table reconstructive procedure [45, 46]. It is only when the right hepatic artery is within the substance of the pancreas that whole pancreas retrieval should be precluded in favor of the liver [59] but the pancreas can still be retrieved for islet cell isolation as the pancreas can still be readily perfused, and on the back table the vessels readily separated, including if necessary taking them from the body of the pancreas [9]. However, if this is undertaken then care should be taken to not damage the parenchyma of the pancreas as this makes the distension of the pancreas with collagenase for digestion more difficult [9]. Over the last 25 years and more than 1000 retrievals the authors have never found any anatomical vascular anomaly to prevent an *en bloc* liver-pancreas retrieval, although this is cited as a common reason to decline pancreas retrieval worldwide.

4.1.5. Back-table separation of the liver-pancreas block and further back-table preparation of the pancreas

The combined liver-pancreas block is taken to the back-table for separation. The aortic segment is divided such that the proximal portion of the SMA remains with the pancreas, whilst the celiac axis remains in continuity with the liver. Superior to the pancreatic head, the portal vein is divided approximately 1 cm from the pancreas, whilst the splenic artery is divided closer to its emergence from the celiac axis [45, 46]. The GDA is ligated and divided prior to entering the pancreas; a longer length remains with the liver. The splenic artery and portal vein associated with the pancreas should be marked with a prolene suture to facilitate identification at the transplant center. The spleen is also routinely removed at the donor hospital, in addition to skeletonization of the pancreas prior to transportation. The Harmonic Scalpel is once again a useful tool that facilitates all pancreas-related back-table work if the graft is to be used for whole pancreas transplantation [41]. Limited back-table perfusion of the pancreas with UW solution is employed to ensure no blood is left within the organ or its vessels, whilst minimizing the risk of graft pancreatitis or edema.

In pancreas retrievals for islet cell isolation, the author's use a similar en bloc technique, with careful mobilization of the pancreas prior to aortic cannulation as per **Figure 3**. However, there is no need for meticulous hemostasis post perfusion and it is not necessary to remove the bulk of the tissues as this can be performed at the islet isolation facility. At the isolation center, the duodenum, spleen, and connective, extracapsular and vascular tissues are removed from the pancreas prior to it being cannulated to allow infusion of the digestive collagenase enzyme for

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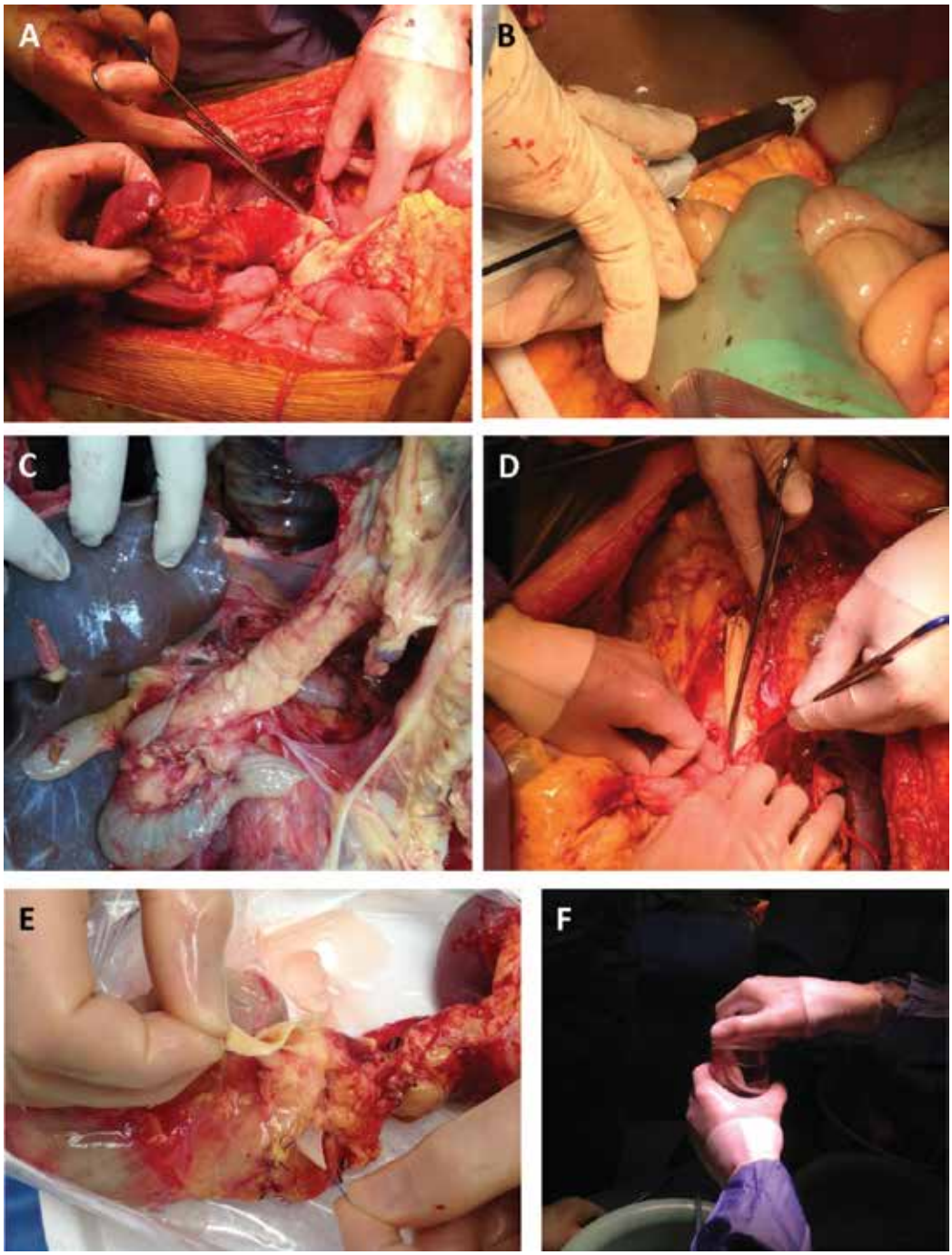

Figure 3. Procurement of the DBD pancreas. (A) Skeletonization of the pancreas, using the spleen as a handle, (B) stapled division of the superior mesenteric pedicle, (C) liver and pancreas ready for en bloc removal, (D) division of the aorta, (E) pancreas appearance after back-table preparation (n.b. Portal vein and superior mesenteric artery), and (F) back-table packing of iliac conduits in preservation solution.

islet cell isolation [9]. As such there is also no need for preservation of pancreas vasculature, which can be given wholly to the liver when separated on the back-table.

4.1.6. Pancreatic inspection and graft assessment

The pancreas must be closely inspected during the retrieval process, and any concerns regarding organ quality and/or integrity should be fully conveyed to the implanting surgeon. Graft assessment should include qualities inherent to the donor pancreas, in addition to any retrieval-related damage, and should be made both *in situ* and on the back-table as per **Figure 3**. The pancreas should be assessed for parenchymal damage, capsular breach, and/or hematoma(s). Furthermore, other important factors that may preclude further transplantation individually or in combination should be identified, including fibrosis, mass(es), high intra-parenchymal fat content, calcification, edema, and/or significantly diseased arteries [40, 46, 59]. It is important to note that much of this assessment is highly subjective, and an "acceptable" pancreatic appearance and/or texture will vary from center-to-center. Obviously some of the co-factors such as high intra-parenchymal fat content, calcification, edema, and/or significantly diseased arteries do not preclude the pancreas from being used for cellular transplantation. As an example, high intra-parenchymal fat content has been shown to be an advantage when performing islet isolation. Additionally, calcification, edema, and/or significantly diseased arteries do not affect the pancreas when used for islet cell isolation as all blood vessels and extraneous tissues are stripped from the pancreas prior to its use. The pancreas should not be discarded without direct consultation with the recipient team and exploration of its use for cellular transplantation if precluded from whole organ transplantation [9].

4.1.7. Packaging the organ for transport

Following perfusion, back-table dissection, and final inspection, the pancreas can then be packed into a suitable transport container along with perfusate solution to ensure ongoing exposure to cold preservation solution. Our unit uses the sterile triple plastic bag technique whereby the organ and a suitable volume of organ perfusion fluid is instilled into the first sterile plastic bag, without dilution from iced slush. All air is removed from the bag, prior to sealing it with a zip-tie or heavy tie. This bag is then placed inside a second sterile plastic bag filled with iced slush, ensuring close and adequate cooling of the perfusate-filled inner bag. These two bags are then placed inside a third sterile plastic bag that is securely sealed, double tied, and appropriately labeled to identify the organ and contents of the bags. Additional vessels retrieved for back-table reconstruction of the whole pancreas may also be packed into the triple sterile plastic bag set with the pancreas, or alternatively are placed inside a sterile vessel jar filled with preservation solution as per **Figure 3**, which is then double-bagged in sterile plastic bags, the first of which contains iced slush. The sealed pancreas and vessels are thence transported in a suitable, insulated iced shipping container. The container is labeled with its contents along with the contact details for both the donor and recipient coordinators.

4.2. In situ perfusion and cold static preservation

The function of *in situ* perfusion of the pancreas, as with other organs, is to achieve rapid removal of residual blood, whilst simultaneously cooling the organ and exposing it to preservation fluid media for subsequent cold static storage (CS).

4.2.1. Perfusion route

In order to achieve adequate *in situ* pancreatic perfusion during abdominal organ perfusion as a whole, the aorta must be securely cannulated and flushed using pressure such that perfusion media can flow into the pancreas via the superior mesenteric, gastroduodenal, and splenic arteries. Once perfusion fluid has traversed the pancreas, it must be allowed to exit the donor's vasculature via the systemic and/or portal routes to prevent graft edema. Aortic-only perfusion is routinely performed by our center, and subsequent venous venting is usually undertaken via the IVC in the thorax. In the event that dual aorto-portal perfusion is employed for combined liver-pancreas retrievals, portal venous access via an inferior mesenteric cannula can impede pancreatic outflow, and reduce the physiologic arterial-portal pressure difference that is required for pancreatic perfusion/flow [42, 60]. As such, in these cases, the portal vein may instead be accessed after dividing it superior to the pancreas, thereby also allowing unobstructed pancreatic venous drainage via the proximal aspect of the transected portal vein [60, 61]. A further back-table flush of the pancreas at the donor center is sometimes conducted via the splenic artery and SMA, although this step may be omitted [45, 62–65]. Evidence for or against either approach is currently lacking in both the case of whole pancreas and cellular transplantation. But preference in the cellular setting appears to favor not having any over-perfusion or edema as this can impede and dilute the infusion of the collagenase used for digestion of the pancreas in the isolation process [66].

4.2.2. Perfusion fluid types

In general, the same final fluid employed for the final *in situ* flush of the pancreas is then utilized for preservation of the organ in a bag of cold preservation fluid (CS). The preservation fluid utilized must maintain the organ at a hypothermic temperature (0–4°C), whilst simultaneously ameliorating the consequences of cold ischemia and prolonged organ immersion in fluid. As such, cold organ preservation fluids should ideally have the following properties that aim to minimize and/or reverse cellular and subcellular processes occurring within the pancreas during CS:

- Disrupted ionic pumps and ion accumulation and/or depletion, with subsequent downstream effects;
- Mitochondrial dysfunction, including reversal of the electron transport chain, and succinate accumulation;
- Altered redox potentials (RP);
- Cellular edema;
- Acidosis;
- Accumulation of reactive oxygen species (ROS);
- Adenosine triphosphate (ATP) depletion; and,
- Disruption in glycolytic pathways [67–69].

There are multiple preservation fluids currently in existence. These can be broadly classified as those that are intracellular and extracellular/intermediate in nature, based largely upon the solution's potassium content, or low viscosity compared to high viscosity solutions [70]. Common components include colloid and/or impermeants to counteract cellular edema, anti-oxidants for protection against ROS generation, ATP precursors to allow replenishment upon reperfusion, and buffers to retard the acidosis attendant with organ ischemia [70].

University of Wisconsin (UW) solution remains the most popular pancreatic preservation fluid, and was initially developed specifically for this purpose [71]. It is an intracellular solution with a high potassium content and high viscosity as it contains hydroxyethyl starch, a particularly important component for pancreas preservation [24]. UW contains other components that fulfill many ideal criteria that should be exhibited by preservation fluids, including the addition of impermeants such as raffinose, the ATP precursor adenosine, and anti-oxidants such as allopurinol. [68] Histidine-tryptophan-ketoglutarate (HTK) is another commonly utilized preservation fluid for the pancreas. In contrast to UW, HTK it is an "intermediate" solution with a significantly lower potassium and sodium concentration, thereby in effect preventing ongoing organ metabolism. HTK also has low viscosity, theoretically allowing higher flow rates, and the histidine component of HTK provides it with significant buffering capacity [68, 70]. The next most commonly studied and clinically utilized pancreas perfusion and preservation fluid is Celsior, which has similar potassium content to HTK in addition to containing histidine as a buffer. It differs from HTK in that it has much higher sodium content; furthermore, it incorporates some of the advantageous constituents of UW, including similar impermeants and one shared anti-oxidant [68, 70]. Most recently, the use of Institut Georges Lopez (IGL-1) solution has been reported in pancreatic transplantation [72]. This solution has similar constituents to UW, except the sodium and potassium concentrations are reversed such that it more closely resembles the extra-cellular environment [68]. A number of other more recently developed perfusion fluids have shown good effect in the preservation of pancreata for islet cell transplantation in particular the ET-Kyoto perfusion fluid. This fluid has a high sodium:low potassium ratio, and contains trehalose to protect the cell membrane against hypothermia and the nitric oxide donor nitroglycerin that facilitates vasodilatation [73].

National guidelines and/or protocols differ with respect to recommended perfusion and preservation fluids for the pancreas [45, 60, 74, 75]. UW and HTK solutions are the two most frequently recommended solutions for pancreas retrieval by such guidelines, although their utilization and volumes vary significantly. UK guidelines stipulate that *in situ* UW perfusion must be undertaken for pancreas retrieval, whilst Eurotransplant, German, and Australia/New Zealand guidelines allow for either UW or HTK. Furthermore, none of these guidelines preclude dual perfusion when the pancreas is being retrieved, although German standards stipulate portal venous perfusion via a catheter inserted directly into the portal vein above the pancreas/duodenum [45, 60, 74, 75]. The use of Celsior or IGL-1 solution has not yet been incorporated into major National or Regional guidelines, although both have been employed in the clinical context [64, 65, 72, 76].

A "pre-flush" is defined as a crystalloid fluid, such as Hartmann's solution, that is perfused *in situ* prior to the final flush/preservation fluid, such as UW. The pre-flush can be employed safely in pancreatic procurement, although it is not commonly utilized. The function of this pre-flush in the context of pancreas procurement is to potentially (1) reduce the amount of UW required, thereby reducing retrieval costs, and (2) to clear all blood from the vasculature such that any residual blood does not aggregate with the hydroxyethyl starch in UW [77, 78].

UW is traditionally perfused in much lower volumes in comparison to HTK, and this is also reflected in the various pancreas retrieval guidelines in existence. This is largely related to the higher viscosity of UW, in addition to the larger volume and time for HTK perfusion to achieve equilibration of electrolyte content with the extracellular milieu [79, 80]. Australian guidelines recommend a 2–4 L crystalloid/low viscosity solution *in situ* pre-flush, followed by a UW flush of at least 1–2 L; a volume range for HTK is not specified [45]. In contrast, UK guidelines state a UW flush of 50–70 ml/kg should be employed via the aorta, whilst Eurotransplant allows for 50–100 ml/kg UW or 150–300 ml/kg HTK [74, 75]. Published reports may deviate from this; perfusion volumes utilized in aortic-only perfusion range from 0.8–5.6 L, 4.9–9.7 L, and 0.8–7.9 L for UW, HTK, and Celsior respectively [81].

4.2.3. Additive(s) to perfusate

Heparin is a standard additive to the *in situ* perfusion fluid during DCD organ retrievals, including for the pancreas. Additionally, thrombolytics such as streptokinase or tissue plasminogen activator (tPA) can be added to the *in situ* perfusion fluid, or alternatively our approach is to directly inject tPA into the aorta before commencement of the cold *in situ* flush; the aim of this is to achieve a higher quality vascular flush through the clearance of microthrombi [82–84]. However no comparative evidence exists for or against the use of thrombolytics in DCD pancreas retrieval, although there is certainly enthusiasm for this approach [83, 85].

4.2.4. Two-layer method

Great focus has remained on improving the quality of pancreas transport to the islet transplant centers, including novel ways to provide oxygen rich media to the graft whilst in cold storage during shipping. In late 1988 Kuroda et al. was the first to report the use of the Two-Layer Method (TLM) for shipping of the pancreas prior to islet cell isolation [86]. The TLM uses a perfluorochemical (PFC) and the organ perfusion fluid; initially Euro-Collins' solution was used but was replaced by UW solution. The benefits of the use of the PFC are due to it being a biologically inert liquid that acts as an oxygen-supplying media. A pancreas preserved using the TLM is theoretically oxygenated through the PFC and substrates are supplied by the UW solution. This allows the pancreas preserved using the TLM to generate adenosine triphosphate during storage, prolonging the preservation time [87]. Strong debate still remains over its benefits, if any, when compared to the use of UW solution during CS [88, 89] and a recent publication of guidelines recommended against the use of the TLM for preservation of the pancreas preceding islet isolation [85].

5. Outcomes

5.1. Whole organ pancreas transplant outcomes

Vascularized pancreas transplantation outcomes have improved considerably over time. Although changes to immunosuppression and post-transplantation care can partly account for this, advances in retrieval surgery and organ preservation, in addition to better donor selection, are significant contributors [90, 91]. When exploring pancreas transplantation outcomes, it is paramount to account for the type of transplant performed, as these are associated with differential graft success and survival rates. More specifically, outcomes must be considered based on whether a simultaneous pancreas-kidney (SPK), pancreas after kidney (PAK) transplant, or pancreas transplant alone (PTA) was performed. An exploration of general pancreas transplantation outcomes is beyond the scope of this chapter, as the focus is on the specific impact of retrieval and preservation practices. Overviews investigating trends and recipient outcomes following pancreas transplantation have been published by others, including Dean et al., and Gruessner et al. [90–94]. In brief, the current expected 5-year graft and patient survival rates for pancreas (SPK) transplantation range from 73 to 82% and 89 to 93%, respectively, in the US, UK, Eurotransplant region, and Australia/New Zealand [91, 94–96]. Outcome differences are seen between SPKs, which have historically provided better results, and PTAs and PAKs, due to important variations in the type(s) of recipients for each transplant type, technical differences in the transplantation procedure, and a differential ability to diagnose and treat rejection episodes [91]. SPK transplantation is by far the most commonly performed type of pancreas transplant but islet cell transplantation has also seen a great increase in acceptance and success.

5.2. Islet cell transplant outcomes

Like its forebear, islet cell transplantation outcomes have improved considerably over time. The most impactful change was seen with advances in immunosuppression, clearly shown by the success of the Edmonton trial [97], one that revolutionized the progress of the cellular transplant. Other factors have also continued to impact the field, including post-transplantation care, advances in retrieval surgery and organ preservation, in addition to better donor selection. In brief, the most recent presentation from the Collaborative Islet transplant registry (CITR), presented the combined world islet cell transplant data where they reported that over 1055 allogeneic islet transplants have now been reported by 50 islet transplantation centers in Australia, Europe, North America, and Asia. Of these cases, islet transplant alone (ITA) was the most frequent procedure (n = 858) followed by islet after kidney (IAK) and simultaneous islet and kidney transplantation (SIK) (n = 197) [98]. More recently, according to outcomes of the Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia, the primary end point of HbA1c < 7.0% was achieved by 87.5% of subjects at 1 year and by 71% at 2 years. The median HbA1c level was 5.6% at both 1 and 2 years. Hypoglycemia awareness was restored, with highly significant improvements in Clarke and HYPO scores ($P > 0.0001$). No study-related deaths or disabilities occurred [99]. This trial clearly demonstrated the significant improvements achieved in the outcomes of islet cell transplantation and its impact on those patients suffering from hypoglycemic unawareness.

5.3. The impact of procurement practices and techniques

Pancreas procurement techniques can significantly impact subsequent transplantation outcomes, and can also prove the difference between organ utilization and discard. In particular, there is ample evidence that factors such as *en bloc* retrieval, retrieval technique and graft handling, type(s) of instruments utilized, and perfusion routes are all important determinants of graft function and transplant-related morbidity. Ensuring that pancreas retrieval is performed by an experienced pancreatic transplant surgeon can significantly minimize such retrieval-related complications and risks [100].

Pancreatic damage during retrieval is not uncommon, and may deem the organ unusable certainly for whole organ transplant. Although the rates are different between centers and of course depends upon the level of training of the surgeons performing the retrievals, a large UK registry analysis showed a greater than 50% rate of surgical damage in retrieved pancreata; furthermore, approximately 10% of grafts were subsequently discarded due to damage sustained at retrieval in this analysis [37]. This was further seen as a significant loss as the grafts were also not utilized for islet cell transplantation due to extended cold ischemic times as a result of ongoing referrals. Within the same series, parenchymal and/or vascular (arterial) damage at procurement contributed to significantly higher rates of subsequent graft loss if the pancreas proceeded to transplantation [37]. In order to minimize surgical retrieval damage it is best to ensure that the staff performing the surgery are at a more senior level, and therefore our unit always sends a senior experienced surgeon to all pancreas retrieval surgeries to ensure adequate training of junior staff and optimize graft quality.

Graft thrombosis is the most important technical cause of whole organ pancreatic allograft loss. Pancreas retrieval and surgical technique is a significant etiologic factor in the incidence of graft thrombosis [101–104]. Graft pancreatitis, which in itself is a significant risk factor for graft thrombosis, is another potentially catastrophic complication associated with morbidity and graft loss that is partly attributable to retrieval technique [100]. Excessive graft handling and poor retrieval surgical technique, including damage to the inferior pancreaticoduodenal artery, are commonly accepted causes of graft pancreatitis in the recipient. [100] The same contributing factors also have an impact on the organs when they are used for islet cell isolation [9].

En bloc procurement of the liver and pancreas is associated with better recipient outcomes owing to faster organ retrieval and therefore shorter warm ischemia times [58, 100]. Interestingly, in the aforementioned UK registry analysis between 2008 and 2012, although the vast majority of liver and pancreas retrievals were not performed *en bloc*, there was a trend favoring the *en bloc* approach with respect to reduced pancreatic retrieval injury [37].

In situ perfusion routes, in particular the utilization of dual aorto-portal perfusion in preference to aortic-only perfusion, can impact both whole organ and cellular allograft outcomes. Dual perfusion is potentially associated with increased retrieval-related pancreatic injury through a combination of flush injury (increased perfusion volumes), and/or an obstruction of pancreatic portal venous outflow secondary to catheter placement within the inferior or superior mesenteric veins [52, 58]. This ultimately impacts on the pancreas that is retrieved for whole pancreas or cellular transplantation as it can cause a significant increase in edema, and

may be associated with a higher rate of graft pancreatitis in whole organ, and poorer isolation results due to collagenase dilution in the islet isolation process. Importantly, an aortic-only perfusion technique does not seem to compromise hepatic allograft outcomes, especially in the standard criteria DBD donors from which pancreata are usually retrieved, and therefore should be considered by retrieval surgeons in these circumstances especially in centers that retrieve grafts for both whole and cellular transplantation [34, 58].

Furthermore, the specific instrument-type employed for pancreatic dissection is an important determinant of the amount of pancreatic bleeding upon reperfusion in the recipient [46]. We have shown that ultrasonic shear (e.g. Harmonic Scalpel) utilization during pancreas retrieval allows the sealing of peri-pancreatic vessels that are otherwise easily missed, thereby contributing to less bleeding and a reduced blood transfusion requirement after transplantation within the recipient [41].

5.4. The impact of perfusion and preservation fluids

Pancreas preservation by cold storage using University of Wisconsin solution has been the mainstay method used for pancreas transplantation over the past two decades. Other solutions, such as HTK, Celsior, and SCOT 15, struggled to demonstrate any advantage for short-term preservation periods. But the advent of clinical islet transplantation and the larger use of controlled DBD donors have prompted the transplantation community to develop methods for increasing pancreas graft quality while preventing ischemic reperfusion damage especially in the cellular arena. It has been thought that oxygenation by 1- or 2-layer methods during pancreas preservation, as well as the use of perfluorocarbons, may increase islet yield. Based on the former methods, there is a renewed interest in machine perfusion and oxygenation in pancreas preservation for pancreas transplantation and islet cell preparation [105].

A recent systematic review and meta-analysis by our group compared the outcomes of whole organ pancreas transplantation based on the *in situ* perfusion and subsequent preservation fluid utilized (UW, HTK, or Celsior) [81]. Ischemia-reperfusion injury of the pancreas, as reflected by post-operative peak lipase levels, was significantly lower when UW was employed as a perfusion/preservation fluid in comparison to HTK, but there was no significant difference in peak amylase. This pancreatic ischemia-reperfusion injury may translate to lower clinical graft pancreatitis rates when UW is used in comparison to HTK, although this is not a universal finding [106]. No significant disparity was observed in biochemical injury markers or graft pancreatitis rates between UW and Celsior [81].

As discussed above, post-transplantation graft thrombosis is a significant cause of graft loss. Thrombotic graft loss rates do not differ based on whether UW, HTK, or Celsior is used for *in situ* perfusion and preservation of the pancreas [81]. Furthermore, cumulative graft survival after first post-transplantation month does not favor UW over HTK, although a distinct trend favoring UW emerges at the 1-year mark [81, 106, 107]. A US registry analysis provided further evidence for this, showing a significant association between HTK perfusion/preservation and graft loss, in comparison to UW [108]. In comparison, the use of Celsior is associated with similar 1-year graft survival rates to UW [64, 76].

The comparative utility of each preservation fluid must also be considered in the context of additional donor and transplant-related factors. One important consideration when considering any possible superior preservation effects of UW is the expected pancreatic graft cold ischemic time (CIT). UW may especially be beneficial when CIT is greater than or equal to 12 hours [106, 108]. Furthermore, as already mentioned previously, pancreas retrieval is usually undertaken in the multi-organ donor setting. The perfusion/preservation fluid utilized must therefore not compromise any abdominal organ additionally procured, especially the liver. There is conflicting evidence regarding the relative efficacy of UW, HTK, Celsior, and IGL-1 for liver preservation. Some authors suggest that HTK results in inferior graft survival in comparison to UW, whilst others have reported similar survival but a reduction in post-liver transplantation biliary strictures when HTK is utilized [109, 110]. Overall, current cumulative evidence does not suggest a significant difference between these four fluids, and further research in this area is required [34].

5.5. Donation after circulatory death (DCD) vs. donation after brain death (DBD) transplantation and the importance of donor selection

With careful selection of donors, excellent whole organ pancreatic transplantation outcomes can be obtained even after DCD transplantation. The Pancreas Donor Risk Index (PDRI) is a tool that incorporates donor and preservation-related risk factors, including DCD donors, prolonged preservation time, and high body mass index (BMI), in a risk model for subsequent graft failure [111]. This model has been utilized in both the North American and European settings [111, 112]. It is important to note however that such models must not be used in isolation, and donor pancreata with one or more risk factors, including DCD donors, can still be used to achieve good outcomes. Indeed, our center's first DCD pancreas transplant was in 2007, and has been followed by a further six DCD pancreas transplants, all displaying good long-term graft function [84, 113]. Meta-analyses have shown equivalent graft and patient survival when comparing DBD and DCD pancreatic transplantation, although graft thrombosis rates are higher when DCD grafts are used [51, 114]. Importantly, this higher graft thrombosis rate can be abrogated when donor therapies such as systemic ante-mortem heparin administration are applied [51]. The use of younger donors, with a lower BMI, and low warm ischemic times, has contributed to the success of DCD whole organ pancreas transplantation [84, 115].

There has, however, been more reserved interest in DCD in pancreas for cellular transplantation as the perceived ischemic insult appears to have a much greater effect on the isolated islets for cellular transplantation than when the whole pancreas is transplanted. This is largely because the entire reserve of islets remains intact in the whole organ graft rather than being removed, and a smaller proportion is transplanted in the cellular graft [66, 99]. However, a number of encouraging studies have shown varying success. Albeit from a more advantageous DCD setting allowing earlier intervention including cannulation of the donor and antemortem heparin administration, which has, been shown to be a distinct advantage in this setting [51]. One such report from the Japanese Islet Registry reported their findings from 65 DCD islet isolations performed for 34 transplantations in 18 patients with T1DM. Despite

the fact that all recipients remained free of severe hypoglycemia, only three patients achieved insulin independence for 14, 79, and 215 days. HbA1c levels and requirement of exogenous insulin were significantly improved in all patients [116]. In the more traditional DCD setting the Edmonton group have recently reported their findings comparing islet isolations from 15 DCD and 418 DBD donors performed between September 2008 and September 2014. Compared to DBD, pancreata from DCD were procured locally and donors required less vasopressive support ($P < 0.001$ and $P = 0.023$, respectively), but the other variables were similar between groups. The metabolic function was similar between DBD and DCD, as well as the mean decrease in insulin requirement at 1-month post-transplantation (DBD: 64.82%; DCD: 60.17% reduction, $P = 0.517$). These results support the broader use of DCD pancreata for islet isolation. However, a much larger DCD islet experience will be required to truly determine non-inferiority of both short and long-term outcomes [117].

6. Future perspectives

There has been considerable interest regarding the utility and advantages of dynamic preservation methods in comparison to CS alone for organs such as the liver, kidneys, heart, and lungs. The pancreas has not remained immune to attempts adapting such techniques during the post-procurement phase, although their current clinical success remains limited. Non-static methods of preservation can potentially:

- Reduce graft discard by allowing more accurate graft assessment after retrieval in comparison to current methods, which are largely subjective; and
- Improve organ quality by reducing ischemia-reperfusion-related damage, including by the targeted delivery of pharmacotherapies aimed against ischemia-reperfusion injury, and also gene therapies and stem cells, into the pancreas.

6.1. Machine perfusion

Machine (*ex vivo*) perfusion (MP) entails cannulation and mechanical perfusion of the pancreas via its inflow vessels; perfusion fluid is re-circulated through the circuit for the duration of perfusion. Broadly, MP can be hypothermic, subnormothermic or normothermic, pulsatile or non-pulsatile, and continuous or for a limited proportion of the preservation/transport phase (e.g. pre-implantation). Current pancreatic MP work is lacking in the sphere of clinical transplantation, and is limited to pre-clinical animal and discarded human pancreas studies; only the latter will be the focus of this section, with experimental animal work summarized in detail elsewhere [118–120].

There are certain pancreas-specific issues that need to be considered with respect to MP that do not apply to other organs such as the kidney. Most importantly, the pancreas is a low-flow organ, and even relatively low pressures in a MP setup can result in significant graft edema and weight gain [121]. Furthermore, higher perfusion pressures can contribute to vascular thrombosis secondary to endothelial damage [120]. However, especially if MP is undertaken

at normal body temperature (normothermic), such risks must then be balanced against the need for adequate perfusion to sustain normal aerobic metabolism. An additional challenge during pancreatic MP is the need to adequately and safely account for the organ's exocrine output, which is stimulated during normothermic perfusion [122].

As a result of these issues, most pancreatic MP studies have been conducted in the field of islet cell transplantation rather than the whole pancreas [120, 123]. Graft edema, is disadvantageous for both whole organ and cellular transplantation. However some groups have studied its use as it theoretically facilitates the enzymatic digestion of pancreatic acinar tissue [124]. Hypothermic MP can potentially be employed to increase human islet yield, viability, and insulin secretion despite an extended CIT (> 12 hours), possibly increasing the number of pancreata that can be used for successful islet isolation [125]. Cases of human islet transplantation following MP are yet to be published, however. Whole organ pancreas MP has been investigated in the context of extended criteria organs that were not utilized for human transplantation. Some authors have shown 6 hours of oxygenated hypothermic MP using UW machine perfusion solution increases the ATP content of DCD pancreata to reach a level that is similar to DBD pancreata at baseline [126]. Graft edema can be kept to a minimum if low pressure hypothermic MP is utilized, even for as long as 24 hours [127]. Subsequent *ex vivo* normothermic perfusion can be used to simulate reperfusion at transplantation after initial hypothermic MP, and has been shown to demonstrate adequate insulin secretion by such pancreata [128].

Normothermic MP is an attractive alternative for whole pancreas preservation, and likely provides better graft viability assessment than hypothermic perfusion. Both endocrine and exocrine graft function can be assessed during perfusion by measuring C-peptide and/or insulin secretion and stimulation in response to glucose, and amylase and lipase release, respectively [122, 129]. Blood flow and resistance parameters can also be assessed using this technique, although this is also possible with hypothermic MP. However it is important to note that no defined cut-offs or validated protocols for human transplantation have been developed, and will require significantly more pre-clinical work.

6.2. Persufflation

Persufflation is a technique in which the pancreas is directly perfused with a humidified gas such as oxygen via the SMA and/or splenic arteries. Non-utilized human DBD pancreata have been perfused by this method, and subsequent graft assessment showed an increase in pancreatic ATP levels [130]. Porcine data from the same group showed significantly improved pancreatic histology after 24 hours of persufflation in comparison to utilization of the TLM [131]. Islet isolation after 24 hours of persufflation, including in human pancreata, is likely increased, compared to other methods such as the TLM [132]. This was confirmed in a later study, whereby islets of sufficient quantity and quality for transplantation were isolated from all five human pancreata that underwent persufflation using 40% humidified oxygen perfused at 10–25 mmHg [133]. Similar to MP however, pancreas persufflation has not yet been followed by clinical islet and/or whole organ pancreas transplantation although some research is now underway by a limited number of groups.

6.3. Normothermic regional perfusion

Normothermic Regional Perfusion (NRP) of the abdomen was initially utilized in Spain in the uncontrolled DCD setting, and has since been utilized in the controlled DCD setting in other European countries and Asia [134–138]. The donor's systemic arterial and venous systems are rapidly cannulated, and an *ex vivo* pump/oxygenator system is used to maintain an effective artificial circulation of the abdominal viscera. Cerebral and thoracic perfusion is avoided by clamping the supra-celiac aorta. This system reduces the organ's warm ischemic insult, and proposed benefits include facilitation of a more effective subsequent *in situ* cold flush, ATP replenishment, and reduced oxidative stress [139]. Current experience for NRP exists mainly in the sphere of kidney and liver transplantation. However, utilization of this technique for DCD pancreas preservation and transplantation is appealing, especially because DCD pancreata can have sustained, long-term graft function (as discussed above). Within the UK, five pancreata have been procured after initial NRP, resulting in two SPK transplants and one islet cell transplantation [136]. In Spain, one NRP pancreas has been transplanted in the context of a controlled DCD donor [140]. Future studies are required to more effectively classify evidence for this strategy, and define its comparative role or efficacy with respect to MP. In the DCD setting, NRP may prove to be a more feasible strategy than MP owing to the aforementioned difficulties of maintaining a pancreas on an *ex vivo* machine circuit, although no direct comparisons exist between the two methods.

7. Conclusions

This chapter outlines the numerous advances that have occurred over the past few decades in pancreas retrieval techniques for both whole organ and cellular transplantation. It clearly demonstrates the improved outcomes in both whole pancreas and islet cell transplantation from significant improvements to organ donor selection and management, and organ perfusion and retrieval surgery. We have seen insulin independence rates for more than 10 years post-transplant in both settings with minimal complications. Whole organ transplantation is obviously now a well-accepted clinical therapy for many patients worldwide. However, islet transplantation still has limited application to the broader population of patients with T1D due to its reliance on the availability of cadaveric donors and selection, isolation results and transplant engraftment, the side effects of immunosuppression and issues associated with the requirement for lifelong immunosuppression. The future holds many interesting potential new therapies that may or may not yield appropriate and safe methods for treatment of type 1 diabetes. From what has been outlined in this chapter we can see that outcomes for patients have improved significantly. If, unfortunately, patients cannot be treated prior to the advent of their type 1 diabetes then they can still be treated by transplantation. Moving forward, researchers and clinicians have numerous fronts and multiple strategies arising at different stages of development in which to be able to offer patients treatments tailored to them and their disease. In the foreseeable future, transplantation and in particular the focus on organ retrieval and organ preservation will play a significant role in further improving outcomes, particularly with newer technologies such as machine perfusion and normothermic regional perfusion. Such technologies are hoped to increase both the

number of suitable whole pancreata, as well as their quality, which will simultaneously lead to improved islet cell numbers and function in the cell therapy sphere of Diabetes care.

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Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

BMI	body mass index
CS	Celsior
CIT	cold ischemic time
DBD	donation after brain death
DCD	donation after circulatory death
PFC	perfluorochemical
T1D	type 1 diabetes
UW solution	University of Wisconsin
SPK	simultaneous pancreas and kidney
TLM	two-layer method
T1D	type 1 diabetes

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Chapter 10

Organ Donation Course in Medical Education Program

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Additional information is available at the end of the chapter

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Abstract

The number of patients waiting for organ transplantation is increasing. Today, living donors and cadaveric donors comprise the donor pool. Although it varies from country to country, organ transplants are mostly based on living donors because the cadaveric donor pool is not enough. There are different alternatives to increase the cadaveric donor pool. One of them is to raise awareness of organ donation in undergraduate medical education. Unfortunately, its effectiveness is controversial. In this section, to increase the effectiveness of the organ donation course given in medical education programs, a method proposal is presented. Medical professionals' knowledge of and attitudes toward donation have an impact on donation rates. It is possible that these attitudes and knowledge are molded during pre-graduation. As such, educating medical students may be an important factor in increasing organ donation. Learners' participation in an educational program is one of the most important factors contributing to learning. Flipped classroom is a student-oriented education method based on the combination of in- and out-of-class activities. With the use of the flipped classroom method in organ donation courses offered in medical education programs, students' knowledge and skills that enable them to discuss the topic of donation with patients can be improved.

Keywords: organ donation, medical education, flipped classroom

1. Introduction

The number of patients waiting for organ transplantation is increasing. Today, living donors and cadaveric donors comprise the donor pool. Although it varies from country to country, organ transplants are mostly based on living donors because the cadaveric donor pool is not enough. There are different alternatives to increase the cadaveric donor pool.

One of them is to raise awareness of organ donation in undergraduate medical education. Unfortunately, its effectiveness is controversial. In this section, to increase the effectiveness of the organ donation course given in medical education programs, a method proposal is presented.

Medical professionals' knowledge of and attitudes toward donation have an impact on donation rates. It is possible that these attitudes and knowledge are molded during pre-graduation. As such, educating medical students may be an important factor in increasing organ donation [1, 2]. Learners' participation in an educational program is one of the most important factors contributing to learning. Today's learner has a good command of digital technology, accesses information easily, and can easily adapt to changing learning styles and needs, and thus they have distinct differences from previous generations in thinking, and processing of information, which necessitates the use of alternative education methods. Flipped classroom (FC) is a student-oriented education method based on the combination of in- and out-of-class activities. With the use of the flipped classroom education method in organ donation courses offered in medical education programs, students' knowledge and skills that enable them to discuss the topic of donation with patients can be improved.

2. What is the flipped class model?

Flipped class model is a model which emerged following transformation of the blended learning as a result of the changes that have taken place in both technology and the notion of education [3]. The flipped learning approach describes a learning process, which progresses counter-wise the teacher-centered and traditional approach that is confined to within the four walls. In the traditional teaching approach, conveyance of a topic is realized through a teacher-centered approach in a classroom setting, while digestion of the topic takes place out of the classroom by the learner and through homework-like practices. Flipped learning approach, on the other hand, is a model of learning that proceeds in the opposite direction of the traditional teaching process, in which the learners watch pre-prepared video lessons before the actual class usually at home, and digest the topic in the classroom through several activities [4]. As an educational technique, the flipped class model is not a novel idea but has gained a reputation with the recent technological advances and the increasing access to computers and mobile devices regardless of time and space.

Flipped class model is defined as "a blended learning model that combines the traditional face-to-face teaching and online components." The learning models blended by Staker and Horn [5] are divided into four groups as the rotation model, flex model, self-blend model, and enriched virtual model. Within this classification, flipped class model falls under the rotation model category. The taxonomy developed by Staker and Horn [5] is presented in **Figure 1** [5].

Flipped class model can be described as an innovative model facilitated by the advances in technology. The objective of this model is to offer learning opportunities independently from time, space, and means and to create settings of active learning where interaction is prioritized. Flipped class model encompasses utilization of all sorts of Internet technologies to enforce

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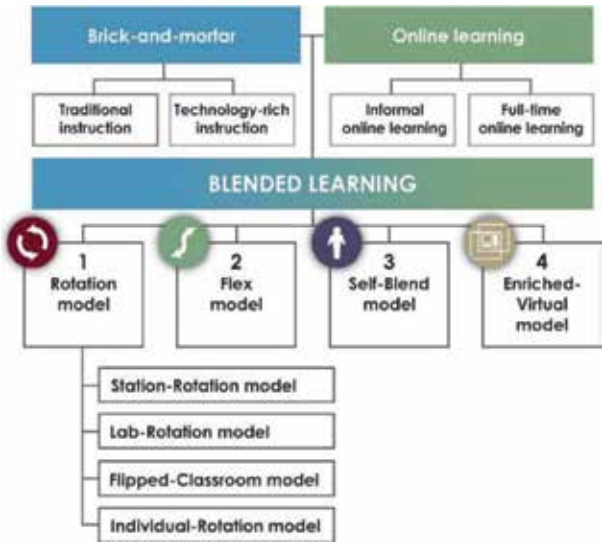

Figure 1. Blended learning taxonomy. Source: Staker and Horn [5].

in-class learnings, and thus the educator spares more time to interact with the student rather than delivering the lecture. In traditional educational settings, students devote their class time to listening to the lecture and, if time remains, practices about the information they have just learned [4, 6, 7]. In this model, on the other hand, the traditional classroom paradigm is reversed to enable students to learn the concepts of the lecture outside the classroom through online educational tools such as video, film, and audio materials. Thus, in-class time is devoted to discussions, problem-solving, and practical training [3, 4, 8, 9].

The activities covered in and out of the classroom for students and instructors in traditional education and flipped class models are presented in **Figure 2** [10].

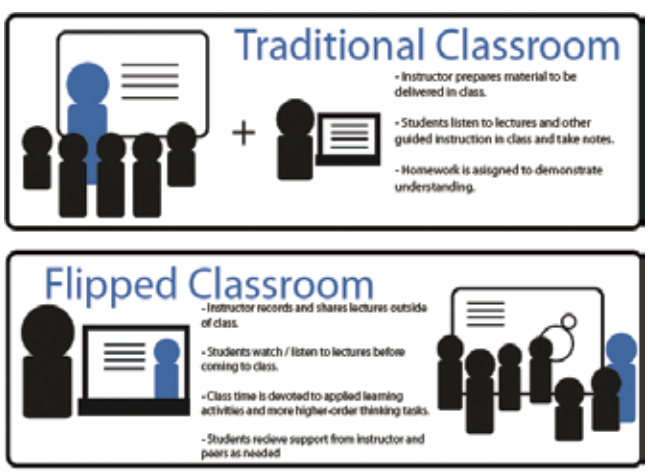

Figure 2. Features of traditional education and flipped classroom. Source: Saint Louis University [10].

As can be seen in **Figure 2**, the flipped class model, which embodies the combination of active education models that require face-to-face communication with educator-centered methods that make less limited use of technology as well as varied opportunities offered through activities in and out of the classroom, will perhaps help us to access today's learners more easily.

The flipped class model involves the students in the learning process and combines the benefits of direct delivery of a lecture used in the traditional learning method with those of active learning. Hence, flipping the classes is being supported as a teaching method that will enable usage of the time to be devoted for delivering the lecture in the classroom much more efficiently through the use of currently available technologies which facilitate access to information. Thus, active learning methods that can be used within the classroom offer the opportunity for teachers to act as a guide while assisting the students to advance their skills such as peer communication, collaborative learning, and taking on their own learning responsibility, thus improving active learning activity. The skill of taking on one's own learning responsibility is reinforced by coming to the class prepared and assuming active roles in in-class activities [11–13]. This method offers advantages to the instructor as well, particularly enabling them to understand and correct the thinking/reasoning errors of the learner and achieve the learning goals with more interactive methods instead of delivering a lesson in the classroom [11, 14]. Besides, although it takes time to prepare, the flipped class model allows the teachers to actualize transformative experiences for their students, build flexible teaching strategies, and make the lessons appealing [15].

The underlying idea of the flipped class model is to devote in-class time to active learning. This is a model that encourages the use of technology in the classroom as well as at home (e.g., video records) for active learning. With the flipped class model, most of the time spent in the classroom is used for communication in comparison to the traditional teaching [16]. This model is student centered. Each student has the responsibility to understand the material at a basic level before coming to the class, and this enables the student to take interest and participate in classroom discussions [17, 18]. Knowledge acquisition takes place at the own pace and guidance of the student; when and how the student will view the content is at their own control. The instructor provides guidance on the content, interactive practices, and creative thinking to facilitate learning, ensures deepening the information, and offers feedback. By offering materials of varying formats, the instructor also makes sure that learners with different learning styles are addressed to [18–20].

The features of the flipped class model are described as follows, emphasizing the word "flip" as an acronym [21]:

- Flexible Environment: It describes a learning setting that allows for the flexibility for students to choose when and where they will learn, which allows the teacher to organize the learning setting in different ways to be personal or cooperative.

- Learning Culture: It is a student-centered approach as opposed the teacher-centered traditional model. Means for profound and creative learning are offered during in-class time. Involved in their own learning processes and evaluating their own learnings, students participate actively to the structuring of information.

- Intentional Content: Educators focus on helping the students improve their conceptual understanding. Educators decide what they will teach and what materials the students will study themselves.
- Professional Educator: The role of educators is more important in the flipped class model. In this model, educators must continuously observe the students, offer instantaneous feedback, and assess the students. Although educators have a less visible role in the classroom in this model, they are the required element for the flipped class model to develop.

With the growing interest toward the flipped class model, Bergmann et al. describe that the model leads to misunderstandings by the practitioners of it. According to the authors, flipped class practices [22]

- are not synonymous with online videos. In this model, interactions emerging during face-to-face time and meaningful learnings are more important
- are not online lessons
- are not replacement of instructors and teaching by videos
- do not mean unplanned, non-programmed, and disorganized work by students
- do not mean that students will spend the whole lesson time looking at a computer screen
- do not mean that students will study alone

3. The advantages and disadvantages of the flipped class model

Flipped class model seems advantageous, in that it encourages single-handed learning, new ideas arise in platforms of discussion, individuals come prepared to the subject, allows flexibility in watching videos, helps understanding of the subject, contributes to pre-learning, motivates learning, and offers the opportunity for the individuals in accordance with their characteristics. Besides, the flipped class model increases student's commitment to the lesson, strengthens team skills, provides individualized student guidance, focuses on class discussions and offers freedom of teaching [23, 24].

On the other hand, the flipped class model appears disadvantageous because of problems with Internet access, students coming to the classroom without necessary pre-work, some students resisting to new applications, and lack of simultaneous feedback [25].

Besides, Bergmann and Sams [4] described two major problems that may be experienced in the flipped class model as the lack of means to check whether the students have watched the video and the vagueness of what the students who have not watched the video prior to the lesson will do in the classroom, and offered the following suggestions for the problems [4]:

- to verify whether the students have watched the video, having the students log in to the page with a username and password, or come to the classroom with questions on the video prepared beforehand.

- for the students who have not watched the video, having them watch it on a computer kept at the classroom. Thus, the students will understand the importance of participating in classroom activities.

4. Theoretical bases of the flipped classroom practice

The flipped class model regards education as a lifelong process. This model is a learning approach that is based on the pragmatist philosophy. Pragmatist approach appraises accuracy or reality only by looking at the consequences of the action and emphasizes benefit. All knowledge and theories are used to make life easier. In this sense, the value of a knowledge or thought is associated with its being beneficial. Therefore, outcomes, or in other words implementations rather than theory, stand out. The student is thus carried to the center of education. The reflection of pragmatism to education manifests as progressivism and reconstructivism advocating the concepts of change, experiences derived by the individual, and learning responsibility.

Progressivism, in reaction to the oppressive and conservative approach of traditional education, considers the essence of truth as change and freedom. In this understanding, training that is intertwined with the evolving life should naturally be progressive. Progressivism places students, who develop themselves and learn to learn through personal experiences, at the center of education. It advocates a teaching approach which preserves the core of change and aim at exhibiting democratic behavior [26, 27].

On the other hand, reconstructivism considers education as a means to achieve a more modern society. The society must renew and reshape constantly. Potential detrimental effects originating from changes would otherwise be unavoidable. Because change is inevitable. In this understanding, the role of actualizing change is entrusted to students who are described as social engineers. Schools should therefore raise social engineers who are able use the fundamental dynamics of change, that is, science and technology. Reconstructivists object to teaching traditional subjects at schools. Methods to solve ever-changing problems should be taught instead. Because only thus can it respond to the requirements of modern life. What is being tried to convey here with the modern life concept is, according to reconstructivists, is group life. From this perspective, school is a special social institution established to prepare students to group living [27].

Contemporary philosophical approaches developed later entrusted schools with the task to reach more modern societies. It was expected from the schools to perform this task to focus on developing problem-solving and learning to learn skills and be able to transform themselves into life settings that support cooperative learning so that the student may be more active in their lifelong learning processes. Also, an emphasis has emerged to use some models when performing this task. As the first of these models, the flipped class model was proposed and began to be used in these schools.

The main reason to use the flipped class model was to have the students at the center, to create a learning setting which involves activities that target research making, creativity, and problem-solving to transform classrooms into a laboratory or a studio to strip teachers out of their information-radiating roles and students out of their roles as actors who are merely takers of

information and to convert them into individuals by processing and shaping them, shortly, to introduce a "constructivist" consciousness. The pedagogic foundations of the flipped classroom approach are based on the constructivist learning theory. According to this theory, students do not take the information as is during the learning process. On the contrary, students take information as active constructive participants throughout the learning process and the responsibility of learning is solely on the student. The process or restructuring information is accomplished through problem-based learning, simulation and pair-share-like active learning strategies. In a flipped classroom, out-of-classroom learning processes depend entirely on self-controlled learning. In-class learning activities comprise higher-order cognitive activities that utilize active learning techniques including decision-making and problem-solving, which students perform through interaction. Constructivist theory does not deny the role of instructor in the learning process. According to the constructivist theory, the instructor is not the wise man who knows everything on the scene but the person who take sides and collaborates with the student during the learning process. Also in a flipped classroom, the instructor does not deliver a lecture but assumes to role of facilitating the learning process in the classroom [28–33].

In summary, the flipped class model encompasses such concepts as constructivist approach, research-based method, active learning, and student-centered learning [13].

5. Flipped class model and Bloom's taxonomy

In 1956, Benjamin Bloom described the incrementally organized "Cognitive Domain" taxonomy. The main theme of this taxonomy was gradual and hierarchical listing of the things the educators want the students to know (learning targets). Bloom's taxonomy—which consists of remembering, understanding, applying, analyzing, evaluating, and creating stages—progresses from simple, concrete, and easy-to-learn behaviors representing the first step of learning to more complex, abstract, and harder-to-learn behaviors. It was also taken into consideration that each behavior is to be the prerequisite for the other, where they pertain to the same subject. In other words, the first behavior is the precondition of the behavior in the second step. Moreover, the first behavior is included in the behavior in the second step.

In the traditional classroom practice, the educator presents the new information by delivering a lecture. In this process, the students are considered to have reached the first two steps of Bloom's taxonomy, that is, remembering and understanding. After the class, students perform by themselves as homework the exercises of the more complicated higher steps. In the flipped classroom, on the other hand, students carry out the part that involves the relatively easier initial steps, that is listening to the lecture, at home by themselves. Practices for the difficult and complicated higher steps are accomplished through active learning methods accompanied by the educator. The students can thus advance up to higher-order cognitive skill stages in the taxonomy (**Figure 3**) [4, 33–35].

When viewed from the perspective of the reorganized Bloom's taxonomy, learnings at the level of the first to steps of understanding and remembering are accomplished by preparing

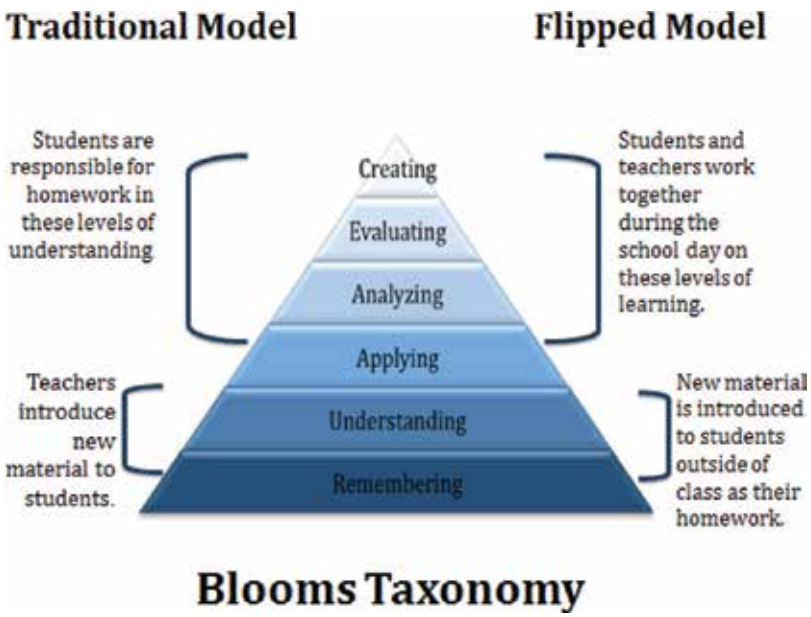

Figure 3. Bloom's taxonomy related to traditional and flipped learning. Source: Ouda and Ahmed [33].

materials at home, while learnings at the levels of applying, analysis, evaluation, and creating are achieved during the active learning processes including in-class practices, discussion, and problem-solving. An important advantage of the flipped class model is that it can incorporate the learning targets of each step in the Bloom's taxonomy.

6. Emergence of the flipped class concept

The conception of traditional teaching describes a process where the educator is deemed as the primary source of information and the action of learning is confined to within school walls and school time. However, the advancing education, technological opportunities, rapidly growing body of knowledge, and changing student profile force learning concepts to change and transform. With the changing educational paradigms, new learning approaches, models, and strategies to enhance the efficacy of learning in learning processes and encourage the learners in this respect are being sought [36–38].

Although referred by a different name, the foundations of the flipped class model were first laid in Miami University by the professors of departments that involve too much reading tasks including law, philosophy, sociology, and psychology because the duration of lectures was not enough to deliver the content [3]. With this model that was first being used in higher education, it was aimed to develop a system that will meet the learning requirements of students with varying styles of learning. Covering different educational resources and appealing to all learning styles, the system was named "Inverted Classroom". When planning the system, Lage et al. [3] first determined the subject and then recorded to videotapes while delivering the determined lecture during the class. The recorded lesson was copied and handed to

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the students. Students who wished so found the opportunity to watch the lecture over and over again and could fill their gaps of the subject. Later, PowerPoint presentations used during the lecture were voiced over and uploaded to web together with complete lecture notes, making them available to students. Getting the printouts of the written resources uploaded to the web, students were able to take the necessary notes on these resources and found the opportunity to undertake a more comprehensive and planned work. Coming to subsequent classes after studying the ready lecture notes they have, students discussed the points they could not understand in the company of their teachers right at the beginning of the lesson and practiced in depth on the subject once the points that could not be understood were eliminated, and engaged in laboratory work where they put their learnings into practice. For all these, a website dedicated for the lecture to access all students and address every learning style was built. The built website was uploaded with materials such as previous exam questions, worksheets, lecture presentations and lecture video records, and were shared with students. Students' questions were answered online in chat rooms created during specific time frames. A virtual library was put together over the established website. This education model was even offered for the appreciation of students and lecturers and a questionnaire and an assessment scale including open-ended questions were applied. While the developed model was received very well by students and lecturers, this first detailed and elaborately planned implementation did not attract the anticipated attention.

The individuals who ensured that the flipped model survived up to the present time and found widespread use were Jonathan Bergmann and Aaron Sams who worked as chemistry teachers in the Woodland Park High School, USA (2007). Bergman and Sams observed that students who could not attend a lesson for various reasons (students taking part in sports games, miscellaneous activities) were unable to fill their learning gaps afterwards. To solve this problem, the duo initially developed a software that could voice over PowerPoint presentations and convert them into a video format and had the students who could not attend a lesson for various reasons watch these videos. The students who could not attend classes learned their missed lessons by these videos. This attracted the attention of other students, and the videos were made available for viewing for all students, regardless of their attendance to the class. By means of the videos, students who missed classes were able to learn the lesson, while those who attended the classes found the opportunity to rehearse and capture the notes they had missed during the lesson. Shortly, these videos were heard of by other schools, students, and teachers who also began to use the videos. With the videos gaining fame in a short span of time, Sams raised the idea of replanning the teaching process. Bergman and Sams [4] asked "When do students really need teachers?" and argued that students needed teachers when they were solving problems at home rather than when the lecture was delivered, and that the students could learn the subject by themselves through videos. Bergman and Sams uploaded other lessons to web in the same way and described students how they should watch videos and take notes before coming to the class. Because when the students arrived at the classroom, the bits that were not understood were now apparent, the parts of the content that were not apprehended were figured out rapidly, and more time was spared for problem-solving and laboratory activities for which students actually needed a teacher [4].

In short, the method of these two teachers has created a great deal of impression and, in 2012, the faculty staff of Northern Colorado University established a database by packing lecture contents

to videos. Later on, Salman Khan, with the math videos he recorded without any commercial purpose, helped the model spread wider and be known as "Flipped Classroom" [39].

The title of the book released by Bergmann and Sams in 2012 was "Flip your classroom; reach every student in every class every day" [4]. As the title suggests, the main objective of the "flipped classroom" method is to make classroom lessons available using technology and to reach all students with different types of learning by making in-class activities more active.

7. Implementing the flipped classroom method

Flipped classrooms is a method in which the learner, viewing the learning topics by means of virtual technologies and videos prior to the classroom lecture and makes preparations and then comes to the classroom, engages in face-to-face interaction with the instructor and peers with the instructor assuming the role of a guide and facilitator. There are two distinct components of the flipped classroom exercise. These are out-of-class and in-class activities. Out-of-class activities is a platform involving technical equipment which aims to form a resource for learning where the learners will access the information they will acquire themselves. In-class activities are the part where interactive methods are used [4, 13, 40–42]. **Figure 4** demonstrates the planning of out-of-class and in-class activities in the flipped classroom exercise.

The cyclical steps in **Figure 5** can be used in structuring lectures in flipped classrooms [42].

Step 1. The content and learning outcomes should be prepared.

- Students should be informed of the flipped classroom method and should be clearly explained what are being expected of them.

Step 2–3. Technical hardware to be used out of and in the class should be prepared, choosing systems that all students can use easily and uninterruptedly (**Table 1**) [40].

- Out-of-class teaching materials suitable for the content and learning outcomes of the chosen lecture should be prepared (**Table 1**). When preparing teaching materials, various resources

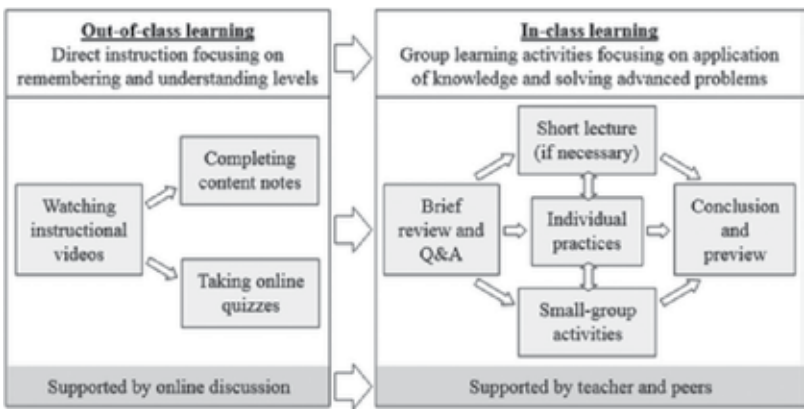

Figure 4. Out-of-class and in-class activities. Source: Lo and Hew [41].

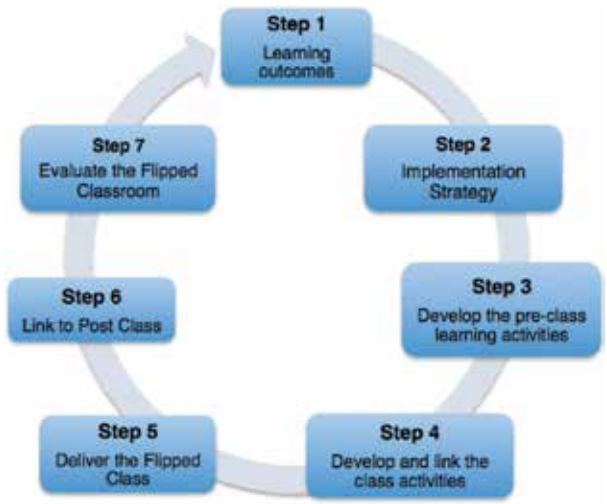

Figure 5. Structuring lectures in flipped classrooms. Source: Karanicolas and Snelling [42].

including lecture notes, research tasks, field work, papers, voiced-over PowerPoint presentations, reports, cases, websites, blog contents, educational games, videoconference recordings, demonstration, and lecture videos can be made use of [43–46].

- For suitable videos, web-sourced open resources may be used, but educators may also make their own videos. Bergmann and Sams [47] recommend that video lessons be prepared by the educator personally [47]. Video lessons prepared by the educator make it possible to initiate student-educator interaction during out-of-classroom activities, resulting in readier acceptance of the model among students [48]. The duration of video lessons is very important. Prolonged video lessons are hard to watch alone, which reduces the success of the exercise, and are also not welcomed by the students [45, 49, 50]. The recommendation is mini video lessons of 20–30 mın in duration [50, 51].

Step 4. In-class activities should be planned. Methods such as discussion sessions, group work, project development work, problem-solving, question-answer exercises, and case discussions can be used in in-class activities. The key is to prefer teaching methods in which knowledge can be used with the highest efficiency. The more in-class activities are supported by the educator, the higher cognitive learning, and reasoning skills will develop [11].

Step 5–6. Flipped class model should be delivered and information on how post-class link will be made should be provided.

Step 7. Assessment and evaluation method should be decided at the very beginning and should be appropriately structured.

- Flipped classrooms possess quite suitable conditions for both formative and summative evaluation [52]. Although studies do not indicate increased success versus the classical system in summative evaluation scales, there is compelling evidence that it improves analytical thinking skills of the learner [4, 6, 13, 32, 49, 50, 53–55]. The assessment and evaluation method should be changed when the model is not improving students' examination success [40]. Also

according to the constructivist theory, assessment and evaluation methods should be changed accordingly after teaching processes in which active teaching strategies are extensively used.

- Formative evaluations are important in monitoring the improvement of the learner and checking the efficiency of the in- and out-of-class activities.

Resource	Features
Externally produced video content	iTunesU: Large online catalogue of free educational content
	TED-Ed: Free videos available which can be customized by educators for use with own students
	Vimeo: Video-sharing website, not restricted to educational content
Presentation software	Microsoft PowerPoint: Most well-known presentation software, slides can be narrated to produce short videos
	Powtoon: Software which allows users to develop colorful animated presentations
Screencasting software	Camtasia: Allows capture and personalization of videos, can be viewed from mobile devices
	ShowMe: Free iPad app that allows educators to create and share whiteboard-style lessons with students
Learning management systems	Moodle: Open source web platform for online learning activities
	Blackboard: Commercially available platform for online learning activities
Other content delivery applications	Edmodo: Virtual learning environment with online discussion and poll facilities
	DropBox: Web-based repository that allows educators and students to share documents and large attachments
	Educlipper: Online social platform that allows educators to collect, store, and share web resources with students
Social networking applications	Twitter: Online social networking platform where "hashtag" categories allow students to hold online, interactive discussions during class
	Wikispaces Classroom: Wiki application which allows students working in small groups to record and playback discussions, either to group or during plenary session
Video-calling and webcast software	Skype: Videoconferencing tool that allows outside speakers to be involved in classroom discussions from a different location
	GoToMeeting: Webcasting tool that allows educators to share screens and interact with an outside speaker
Audience response systems	TurningPoint: Commercially available "clicker"-operated system, allows real-time feedback and polling
Other polling applications	Poll Everywhere: Web-based alternative to clickers. Free for audience size up to 40 people

Source: Moffett [40]

Table 1. Learning resources that can be used in a flipped classroom [40].

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8. What should be paid attention to in a flipped classroom?

It is possible to say that the flipped learning approach contributes to more active involvement of learners in the learning process and help them learn better by increasing their motivation. However, it also appears that very diligent and meticulous work is required in implementing it. There are many points to consider, especially by the educator, in implementing the flipped classroom concept. Foremost among these is the preparation of videos that are included in out-of-class activities. When preparing videos, having an extended video length causes students to get distracted and reduces the viewability of the video, decreasing the success of the practice. Therefore, the recommended duration for the videos was limited as 20–30 min [38, 50, 51]. It may also be useful to add motivating elements to the videos and to add worksheets or questions at the end of the videos to be able to understand whether students have watched the video [45, 47, 51]. Videos prepared by the educator will improve acceptance of the model by the students. This is because flipped classroom exercise should be adopted not only by the educators but also by the students [37]. The practice should be clearly explained to the students for them to adopt the model. Studies report that students find it difficult to internalize the practice when they are not open to change and sometimes because of cultural habits, especially in Asian countries [46, 56]. Students and educators who are equipped with knowledge about their duties and responsibilities surrounding this practice will contribute to successful implementation of it.

Another point to consider is to encourage students to come to the class prepared. For this, in- and out-of-classroom activities should be defined and planned very well and executed according to the plan. Effective use of time and material and the qualities of in- and out-of-classroom activities are the factors that directly affect learning [49, 50]. For example, out-of-classroom activities should be planned so as not to place excessive burden on the student and care should be taken to ensure that materials are comprehensible, especially for difficult subjects. In addition, out-of-classroom activities should be arranged to cover not more than 60 min a day. In in-class activities, on the other hand, approaches should be offered to learners, which allow them to ask questions to improve their deep learning skills, to make associations by following the flow of thoughts, and to make assessments and estimations. Learnings should be supported by proper feedback at proper times and opportunities should be offered for the learner to reflect so that they can comprehend their own learnings. This not only supports learning efficacy but may also be used to formatively evaluate student's knowledge [43–45]. Methods to be used in assessment and evaluation should also be structured to the content and targets of education. Quizzes measuring conventional knowledge should not be maintained when the classrooms are flipped.

9. The place of flipped classrooms in medical education

The future of medical education lies in technology. The cost and efficiency technology allows represent a paradigm shift in how we teach and utilize faculty, space, finances, and other resources [57]. Today's physicians who completed their education in the previous century are

now face to face with new medical students with different ways of thinking and learning. Today's medical students' characteristics are their good command on digital technologies, easy access to information, and changing learning styles and needs, and they exhibit differences in thinking and processing information compared with the generations before them [4, 12]. This makes it imperative to develop alternative teaching-training methods in medical education.

Looking at the medical education literature, examples of implementing the flipped classroom model are seen in the medical faculties in the USA and Central Asian countries. The most radical declaration for the method took place in the Medical Faculty of Stanford University. A model for medical education before graduation based on the flipped classroom philosophy was described by faculty members, Prober and Khan [58]. The two faculty members declared that the current systems were not flexible and did not support individual learning, and that they intended to make the method more common and increase its utilization [56]. There are also attempts to implement the model in Hawaii, as the joint major project of the Accreditation Council for Graduate Medical Education (ACGME) and local medical faculties [59]. Gillous et al. [60] reported that this method was used in the first year of educational programs in medical faculties in France since 2006 [60]. Interestingly, favorable results of the studies performed with the flipped classroom approach in medicine, nursing, and pharmacy domains have been influential in regarding the flipped classroom concept as a pedagogical model.

It can also be seen in the literature that flipped classrooms is a method, which can be used in medical education. Attention of students with intensive course load particularly during the preclinical education period can be grabbed and their motivation can be increased. Also, this method seems to be an opportunity in learning basic mechanisms and integrated pathophysiologic information in the preclinical phase. And in clinical education, flipped classroom can be used in acquiring higher-order cognitive skills such as critical thinking and evidence-based reasoning and application of clinical knowledge including deep learning strategies (approaching patients, initial diagnosis, differential diagnosis, treatment, complementary approach, etc.).

Current evidence suggests that the flipped classroom approach in health professions' education overall yields a statistically significant improvement in learner performance compared with traditional teaching methods. In addition, the flipped classroom would be more effective when instructors use quizzes at the start of each in-class session [61].

In summary, flipped classrooms can be used in medical education in courses and applied training which require conveying the knowledge to a learning level of and above analysis in Bloom's taxonomy. This should therefore be taken into account when determining the content and targets of learning.

10. Using flipped classrooms in organ donation courses

Many organs should function in certain harmony for the human organism to work perfectly. Diseases occur when dysfunction appears in any of these organs. If the dysfunction is left untreated or becomes irreversible, this means that a threat to life has begun to shape, and life expectancy decreases as functional loss progresses. A person at this stage requires a new organ to sustain life. If that organ is the kidney, the person must be confined to dialysis machines for

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the rest of his/her life but if the organ with failure is the heart, lungs, or liver, death is inevitable. Unfortunately, all these patients could lose their lives if donation is not found in good time. One should bear in mind that organ transplantation is the only hope for these patients to survive. Excellent results can be achieved and lives can be saved with organ transplantation, but, unfortunately, people seem not to be sensitive enough regarding the topic of organ donation. When cadaveric organ donation falls short, the frequency of transplantations from living donors increases to prevent life losses. Under these circumstances, individuals with brain death are buried along with their organs, while healthy people are forced to give their organs to their significant other.

There is globally an increasing number of patients whose lives depend solely on organ or tissue transplantation. This increase also escalates the importance of organ and tissue transplantation. Many studies are being performed on this topic, particularly to raise awareness, but the effectiveness of these studies are disputable. People's awareness needs to be raised to increase cadaveric organ transplantations. The major role here belongs to physicians. Raising awareness is only possible by ensuring that physicians or candidate physicians understand the importance of topics such as cadaveric transplantation, donation, and brain death. By including organ donation as a topic in continuing professional development and undergraduation, medical education programs may emphasize the importance of the subject for physicians or candidate physicians. However, the effectiveness of these activities will still be disputable as long as they remain as didactic courses.

The flipped classroom model, which is an active teaching method, can be used in organ donation courses in medical education programs. Learners' participation/contributions in an education program are one of the key factors that maximize learning/teaching. With the use of the flipped classroom education method in organ donation courses offered in medical education programs, students' knowledge and skills that enable them to discuss the topic of donation with patients can be improved. Because when awareness develops, a number of new cognitive schemes occur in an individual's mind. The way individuals react to their experiences may be enriched by increasing the awareness on the thoughts and feelings that drive behaviors.

The objective and learning targets should be identified first when designing organ donation courses using the flipped classroom model in medical education programs. Students should then be informed of the model and be explained in detail what is expected of them. Technical equipment and educational materials that will be used out of the classroom and in the classroom should be prepared. It may be ensured that students understand the topic of organ donation by using voiced over PowerPoint presentations as educational materials. It may also be useful to add videos developed by the educator or presently available videos on organ donation as educational materials. In short, students may be asked to come to class prepared after learning about organ donation topic from videos and taking online quizzes before the actual lesson. During class time, students may be given the opportunity to practice what they have learned by the use of active learning methods as well as case scenarios, discussion sessions, question-answer exercises, project development work, and/or patient simulations. Studies emphasize the importance of in-class activities. Therefore, activities which allow students to practice their pre-class learnings and advance their professional competency should be planned for in-class activities, particularly in clinical case analyses. It should, however, be borne in mind that students not rehearsing their pre-class learnings in the class as part of the

lesson (double course) and absence of a connection between pre-class learnings and in-class practices will result in failure. Testing of the students at the end of the class should aim to evaluate their higher-order cognitive learnings on organ transplantation topic such as practice, analysis, and assessment. Evaluation and assessment activities may utilize case analyses or simulated patient communications.

11. Conclusion

The flipped classroom approach involves problem-solving and cooperative learning as well as hybrid and blended learning activities and focuses on more active involvement of the learner in the learning process. With the flipped learning approach, learners find the opportunity to practice their learnings with both the educator and their peers through activities in the classroom such as discussion and project work and that the learning process continued out of the class as well. The role of the educator in this approach is to ensure that the contents are prepared before the class in a format that can be viewed online, to answer students' questions during the class, to provide feedback, and to encourage them toward active learning.

The flipped classroom model is preferred especially because it supports student-centered approaches and enriches curriculums. For educators, it seems to be important in being a setting in which students may be taught critical and independent thinking and gives them the opportunity to develop their learning strategies throughout their lives.

It is important to state that the flipped classroom cannot be the only form of education. Medicine is still very hands-on, and nothing can replace that experience. Thus, the flipped classroom and case-based instruction cannot be the only form of instruction. However, this model represents a potential future as a means for improved instructional efficiency [57].

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Section 2

Transplantation

Chapter 11

Anesthesia for Liver Transplantation

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Additional information is available at the end of the chapter

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Abstract

Liver transplantation is a high-risk surgery performed on a high-risk patient and is the only treatment for end-stage liver disease. Ever since the first successful liver transplant performed, patient survival increased due to improvement of surgical technique and anaesthetic management as well as the emergence of new generations of immunosuppressants. The pre-anaesthetic evaluation is mandatory and plays an important role in patient inclusion on the transplant list. Liver transplantation is performed under general anaesthesia, and the anaesthetic monitoring is very important for a successful liver transplantation as it can expose problems before irreversible damage occurs. Haemodynamic instability is common during surgery, requiring complex invasive haemodynamic monitoring. Continuous assessment of the patient's volemic status and the amount of perfused fluids represent the key to a successful liver transplantation. Inadequate fluid therapy can lead to pulmonary oedema, abnormal gas exchange, congestion, decrease in perfusion and oedema of the graft. Liver reperfusion takes place in the neohepatic phase and is the most unstable period during liver transplantation, representing a real challenge for the anaesthetist. It can have severe consequences due to a decrease in cardiovascular function with haemodynamic instability, abnormal acid base balance and metabolic abnormalities.

Keywords: liver transplant, cirrhotic cardiomyopathy, postreperfusion syndrome

1. Introduction

Liver transplantation is the only treatment for end-stage liver disease regardless of its aetiology as well as for other categories of liver failure. The procedure is performed on a high-risk patient with impairment of cardiovascular, pulmonary, renal and coagulation systems. Due to increasingly good results, transplant candidates are older and frequently have co-morbidities.

Since the first transplant interventions in the 1960s, postintervention morbidity rate decreased and patient survival increased. This is due to the improvement of surgical technique and anaesthetic management as well as the emergence of new generations of immunosuppressants. Medical care of pre-transplant patients has also experienced a favourable evolution.

The outcome of patients undergoing liver transplantation depends on the perioperative management. Dedicated and specialized teams for liver transplantation have a major role on the outcome of these patients.

2. Preoperative assessment

Liver transplantation is a high-risk surgery performed on a high-risk patient, the cirrhotic patient with end-stage liver disease. The pre-anaesthetic evaluation is mandatory and plays an important role in accepting the inclusion of the patient on the transplant list. Today, patients eligible for transplant are older and often associate co-morbidities.

2.1. Cardiac evaluation

2.1.1. Coronary artery disease (CAD)

The cirrhotic patient has long been considered to be protected from coronary artery disease (CAD) due to his/her haemodynamic profile associated to a low serum cholesterol level. However, recent studies show that CAD has the same prevalence in this group compared to the general population. Patients frequently associate risk factors for CAD such as obesity, diabetes and hypertension.

The incidence of CAD does not seem to be influenced by the aetiology of cirrhosis except for non-alcoholic steatohepatitis (NASH), in which case it is twice as high, NASH associating with the metabolic syndrome [1]. The importance of CAD detection is due to the haemodynamic high stress during liver transplant leading to exacerbation of the cardiac suffering during surgery or generating postoperative cardiac complications [2, 3]. Recognizing CAD is very important, but the best therapeutic approach in case of significant coronary stenosis is not well defined.

Coronary angioplasty may be recommended, but due to the need for heavy anti-aggregation, this may increase the risk of bleeding in the cirrhotic patient. When required, bare metal stents are preferred to pharmacologically active ones because of a shorter period of anti-aggregation. Surgical intervention for myocardial revascularization is not recommended due to a very high mortality risk in the cirrhotic patient [2, 4].

2.1.2. Cirrhotic cardiomyopathy

Cirrhotic patients have myocardial dysfunction secondary to hepatic impairment [5].

Regardless of the aetiology of cirrhosis, cardiomyopathy is characterized by

- increased cardiac output at baseline,
- impaired contractile reserve in response to stress; this is due to a lower density of beta adrenergic receptors as well as to the negative inotropic effect of excess nitric oxide production,

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- diastolic dysfunction; it is more pronounced in patients with ascites,
- electrophysiological alterations like prolongation of the QT interval [3, 6].

The prolonged QT interval is associated with the severity of liver disease and the degree of portal hypertension as well as mortality [2]. A QTc interval of >440 ms correlates with an increased risk of ventricular arrhythmias [7].

Both diastolic and systolic dysfunction can be causes of postoperative pulmonary oedema. Right heart dysfunction, when present, has a higher predictive value for postoperative cardiac complications.

2.1.3. Other causes for cardiac impairment

Chronic consumption of ethanol may cause dilated cardiomyopathy; it is characterized by left ventricular dilatation with altered systolic function. In the initial stages of heart disease, abstinence from alcohol can significantly improve the symptoms.

In the case of haemochromatosis, excess iron will be deposited in the myocardium, leading to restrictive cardiomyopathy [2].

2.1.4. Steps of cardiac evaluation

Investigating a patient will begin with the existence of a history of heart disease as well as symptoms suggestive of cardiovascular events such as rhythm disorders or angina. Because of their low exercise capacity due to cirrhosis, the incidence of angina is low and frequently coronary heart disease can be underestimated.

The baseline assessment includes an electrocardiographic (ECG) recording at rest and an echocardiography.

The echocardiography evaluates the following [8]:

- measurements of cardiac chambers
- valvular characteristics and function
- evaluation of systolic function of the left ventricle (LV) expressed as ejection fraction; we must not forget that these values are obtained under the conditions of low blood pressure due to the vasodilatation of the cirrhotic patient, so they can underestimate the real ejection fraction
- evaluation of diastolic function of the left ventricle by measuring early and late diastolic velocities through the mitral valve to determine the E/A ratio (ventricular filling phases = initial E vs. tardive A); E/A < 1 reflects a diastolic dysfunction; diastolic dysfunction is more pronounced in patients with ascites [6]

If electrocardiogram and echocardiography are two mandatory pre-transplant investigations, the question is what other methods should be used in those patients who require additional methods.

According to AASLD (American Association for the Study of Liver Disease), AHA (American Heart Association) and ACCF (American College of Cardiology Foundation), additional

investigations should be undertaken for patients who have three or more associated risk factors of the following: diabetes, left ventricular hypertrophy, history of CAD, age > 60 years, smoking, hypertension, dyslipidaemia and obesity [4, 9].

Several types of investigations have been proposed:

- stress echocardiography using dobutamine, adenosine or dipyridamole,
- myocardial perfusion scintigraphy,
- computerized cardiac tomography which allows calculating the calcium score; this is a rapid and non-invasive way to measure calcium deposits in the coronary vessel wall as an expression of coronary stenosis; results are measured in Ca scores: a Ca of >100 score carries a moderate risk of cardiac events and a score of >400 a high risk [10].

Valvular dysfunctions in the cirrhotic patient on the transplant list are poorly studied. The evaluation of such a patient should include the severity of the valve dysfunction, either stenosis or regurgitation, the degree of alteration of myocardial contractility and the clinical presence of signs of insufficient cardiac output. Several cases of simultaneous liver transplantation and aortic valve replacement for tight aortic stenosis were reported in the cirrhotic patient [6].

2.2. Pulmonary system

Chronic liver disease can affect both the pleural space and the pulmonary parenchyma. The two pulmonary conditions characteristic of the cirrhotic patient are hepatopulmonary syndrome and pulmonary hypertension. The two syndromes exclude each other, and their pathophysiology depends on predominant vasodilator or vasoconstrictor elements resulting from liver dysfunction.

Portopulmonary hypertension is a pulmonary hypertension syndrome with vascular obstruction, coexisting with portal hypertension. The portopulmonary syndrome has important haemodynamic consequences with minor changes in blood gases.

All patients proposed for transplantation should be screened for portopulmonary hypertension as the postoperative evolution depends on it. It might be suspected in the case of a right branch block on ECG. Echocardiography can detect pulmonary hypertension and can evaluate it, but correct values are obtained by right heart catheterization. Depending on the mean pressure in the pulmonary artery (PAPm), hypertension is classified in mild (PAPm 25–34 mmHg), moderate (PAPm 35–44 mmHg) and severe (PAPm >45 mmHg).

Severe pulmonary hypertension excludes the patient from liver transplantation; moderate form may benefit from vasodilator drug treatment in pre-transplant [4]. Decision to perform liver transplant in this case depends on the response to therapy and is taken by the transplant team on an individual basis.

Hepatopulmonary syndrome is characterized by hypoxemia secondary to intrapulmonary shunt due to vascular dilation. In contrast with pulmonary hypertension that may be

aggravated by surgery for liver transplant, the hepatopulmonary syndrome's evolution is extremely favourable post transplantation with net amelioration or even complete resolution.

The cirrhotic patient might develop **pleural effusions**. The hydrothorax appears most frequently on the right side and generally accompanies ascites. It is due to an anatomical defect in the right hemidiaphragm. Sometimes, it might need drainage prior to surgery [11–13].

The cirrhotic patient may suffer from any other pulmonary disease not related to the chronic liver failure. Chronic obstructive pulmonary disease may be associated with cirrhosis, especially in smoker patients, resulting in obstructive respiratory insufficiency, which together with restrictive ascites dysfunction may greatly compromise respiratory function. Patients will be evaluated by a pneumologist for treatment and encouraged to quit smoking.

Since the necessary immunosuppression after transplantation may lead to reactivation of a dormant latent tuberculosis, it is mandatory to test transplant candidates for latent tuberculosis. This is done either with the tuberculin skin test or with the quantiferon TB test, a cell immune response assay using a *Mycobacterium tuberculosis*-like protein substrate.

Depending on the lung's status, investigations will be limited to a chest X-ray or will go further to pulmonary ultrasound or computed tomography (CT) scan of the thorax [14, 15].

2.3. Evaluation of renal function

Renal dysfunction in the cirrhotic patient is due to a decreased blood volume due to vasodilation, with a decrease in glomerular filtration. It may not be reflected correctly in serum creatinine levels; the end-stage liver disease patient often has a less muscle mass and a low creatinine production. Higher sensitivity tests are cystatin C or NGAL (neutrophil gelatinase-associated lipocalin).

Two types of kidney dysfunction are related to cirrhosis: hepatorenal syndrome is type 1 with rapid deterioration in renal function (doubling serum creatinine or increasing it to >2.5 mg/dl in less than 2 weeks) and a type 2 with slower evolution.

The occurrence of renal dysfunction can be precipitated by haemorrhage and infection [4, 16]. Preoperative renal dysfunction increases the risk of adverse development of postoperative complications.

2.4. Coagulation status

Coagulation abnormalities are caused by reduced concentrations of vitamin K-dependent factors and an imbalance between procoagulant and anticoagulant factors. Standard coagulation tests do not reflect rebalanced haemostasis and must not be used to predict the risk of bleeding. Procoagulant factors must not be administered unless signs of bleeding are present [17].

The Guidelines of the European Society of Anaesthesia regarding cirrhotic patients do not recommend routine preoperative correction of international normalized ratio (INR) (1.5–5) using fresh-frozen plasma but advise correction through point of care tests: rotational thromboelastometry or thromboelastography [18].

3. Conduct of anaesthesia

Despite recent advances, liver transplantation remains a major challenge to the anaesthetist due to the important cardiovascular changes throughout surgery: important changes in preload, afterload, arrhythmias, hypotension and hyperkalaemic events. All these increase the chances of severe cardiac dysfunction.

Liver transplantation is characterized by haemodynamic instability and various complications that can arise throughout the three important surgical phases: preanhepatic, anhepatic and neohepatic.

- Preanhepatic: dissection and liver mobilization takes place; usually massive bleeding can occur that can lead to hypovolaemia and haemorrhagic shock.
- Anhepatic: between clamping hepatic inflow and before graft reperfusion; consists of clamping of the inferior vena cava (IVC); significant decrease in cardiac output (CO) occurs.
- Neohepatic: characterized by liver reperfusion, reappearance of flow in the vena cava and vena porta, blood volume goes back to normal; can be complicated by reperfusion syndrome or bleeding from vascular anastomosis (hepatic artery, portal vein).

3.1. Patient monitoring during liver transplantation

Liver transplantation is performed under general anaesthesia, and the anaesthetic monitoring plays an important part in a successful liver transplantation as it can expose problems before irreversible damage occurs.

Haemodynamic instability is common during liver transplantation, and that is why the anaesthetic monitoring is complex, being divided into standard monitoring, haemodynamic (invasive and non-invasive), neurologic and neuromuscular monitoring.

3.1.1. Standard monitoring

According to the American Society of Anaesthesia (ASA) protocols, standard monitoring applies to all patients during all types of anaesthesia with the aim of increasing patients' quality of care. Trained personnel must be present in the operating room during the entire surgery, while continuous monitoring of the oxygenation, ventilation, circulation and temperature is mandatory [19].

> **Oxygenation***:* gas monitoring as the level of inspired oxygen (FiO_2) and the level of expired CO_2 ($ETCO_2$ capnography) and blood oxygenation in a continuous form as peripheral capillary oxygen saturation (SpO_2 pulsoxymetry).
>
> **Ventilation***:* chest movements and lung auscultation but also the volume of expired gas and capnography.
>
> **Circulation:** continuous electrocardiogram (ECG) for rhythm, frequency, signs of ischaemia (ST segment), QT interval and measurement of blood pressure in a non-invasive way before induction of anaesthesia.

Temperature: central temperature is the blood temperature which bathes the vital organs (heart and brain). Important sites of measurement are the following: tympanic membrane, nasopharynx, distal oesophagus, blood, urinary bladder and rectum [20].

3.1.2. Haemodynamic monitoring

Patients with chronic liver disease usually present many other systemic complications including portal hypertension, ascites, coagulopathy, hyperdynamic circulatory syndrome (high cardiac index and low systemic vascular resistance) and cirrhotic cardiomyopathy. Due to all these changes, advanced haemodynamic monitoring is necessary. Monitoring parameters and normal values are shown in **Table 1** [21].

3.1.2.1. Invasive blood pressure

Invasive arterial pressure monitoring represents standard practice during liver transplantation. The arterial catheter is usually inserted in the radial artery and is being used for continuous pressure monitoring, blood gas analysis and other blood tests. Due to variations of radial artery pressure which may sometimes underestimate aortic pressure in hypotensive states, when high dose vasopressors are used and after reperfusion of the liver, some anaesthetists prefer using the femoral artery. Despite all these, the mean central and peripheral arterial pressures are usually the same [22]. An important problem when deciding the sites for catheter insertion is the cirrhotic coagulopathy which can lead to important puncture site bleeding. The number and sites of line insertion vary according to the transplant centre and experience.

3.1.2.2. Central venous pressure

Central venous pressure (CVP) monitoring is essential during liver transplantation. Central venous pressure is measured via a central catheter inserted in the superior vena cava system. Maintaining a low CVP in a normovolaemic patient can reduce the risk of bleeding but can increase the risk of vital organ hypoperfusion in case of a hypovolaemic patient or a massive bleeding [23].

3.1.2.3. Cardiac output and pulmonary artery pressures

Since its discovery, the pulmonary artery flotation catheter (Swan Ganz) has been considered the gold standard for cardiac output measurement. Swan Ganz catheters are still used routinely in some transplant centres around the world. A pulmonary artery catheter is mandatory if pulmonary hypertension is diagnosed or suspected.

Pulmonary artery pressures, cardiac output measurements and mixed venous oxygen saturation help in the diagnosis and management of haemodynamic instability during surgery. The new modified Swan Ganz catheters help anaesthetists with continuous cardiac output measurements and right ventricular end-diastolic volume as a more accurate parameter of preload.

Despite the massive use in the last 40 years, its high cost and patient safety have led to question its utility [24].

Parameter	Abbreviation	Formula	Normal value
Median arterial pressure	MAP	SBP+(2 × DBP)/3	75–105 mmHg
Arterial oxygen concentration	CaO_2	(0.0138 × Hb × SaO_2) + 0.003 × PaO_2	16–22 ml/dl
Venous oxygen concentration	CvO_2	(0.0138 × Hb × SvO_2) + 0.003 × PvO_2	15 ml/dl
Peripheral capillary oxygen saturation	SpO_2	Measurement	95–100%
Arteriovenous oxygen difference	$C(a\text{-}v)O_2$	CaO_2-CvO_2	4–6 ml/dl
Stroke volume	SV	CO/HR × 1000	60–100 ml/beat
Stroke volume index	SVI	CI/HR × 1000	33–47 ml/m^2/beat
Cardiac output	CO	HR × SV/1000	4–8 l/min
Cardiac index	CI	CO/BSA	2.5–4 l/min/m^2
Central venous pressure	CVP	Measurement	2–6 mmHg
Central venous oxygen saturation	$ScvO_2$	Measurement	70–80%
Extravascular lung water	EVLW	CO × DSt-0.25GEDV	
Extravascular lung water index	EVLWI	EVLW/PBW	0–7 ml/kgc
Global ejection fraction	GEF	SV × 4/GEDV	>20%
Global end-diastolic volume	GEDV	CO × MTt × f(S1/S2)	
Global end-diastolic volume index	GEDI	CI × MTt × f(S1/S2)	650–800 ml/kgc
Intrathoracic blood volume	ITBV	1.25 × GEDV	
Intrathoracic blood volume index	ITBI	1.25 × GEDI	850–1000 ml/m^2
Left ventricular stroke work	LVSW	SI × MAP×0.0144	8–10 g/m^2/beat
Left ventricular stroke work index	LVSWI	SVI× (MAP-PAOP) × 0.0136	50–62 g/m^2/beat
Mean pulmonary artery pressure	MPAP	PASP+(2 × PADP)/3	9–18 mmHg
Oxygen consumption	VO_2	$C(a\text{-}v)O_2$ × CO × 10	200–250 ml/min
Oxygen delivery	DO_2	CaO_2 × CO × 10	950–1150 ml/min
Pulmonary artery occlusion pressure	PAOP	Measurement	6–12 mmHg
Pulmonary artery systolic pressure	PASP	Measurement	15–30 mmHg
Pulmonary artery diastolic pressure	PADP	Measurement	8–15 mmHg
Pulmonary vascular resistance	PVR	80× (MPAP-PAOP)/CO	<250 dynes/s/cm^5
Pulmonary vascular resistance index	PVRI	80× (MPAP-PAOP)/CI	255–285 dynes/s/cm^5/m^2
Stroke volume variation	SVV	SVmax-SVmin/SVmean×100	10–15%
Systemic vascular resistance	SVR	80× (MAP-RAP)/CO	800–1200 dynes/s/cm^5
Systemic vascular resistance index	SVRI	80× (MAP-RAP)/CI	1970–2390 dynes/sec/cm5/m2

Table 1. Haemodynamic parameters.

3.1.2.4. Other ways of measuring cardiac output

The pulse contour analysis is useful for continuous monitoring of cardiac output and needs frequent calibration via thermodilution technique:

- PiCCO system (Pulse-induced Contour Cardiac Output): this consists of a thermistor catheter placed in the femoral artery and can estimate stroke volume. A central venous catheter inserted in the superior vena cava circulation is needed for the pulmonary calibration which must be done every 8 h in a haemodynamic stable patient and more frequent for unstable patients [25].
- LiDCO: it uses the same algorithm for stroke volume monitoring. Lithium chloride is used for transpulmonary calibration and can be injected into a peripheral vein.
- Transoesophageal echocardiography gives continuous information on ventricular function and volume status and allows immediate diagnosis of air or thrombus embolization. It also provides information regarding contractility, valvular function, pericardial or pleural effusion. Its main advantage is the ease of continuous use during surgery due to the anaesthetized and intubated patient [26].
- Thoracic bioimpedance: it estimates cardiac output and other haemodynamic parameters based on the electric properties of the thorax produced by blood movements during cardiac cycle; rarely used in liver transplantation.

3.1.3. Neurologic monitoring

- Bispectral index: Fourier analysis of a fronto-parietal electroencephalogram (EEG). It varies between 0 (coma) and 10 (normal cortical activity) with an adequate value between 40 and 60 in an anaesthetized patient [27].
- Transcranial Doppler echography can diagnose vasospasm, intracranial hypertension and cerebral death.
- Oxygen saturation in the jugular bulb ($SjvO_2$): continuous monitoring of venous oxygen saturation via a central catheter inserted in the internal jugular vein that shows the balance between oxygen intake and consumption. Values below 50% show ischaemia while values above 75% show cerebral hyperaemia.
- Transcerebral cranial oximetry: resembles $SjvO_2$.
- Electroencephalogram: rarely used for continuous monitoring during surgery; requires a trained anaesthetist who must differentiate pathological changes from normal changes in a cerebral activity during anaesthesia.

3.1.4. Neuromuscular monitoring

Residual neuromuscular block after the use of neuromuscular blockade agents can have a detrimental effect in patients after surgery causing inadequate hypoxic ventilatory response, depressed pharyngeal tonus leading to an increased risk of airway obstruction and death.

Peripheral nerve stimulation and depth of block can be assessed using single twitch, train of four, tetanic stimulation and double burst stimulation.

3.1.5. Other parameters monitored during liver transplantation

Other parameters monitored during liver transplantation include the following:

- hourly diuresis,
- haemoglobin and haematocrit,
- electrolytes,
- base excess and lactate,
- coagulation parameters via rotational thromboelastometry (ROTEM).

3.2. Haemodynamic management during the three phases of liver transplantation

3.2.1. Volemic resuscitation

Ever since the first successful liver transplantation in 1960, the surgery has been associated with significant bleeding and an increased amount of blood products transfused [28]. Blood products used during liver transplantation have declined significantly in the last 20 years. Even if bleeding risk has decreased over time, it still can induce a volemic stress.

Clamping of the inferior vena cava during the second phase (anhepatic) of the liver transplantation leads to an important decrease in preload, CO and arterial pressure which need a quick diagnosis and management. Normal response of right ventricle (RV) and left ventricle (LV) to stress does not take place due to all the substances released from the liver in the anhepatic phase.

Continuous assessment of patient's volemic status and the amount of perfused fluids represent the key to a successful liver transplantation. This can be done using dynamic measurements of CVP and pulmonary capillary wedge pressure (PCWP), but these parameters do not correlate with changes in CO [29]. Another way of assessing fluid responsiveness is the use of stroke volume variation (SVV) and global end-diastolic volume index (GEDI). Inadequate fluid therapy can lead to pulmonary oedema, abnormal gas exchange, congestion, a decrease in perfusion and oedema of the graft [30].

This study does not offer an answer for the ideal monitoring system and guiding of fluid therapy [31]. In our clinic, the guidance of fluid management is done with the pulmonary thermodilution technique and pulse contour wave analysis via the PiCCO system.

Using adequate vasoactive substances, which protect the brain, heart and kidney, led to a greater haemodynamic stability, adequate CO and renal perfusion [31]. The CVP can also be correlated with the severity of the post-reperfusion syndrome [32].

Specific fluid management during liver transplantation can be described according to the three surgical phases:

- Preanhepatic phase: cirrhotic patients usually have variate quantities of ascites; the anaesthetist must try and compensate it in order to reach normovolaemia and avoid hypovolaemia during the next phase (anhepatic) when clamping the inferior vena cava. Albumin of 20 or 5% is used for volemic resuscitation.
- Anhepatic phase: fluid restriction is the best solution in this phase while maintaining adequate arterial pressure with the help of vasopressors. The vasopressor of choice is noradrenaline, and the parameters measured with the PiCCO system can guide us to using inotopes such as dobutamine.
- Neohepatic phase: in this stage, the patient needs adequate volemic resuscitation guided by the PiCCO parameters; an important decrease in vasopressors takes place.

Albumin determines the oncotic pressure that keeps fluids in the intravascular space. Cirrhotic patients usually have low albumin levels [32]. Studies have shown that the use of albumin during liver transplantation decreases the amount of intraoperative fluids used and the frequency of pulmonary oedema in cardiac and noncardiac surgery [33]. It has also been proved that albumin decreases mortality in cirrhotic patients, decreases the incidence of postreperfusion syndrome and the use of vasopressor agents [34].

The use of Hetastarch is not recommended as it affects platelet aggregability and increases the risk of bleeding by decreasing the concentration of coagulation factor 8 [33]. Gelatines can have numerous side effects: anaphylactic reactions, a decrease in thrombin generation and worsening of fibrinolysis which is specific in the anhepatic phase [35].

Manitol can be used in the anhepatic phase before clamping of the IVC (0.5 g/kgc) in order to avoid blood congestion in the liver and intraabdominal organ oedema [36].

PiCCO monitoring has a number of advantages: accurate CO calculations and guiding fluid management. ITBV is an accurate parameter of preload even when IVC is clamped [37]. We can also use SVV in order to predict fluid responsiveness [38].

Fluid management is done using PiCCO parameters determined during the three phases of the liver transplantation. This guidance of fluid therapy decreases the post-anaesthesia care unit stay and mortality [39]. The decisional tree regarding fluid management is presented in **Table 2**.

TOE can also be helpful in high-risk patients with various cardiac problems. It has the advantage of direct assessment of cardiac contractility and assesses response to inotropes and volemic status. It can diagnose embolic complications and cardiac tamponade [40].

The use of different monitoring techniques is the key to successful management of haemodynamic instability and fluid guidance during liver transplantation.

3.2.2. Vasoactive substances and inotropes

There are a variety of substances that can be used when haemodynamic instability takes place during liver transplantation. Noradrenaline is most often used, followed by adrenaline and dobutamine. Indications are shown in **Table 3**.

CI < 3 l/min/m²			
GEDI <700 ml/m² or ITBI <850 ml/m²		GEDI >700 ml/m² or ITBI >850 ml/m²	
ELWI <10	ELWI >10	ELWI <10	ELWI >10
Administer fluids	Administer fluids (limited) Vasopressor	Vasopressor	Vasopressor Fluid restriction
CI > 3 l/min/m²			
GEDI <700 ml/m² or ITBI <850 ml/m²		GEDI >700 ml/m² or ITBI >850 ml/m²	
ELWI <10 ml/m²	ELWI >10 ml/m²	ELWI <10 ml/m²	ELWI >10 ml/m²
Administer fluids	Administer fluids	No measure	Fluid restriction

Table 2. Fluid therapy decisional tree.

3.2.3. Postreperfusion syndrome

Liver reperfusion takes place in the neohepatic phase and is the most unstable period during liver transplantation, representing a real challenge for the anaesthetist. The postreperfusion syndrome is defined as a 30% decrease in the mean arterial pressure that lasts for at least a minute and appears in the first 5 min after the unclamping of the inferior vena cava (IVC) [41].

It can have severe consequences due to a decrease in cardiovascular function with haemodynamic instability, abnormal acid base balance and metabolic abnormalities. Haemodynamic instability is the consequence of severe vasodilation, negative inotropic effects and massive bleeding which may appear during surgery. Graft reperfusion can have fatal consequences such as severe arrhythmias or asystole [42].

The incidence of PRS varies between 5.9 and 60% due to different surgical techniques, haemodynamic intraoperative differences and geographic factors (in some countries, the number of reduced donors led to marginal graft use-expanded criteria donors) [43].

Causes of PRS are not yet clear, but there are several theories. Dyselectrolytemia, cold solutions used for graft preservation and severe vasodilation due to NO release may contribute to PRS. Unclamping of the inferior vena cava leads to vasoactive pro-inflammatory substance released from Kupffer cells and which are the result of the postischaemic graft [44].

Substance	Indications	Dose
Noradrenaline	Arterial hypotension	0.2–1 μg/kgc/min
Adrenaline	Asystole, severe arterial hypotension	0.01–0.5 μg/kgc/min
Dobutamine	Cardiogenic shock	2.5–10 μg/kgc/min
Ephedrine	Arterial hypotension Before unclamping IVC	5–25 mg i.v. bolus every 10 min

Table 3. Main indications of inotropes during liver transplantation.

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One of the most important elements released from the graft is potassium as a result of the portal venous congestion. All these substances can contribute to postoperative ischaemia and reperfusion lesions [45]. Another theory is represented by the pro-inflammatory cytokines (IL 1B, IL 2, IL 8, TNF-α) produced by ischaemia and released during the reperfusion of the graft leading to a marked inflammatory response and cellular death. They also have vasodilation and negative inotropic effect. The amount of pro-inflammatory cytokines released does not correlate with the duration of cold ischaemia time (CIT) [46].

Factors that may predict PRS are the following:

- donor-related: age, obesity, hypernatraemia (>155 mEq), hepatic steatosis, prolonged intensive care unit (ICU),
- intraoperative factors: duration of the surgery,
- cold ischaemia time exceeding 6 h.

Risk factors include

- patient's volemic status before reperfusion,
- myocardial depression (due to cold solutions released in circulation, abnormal acid base status),
- severity of metabolic acidosis.

Studies have shown that patients who had PRS have a higher risk of postoperative renal dysfunction and 15 days of mortality [43].

Postreperfusion syndrome leads to

- a decrease in arterial pressure and SVR,
- a decrease in CO,
- a moderate increase in PAP,
- frequent malignant arrhythmias.

Therapeutic options include the following:

- optimizing volemic status,
- adequate haemodynamic management,
- adequate graft perfusion; vasodilators are rarely used (calcium channel blockers, prostaglandins),
- liver graft wash with flush fluid of albumin 5% before IVC unclamping,
- correction of hypocalcaemia and hyperkalaemia,
- give ephedrine bolus 5 min before unclamping of the IVC in order to obtain a MAP of 85–100 mmHg [47].

3.3. Coagulation management

Cirrhotic patients have been regarded as having a high risk of bleeding due to standard coagulation test abnormalities caused by cirrhotic coagulopathy. Recent studies have shown that bleeding episodes are caused by vascular abnormalities and portal hypertension [48].

Standard coagulation tests (prothrombin time, INR and activated partial thromboplastin time (aPTT)) reveal the deficit of procoagulant factors without showing the status of the anticoagulant factors.

In order to have an optimal view regarding cirrhotic coagulopathy, global coagulation tests (viscoelastic tests) are recommended [49].

Intraoperative blood transfusion has a negative impact on patient outcome. Intraoperative packed red cells and platelet transfusion are independent predictors of 1 year mortality [50].

There are a few measures that can decrease the need of blood transfusion:

- maintaining a low CVP,
- use of antifibrinolytic agents,
- use of recombinant activated factor 7,
- use of point of care tests for transfusion guidance.

The most important coagulation problems that can appear during the three transplant phases are specific for each phase:

- Preanhepatic phase: bleeding occurs due to extensive dissection, collateral circulation and portal hypertension. Maintaining a normal volemic status may lead to dilutional coagulopathy and thrombocytopaenia [51]. A low CVP must be maintained in this phase. Cell saver can also be used after evacuation of ascites and before biliary anastomosis.
- Anhepatic phase: between clamping hepatic inflow and before graft reperfusion. Usually, minimal bleeding takes place here, but the risk exists. Platelets and coagulation factors are low due to loss and consumption from the previous phase. Synthesis and liver clearance do not exist. An increase in the release of tPA from endothelial cells and the absence of hepatic clearance can lead to hyperfibrinolysis and bleeding. Viscoelastic tests are mandatory for the diagnosis and management of hyperfibrinolysis.
- Neohepatic phase: bleeding can occur due to surgical problems or haemostatic abnormalities. Usually, hypothermia, metabolic acidosis and hypocalcaemia must be corrected before any further decision is taken. Platelets are seized in the liver graft after reperfusion causing important thrombocytopaenia. Some platelets are partially activated in the liver graft and released in the circulation as inefficient.

Hyperfibrinolysis can often appear during liver transplantation, but is self-limited as long as the liver graft is viable.

Antifibrinolytic agents can be used when diffuse bleeding occurs or when point of care tests show hyperfibrinolysis. In case of diffuse bleeding, the anaesthetist must correct hypothermia, acidosis, hypocalcaemia, treat hyperfibrinolysis and then administer fibrinogen 24 mg/kgc and platelets.

4. Conclusion

Anaesthesia for liver transplantation is one of the most difficult anaesthesias. This is due to the haemodynamic problems that can occur in the intraoperative, related both to the status of the patient (cardiomyopathy of the cirrhosis, etc.) and to the surgical moments (bleeding and reperfusion syndrome) as well as to the blood coagulation pattern of the patient.

A good understanding of the pathophysiology of the cirrhotic patient is very important for best decision making. Adequate perioperative management is extremely important for a successful liver transplantation with a good outcome.

Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 12

Anesthetic Considerations in Transplant Recipients for Nontransplant Surgery

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Additional information is available at the end of the chapter

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Abstract

As solid organ transplantation increases and patient survival improves, it will become more common for these patients to present for nontransplant surgery. Recipients may present with medical problems unique to the transplant, and important considerations are necessary to keep the transplanted organ functioning. A comprehensive preoperative examination with specific focus on graft functioning is required. The anesthesiologist needs to pay close attention to considerations of immunosuppressive regimens, blood product administration, drug interactions as well as the risk and benefits of invasive monitoring in these immunosuppressed patients. This article reviews the post-transplant physiology and anesthetic considerations for patients after solid organ transplantation.

Keywords: anesthesia methods, intraoperative monitoring, organ transplantation physiology, perioperative care, solid organ transplantation

1. Introduction

The number of solid organ transplants performed worldwide is ever increasing. The improved survival rates are the result of well-established surgical techniques and effective immunosuppressive therapy. All this has led to an increase in number of patients who present for either elective or emergency non-transplant surgery [1, 2]. Laparotomy for small bowel obstruction, hip arthroplasty given the increased risk of fracture and avascular necrosis as a result of chronic steroid use causing bone demineralization and osteoporosis, lymph node excision and biopsy because of increased risk of lymphoproliferative disease, native nephrectomy in kidney transplant recipients, bronchoscopy in lung recipients, biliary tract interventions in liver recipients, and abscess drainage because of increased risk of infection are just a few of

the increased surgical needs in this population. These patients cannot always return to the transplant facility for surgery, so it is incumbent on all anesthesiologists to review perioperative issues associated with transplantation.

Perioperative anesthetic management in majority of recipients is similar to the standard practice for any patient. However, we must bear in mind some essential considerations: problems of allograft denervation, the adverse effects of immunosuppression and its interaction with anesthetic drugs, the risk of infection, and the potential for organ rejection. When transplant recipients require nontransplant surgery, immune competence can be altered from the stress of surgery, acute illness, or disruption of the regimen by inexperienced providers [3].

Preoperative assessment of any transplant recipient undergoing non-cardiac surgery should focus on graft function and rejection, risks of infection, and function of other organs, particularly those that may be compromised due to either immunosuppressive therapy or dysfunction of the transplanted organ itself and drug interactions. There is no ideal anesthetic for use in organ transplant recipients. However, certain principles can be applied to all transplant patients who undergo anesthesia and surgery [4].

In this chapter, we will give an overview of immunosuppressive therapy and its interaction with anesthetic drugs as well as considerations regarding specific transplanted organs (heart, lungs, liver, kidney, pancreas, and intestine).

2. Immunosuppression

2.1. Immunosuppression protocol

Transplant patients are always under various regimens of immunosuppressive therapy (**Table 1**). Immunosuppression trends for solid organ transplantation have undergone a perceptible shift over the past decade. There are broad therapeutic patterns and numerous immunosuppressive protocols depending on transplanted organ, as well as the regional differences (country, hospital). However, some strategies are similar. We distinguish induction immunosuppressive therapy and maintenance of immunosuppressive therapy. Induction of immunosuppression begins immediately before the organ implantation. Antibodies are prescribed for the majority of kidney, pancreas, and intestine recipients and for just under half of thoracic organ recipients. It is extremely uncommon in liver transplantation [5]. Maintenance of immunosuppression involves one of the drugs from each group: calcineurin inhibitors (CNI), antimetabolites, and steroids.

The immunosuppressant strategy has been changing over the years. CNIs are still being used for the maintenance of immunosuppressive therapy, though shifting from cyclosporine to tacrolimus is being observed [6]. Modifications are also made among antimetabolites, from azathioprine to mycophenolate mofetil, and it is more common to decrease corticosteroid use or even implement steroid-free protocol in suitable transplant recipients [7, 8].

Most of the commonly used immunosuppressants have a narrow therapeutic index and display significant variability in blood concentrations between individuals. In transplant recipients,

General names	Generic names	Brand names
Corticosteroids	Prednisone	
	Methylprednisolone	
Calcineurin inhibitors	Tacrolimus (or FK-506)	Prograf
	Cyclosporine (or cyclosporine A)	Sandimmune, Neoral, Gengraf, Eon, SangCya, generic cyclosporine
Antimetabolites	Azathioprine	Imuran
	Cyclophosphamide	Cytoxan, Neosar
	Mycophenolate mofetil	CellCept
	Mycophenolate sodium	Myfortic
Polyclonal antibodies	Antithymocyte globulin (rabbit)	Thymoglobulin
	Antithymocyte globulin (equine)	ATGAM
	NRATG, NRATS, ALG	
Anti-CD3 monoclonal antibodies	Muromonab-CD3	Orthoclone OKT3
Anti-CD52 monoclonal antibodies	Alemtuzumab	Campath
Anti-IL-2 receptor monoclonal antibodies	Basiliximab	Simulect
	Daclizumab	Zenapax
TOR inhibitors (or rapamycin)	Sirolimus	Rapamune

Table 1. Most commonly used immunosuppressive drugs.

both supratherapeutic and subtherapeutic drug concentrations can have devastating results. Subtherapeutic levels increase the risk of transplant rejection, and supratherapeutic levels (overimmunosuppression) can lead to infection and/or drug-specific side effects. Importantly, the incidence of acute rejection has declined over the past decade. Treatments for acute rejection continue to include high-dose corticosteroid and antibody therapies [9].

2.2. Side effects and drug interactions

Chronic immunosuppressive therapy has its adverse effects such as lowered seizure threshold, diabetes, hypertension, hyperlipoproteinemia, decreased glomerular filtration, hyperkalemia, hypomagnesemia, increased risk of infection and tumors, pancytopenia, osteoporosis, and poor wound healing. This may have some impact on perioperative management and choice of anesthetic agents (**Table 2**) [10].

The blood level of both cyclosporine and tacrolimus must be kept within the indicated therapeutic range to get the desired effect. The perioperative fluctuation of the plasma level of these two drugs should be strictly monitored. There is a significant reduction of drug blood level by dilution with volume infusion or cardiopulmonary bypass in cardiac surgery [11]. Both these drugs are metabolized by cytochrome P-450 system of liver, and therefore many of the drugs administered perioperatively can affect their plasma levels [12, 13]. A better understanding of

CyA	Tac	Aza	Ster	MMF	ATG	OKT3	
Anemia	–	–	+	–	+	–	–
Leucopenia	–	–	++	–	+	+	+
Thrombocytopenia	–	–	–	–	+	–	–
Hypertension	++	+	–	+	–	–	–
Diabetes	+	++	–	++	–	–	–
Neurotoxicity	+	+	–	+	–	–	–
Renal insufficiency	+	++	–	–	–	–	–
Anaphylaxis	–	–	–	–	–	+	+
Fever	–	–	–	–	–	+	+

ATG = anti-thymocyte globulin; Aza = azathioprine; CyA = cyclosporine A; MMF = mycophenolate mofetil; OKT3 = monoclonal antibodies directed against CD-3 antigen on the surface of human T-lymphocytes; Ster = steroids; and Tac = tacrolimus.

Table 2. Side effects of immunosuppressive that have direct impact on anesthetic and perioperative management [1].

pharmacokinetics over the years, allows now days a reduction of immunosuppressant dose in elderly patients while maintaining the therapeutic level [14].

In the hospital settings, almost 3–5% of adverse drug reactions are due to drug-drug interactions. It is estimated that the percentage is even higher in solid organ transplant recipients dependent on immunosuppressive therapy. Reactions can be among two immunosuppressants or between the immunosuppressant and other drugs [15]. Due to the fact that the administration of cyclosporine (CyA) and tacrolimus (Tac) is ever increasing, we give an overview of their interaction with other drugs (**Table 3**).

During anesthesia, we provide a range of drugs from different groups, which increase the possibility of drug interaction and thus potentially endanger the patient in the perioperative period. One should keep in mind that many pharmacokinetic and pharmacodynamic interactions can occur among the drugs that patients take after transplantation, due to cytochrome P450 enzyme system induction or inhibition by another drug. There are few data concerning interaction between immunosuppressive drugs and anesthetic agents. Most of the evidence is based on clinical practice and occasional case reports or case studies. Large randomized controlled trials involving either general or regional anesthesia are still lacking. **Table 4** describes the effect of various anesthetic agents on immunosuppressant and vice versa.

Data on the effects of general anesthesia on cyclosporine or tacrolimus pharmacokinetics in humans are limited. Most of the inhalational anesthetic agents are well tolerated unless there is a significant heart failure. However, caution is advised in concurrent administration of oral cyclosporine and isoflurane anesthesia. Subtherapeutic blood levels have been reported in patients who received peroral drug form less than 4 hours preoperatively. It is probably due to a reduction in gastric emptying and absorption from the proximal small bowel, which can occur during isoflurane anesthesia [16]. Steady-state blood levels of cyclosporine and cyclosporine clearance are not altered by isoflurane/nitrous oxide anesthesia in animal model [17].

Drug class	Drug	Effect on blood level Adverse effect
Benzodiazepines	Diazepam, midazolam, alprazolam, flurazepam, clonazepam	↑ Benzodiazepines
Antibiotics	Erythromycin, metronidazole, norfloxacin, levofloxacin	↑ CyA and Tac level
Antimicrobial	Rifampicin	↓ CyA and Tac level
Antimalarial	Chloroquine, mefloquine	↑ CyA and Tac level
Antifungal	Ketoconazole, fluconazole, itraconazole, voriconazole, amphotericin B	↑ CyA and Tac level Renal dysfunction
Anti-retroviral	Ritonavir, atazanavir, darunavir, cobicistat, delaviridine	↑ CyA and Tac level
Cardiovascular drugs (antiarrhythmics and calcium channel blocker)	Amiodarone, lidocaine, quinidine, verapamil, diltiazem, amlodipine, felodipine	↑ CyA and Tac level QT prolongation by amiodarone and quinidine
Statins	Simvastatin, atorvastatin, lovastatin, pravastatin	↑ Statin concentration
Anticoagulants	Apixaban, dabigatran, rivaroxaban	↑ Anticoagulant concentration
Oral hypoglycemics	Sulfonylurea, biguanides	↑ CyA level
Gastrointestinal	Metoclopramide, omeprazole, lansoprazole, octreotide, cimetidine, ranitidine	↑ CyA and Tac level Renal dysfunction QT prolongation by octreotide with Tac
Analgesics	Nonsteroidal anti-inflammatory drugs	↑ CyA and Tac level Renal dysfunction
Antipsychotics	Haloperidol, desipramine, fluoxetine, trazodone, pimozide	↑ CyA and Tac level ↑ Pimozide level
Hormones	Estrogen and testosterone preparation	↑ CyA and Tac level
Others	Bosentan, carbamazepine	↓ CyA and Tac level

CyA = cyclosporine A; Tac = tacrolimus.

Table 3. Drugs that interact with cyclosporine A and tacrolimus.

Cyclosporine and tacrolimus increase blood level of benzodiazepines. Transplanted patients need dose modification [12, 13]. Propofol infusion does not modify the cyclosporine concentration. It is considered to be a suitable agent for intravenous anesthesia in cyclosporine-treated patients, provided a close postoperative monitoring of cyclosporine blood concentrations is maintained [18]. Cyclosporine tends to increase the analgesic effect produced by fentanyl, but the mechanism is unclear [19].

Most of relaxants can be used safely. Cyclosporine enhances the effects of muscle relaxants. Prolonged neuromuscular block in patients receiving cyclosporine after vecuronium and pancuronium administration has been described [20, 21]. Atracurium and cisatracurium

Anesthetic agent	Effect with immunosuppressive drugs
Isoflurane	↓ Clearance of oral CyA
Thiopental	Nil
Benzodiazepines	↑ Blood level of benzodiazepines
Propofol	Nil
Etomidate	Nil
Opioids	CyA ↑ analgesic effect produced by fentanyl
Muscle relaxants	Prolonged neuromuscular blockade
Neostigmine	Caution in heart transplant patients
Local anesthetics	Bupivacaine and ropivacaine can be safely used

Table 4. Effect of specific anesthetic agent on immunosuppressive drugs.

are preferred agents because their elimination is not affected by renal or hepatic function. Therefore, patients receiving cyclosporine as immunosuppressive therapy may require a smaller dose of nondepolarizing muscle relaxant and the recovery time may be prolonged [22]. Neostigmine can lead to bradycardia and cardiac arrest in patients with heart transplantation despite the concurrent use of an antimuscarinic agent [23]. Bupivacaine and ropivacaine can be safely used through regional routes without any side effects [24, 25].

3. Anesthesia and preoperative care

3.1. Preoperative assessment of transplant recipients

Many transplant recipients live relatively normal and productive lives, but often have limited physical reserves. A successful transplant abolishes the symptoms and replaces function of the failed organ, but often there are persistent abnormalities from the underlying or preexisting illness that may have caused the organ failure or chronic physiologic abnormalities resulting from the organ failure itself. The preoperative evaluation of transplant recipients undergoing non-transplant surgery should include graft function, signs of rejection, presence of infection, and function of other organs.

Allograft rejection may occur at any time during the post-transplant period, especially when discontinuing the use of immunosuppressants. Chronic rejection is the most significant medical obstacle to long-term morbidity-free allograft survival. The incidence is thought to progressively increase with time after transplantation, but after a period of 5 years, it affects about 10% of liver to around 60% of lung allograft recipients [26]. Chronic organ rejection results in a progressive deterioration of organ function (assessed trough laboratory tests) and is the main cause of late mortality in the transplant recipients. Mortality rate is high if rejection remains untreated before surgery [27]. Therefore, the presence of any degree of rejection should be ruled out and urgently managed preoperatively with increased immunosuppression.

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Immunosuppressed patients are at risk of infections that may be bacterial, viral, fungal, or protozoan. Infection is a significant cause of morbidity and mortality after transplantation [28]. Its presence should also always be preoperatively ruled out (by obtaining laboratory, radiologic, and microbiology tests). It is imperative to emphasize that the immunosuppressed patient may not present the typical signs and symptoms of infection (i.e. fever, leukocytosis). Microbiology advice should be strongly sought for prevention as well as strict control of infection. Any infection should be treated preoperatively [29].

Although transplant patients are considered as risk hosts for infection because of their immune status, there is no evidence to suggest different bacteriology of surgical site infections than for the general population. Antimicrobial coverage need not be expanded to include atypical or opportunistic organisms as long as active infection with such an organism is not present or suspected [30]. Prophylaxis with broad spectrum antibiotic should be administered 1 hour before surgical incision (depending on hospital protocol) (**Table 5**).

Transplanted patients may also suffer from diabetes, hypertension, epilepsy, renal dysfunction, bone marrow suppression, lymphoproliferative disorders, and adrenal insufficiency as the side effects of chronic immunosuppressive therapy [31]. Hepatobiliary and pancreatic diseases are relatively common after transplantation, as well as the upper gastrointestinal bleeding secondary to peptic ulcer disease. Surgical stress, corticosteroids, and mycophenolate may contribute to gastrointestinal ulcers [32]. So, it is absolutely necessary to provide stress ulcer prophylaxis for transplanted patients.

The transplant patient population is considered as high-risk group for developing venous thromboembolism (VTE) given the fact that most of these patients have multiple identifiable risk factors [33]. However, the exact risk of developing VTEs in these patients is not clearly defined in the literature, nor there are clear guidelines regarding the appropriate use of thromboprophylaxis in transplant recipients [34]. In our opinion, VTE prophylaxis should be tailored to the patient's specific needs in accordance with current guidelines [35].

Operation	Recommended antibiotic prophylaxis
Cardiothoracic surgery	Cefazolin, cefuroxime, or cefamandole. If patient has a β-lactam allergy: vancomycin or clindamycin
Vascular surgery	Cefazolin or cefuroxime. If patient has a β-lactam allergy: vancomycin with or without gentamycin, or clindamycin
Colon surgery	Oral: neomycin plus erythromycin base, or neomycin plus metronidazole. Parenteral: cefoxitin or cefotetan, or cefazolin plus metronidazole
Hip or knee arthroplasty	Cefazolin or cefuroxime. If the patient has a β-lactam allergy: vancomycin or clindamycin
Vaginal or abdominal hysterectomy	Cefazolin, cefotetan, cefoxitin or cefuroxime

Table 5. Antimicrobial prophylaxis for selected surgical procedures [30].

3.2. Preoperative assessment and premedication

The transplant team as well as the attending anesthesiologist and surgeon should have a good coordination during perioperative period, especially if a major surgical procedure is planned. A comprehensive preoperative evaluation by the anesthesiologist should include: evaluation of the graft function, presence of infection, function of other organ systems, the presence of concomitant diseases as well as the preoperative performance or functional status. Adherence to the fundamental principles of preoperative evaluation along with a high level of vigilance is required. Information and medical history should be gathered from the medical records, interview with the patient and/or next of kin or guardian. If medical information is unavailable, attempts should be made to contact the transplant center for pertinent history. Other useful information from the transplant center includes their most recent evaluations and recent data on graft function and general health of the patient. Close communication with the transplant team may be the single most important step in preparing the patient for surgery and developing a perioperative anesthetic plan.

A thorough review of systems along with a physical examination is essential in this population. Findings such as recent weight gain, edema, dyspnea, sweats, malaise, fever, rashes, abdominal pain, abnormal breath sounds on auscultation, and changes in stool or urine output are some of the potential signs and symptoms of infection or rejection.

The following investigations should be available preoperatively.

1. Laboratory parameters:
   - **a.** Complete blood count (to rule out bone marrow suppression)
   - **b.** Electrolytes
   - **c.** Renal function tests
   - **d.** Liver function tests
   - **e.** Coagulation tests
   - **f.** Biomarkers (i.e. brain natriuretic peptide)
2. Chest radiograph
3. Electrocardiogram
4. Echocardiography
5. Miscellaneous: depending on the type of surgery and transplanted organ (i.e. coronary angiography, stress test, spirometry, biopsy)

Each preoperative evaluation and testing should be considered individually based on the target organ system(s) to be evaluated, the patient's medical history, and the inherent risks of the upcoming surgical procedure.

Cardiovascular disease is a major cause of mortality and morbidity among organ transplant recipients, especially in those with chronic kidney disease or previous heart transplant, making

the risk of a perioperative cardiovascular event a legitimate concern. Many transplant recipients have undergone complete cardiac testing and in some cases, interventions, before their transplant surgery. Records of the testing and interventions can be easily obtained from the transplant center to be used for comparison and consideration before the upcoming surgery. We must also bear in mind that many of these patients may have asymptomatic coronary disease as a result of diabetes or the transplant itself [36].

If unexpected abnormal findings are identified on physical examination or laboratory testing, symptomatic changes outside the patient's baseline are documented, or suspicion for rejection or infection exists during the preoperative evaluation, it should be considered to postpone any surgery that is non-urgent or elective. The patient should also be expeditiously referred back to the transplant center, cardiologist, or other consulting physician as indicated.

Standard premedication may be used, as in non-transplant patients. However, dose adjustment for some drugs is needed (**Table 3**). Antibiotic, stress ulcer and VTE prophylaxis administration is recommended (as mentioned above). Supplemental steroids are not necessary for stress coverage except in post-transplant recipients in whom steroids are recently withdrawn [37].

Post-transplantation diabetes mellitus is a common metabolic consequence of the agents of immunosuppressive therapy agents [38]. It is imperative to institute a glycemic control plan before the surgery with closely managing intraoperative and postoperative glucose control.

The dose of immunosuppressive drugs should not be altered and should be continued postoperatively to reduce the risk of rejection. Daily monitoring of the steady-state blood level is recommended. Oral cyclosporine should be administered 4–6 h before surgery to maintain therapeutic blood levels. The alteration of dose of other immunosuppressive drugs dose is not required unless the route of administration needs to be changed from oral to intravenous [39].

3.3. General anesthetic considerations

There is no ideal anesthetic plan that can be used for all transplant recipients undergoing nontransplant surgery. A variety of anesthetic techniques have been successfully used in patients with a transplant history including general (inhalational, balanced, and total intravenous), neuraxial, and regional anesthesia.

3.3.1. Monitoring

Generally, invasive monitoring is not mandatory and anesthesia should be performed using standard European Society of Anesthesiology's monitoring guidelines [40]. The decision to use invasive hemodynamic monitors, placement of central venous access, pulmonary artery catheters, or other procedures such as transesophageal echocardiography should be made on a case-by-case basis. It should be guided by consideration of the patient's comorbidities, hemodynamic stability, the expertise of the anesthesiologist in placing the invasive devices, and by the type of surgery and anesthesia planned. Aseptic technique is of utmost importance to minimize exposure to infectious organisms and bacteremia when attempting any invasive procedures in this population [41].

3.3.2. Airway management

Airway management of transplant patients may pose a concern for several reasons. Many patients may have pre-existing diabetes mellitus before transplant or acquire diabetes after transplant. Diabetic patients can develop limitations in joint mobility caused by glycosylation of the connective tissue within their joints [38]. This population is also at increased risk for lymphoproliferative disorders secondary to immunosuppressant drugs, and lymphoproliferative growth may compromise any part of the airway or mediastinum and cause life-threatening airway obstruction during sedation and anesthesia [41]. Gingival hyperplasia is present at times in patients taking cyclosporine and it may lead to bleeding during airway manipulation. Aspiration risk may be increased in transplanted patients as a result of delayed gastric emptying and gastropathy [32]. These potential problems should all be taken into consideration when constructing the anesthetic plan for airway management.

Oral endotracheal intubation is preferred over nasal intubation because of the potential of infection caused by nasal flora [42]. The use of a laryngeal mask is acceptable (within its indications) [43]. Keep in mind that laryngoscopy and tracheal intubation may not produce a sympathetic response secondary to the loss of cardiac baroreceptor reflexes in heart transplanted patients [44]. Avoid hyperventilation in patients taking cyclosporine and tacrolimus because of a decrease in seizure threshold with these two drugs. Early postoperative extubation is preferred if possible to prevent the development of nosocomial or ventilator-associated pneumonia [45].

3.3.3. General anesthesia

All inhalational and intravenous anesthetics have been used with success in transplant recipients. The choice of anesthetics and adjunctive drugs should be determined by the type of surgery and condition of the patient. As a general guideline, if hepatic and renal functions are normal, all standard anesthetic medications and adjuncts may be used. Some special considerations for each type of organ transplant are discussed in the section on organ-specific considerations.

3.3.3.1. Intravenous anesthetics

The selection and administration of intravenous anesthetics should be guided by the patient's hemodynamic status, the drug's cardiovascular effects, and pharmacokinetic properties. Premedication with benzodiazepines is acceptable. Caution should be used in patients with hepatic or renal insufficiency as effects may be prolonged. Also, the dose of barbiturates should be adjusted in patients with hepatic insufficiency to avoid prolonged effects.

Propofol is extensively metabolized by the liver to inactive glucuronic acid metabolites that are excreted by the kidneys. Nevertheless, there seems to be no need for dose adjustments in patients with hepatic or renal failure indicating an extrahepatic route of elimination as well [46]. Caution should be used in patients with cardiovascular compromise as propofol can worsen cardiac contractility, compromise cardiac preload, cause bradycardia, and lower systemic vascular resistance culminating in diminished cardiac output and mean arterial pressure.

Etomidate does not have the cardiac depressant effect of barbiturates and propofol. Etomidate is metabolized rapidly by hydrolysis within the liver and by plasma esterases and also does not require dosage adjustment in renal or hepatic disease. One unique characteristic of etomidate is its ability to inhibit enzyme necessary for the synthesis of cortisol [47]. This may have clinical significance in patients who already have adrenal suppression as a result of exogenous corticosteroid use.

Ketamine is metabolized via the hepatic cytochrome P-450 system. Therefore, the clinical effects of ketamine are prolonged in the presence of hepatic insufficiency. The usual cardiac stimulating effects caused by central stimulation of the sympathetic system are not present in the denervated heart, but ketamine can still increase systemic vascular tone. Ketamine has neuroexcitatory effects and is known to cause myoclonic activity. It would be wisely to proceed with caution if the katamine is administered in patients who simultaneously take cyclosporin due to its potential for neurotoxicity.

3.3.3.2. Inhalational anesthetics

All inhaled anesthetics have been used in transplanted patients with success. Although halothane is nowadays rarely used, nevertheless it is necessary to mention its potential for hepatotoxicity and direct cardiac depressant effects. Most commonly used volatile anesthetics are isoflurane, sevoflurane, and desflurane. There does not seem to be a significant clinical advantage or disadvantage of one over the others. The choice of inhaled anesthetic can be dictated by the anesthesiologist's preference, experiences, and comfort with the anesthetic [48]. It is probably prudent to avoid prolonged use of N2O because of the potential risk of bone marrow suppression and the potential for altered immunologic response [49].

3.3.3.3. Opioids

Fentanyl is suitable and safe for short-term use during surgery. However, if used for long duration, the pharmacodynamic effects should be monitored due to accumulation effect. Reduced renal and liver function does not significantly alter the clearance and half-life of sufentanil. Tissue and blood esterases mainly metabolize remifentanil and its metabolite, excreted via kidneys, has low potency [50].

Among opioids used for postoperative pain treatment (morphine, codeine, oxycodone, and tramadol) have to be used with caution. Some of their active metabolites accumulate in renal failure and can mediate CNS and respiratory depression. Transdermal buprenorphine and methadone appear to be safe to use even in patients with renal dysfunction [51].

3.3.3.4. Neuromuscular blockade

The decision to use neuromuscular blockade should be based on the type of surgery and actual need for muscle relaxation during the procedure or the need to optimize intubating conditions. The choice of specific neuromuscular blocking agent should be dictated by length of surgery, underlying medical illnesses (i.e. myasthenia or other neuromuscular disorders), history of malignant hyperthermia, and the functional state of the patient's kidney and liver.

In the group of non-depolarizing drugs, it is preferable to use short-acting relaxants (mivacurium) or intermediate-acting agents independent of kidney and liver function (cisatracurium, atracurium). Vecuronium, rocuronium, and pancuronium can have prolonged effects in the face of hepatic or renal insufficiency. They require dose adjustments, close neuromuscular monitoring, and evidence of full reversal before extubation [52]. Some immunosuppressive drugs (i.e. azathioprine and cyclosporine) can prolong the action of the neuromuscular blocking agents [22].

Succinylcholine, the only depolarizing agent available, can be used in organ transplant recipients in the need for rapid sequence intubation and rapid airway control. It should be avoided only if there are other clinical reasons, such as hyperkalemia, muscular dystrophy, or history of malignant hyperthermia [53].

3.3.3.5. Anticholinesterase drugs

Most of the cholinesterase inhibitor drugs are eliminated through the kidneys (neostigmine, edrophonium, and pyridostigmine). Caution is advised in renal failure. Several reports described that neostigmine may produce a dose-dependent life-threatening bradycardia in heart transplant recipients, whereas another publication described the safe use of neostigmine [54]. Reversal of neuromuscular block with sugammadex is another possibility, but limited data exist in literature [55].

3.3.4. Regional and neuraxial anesthesia

The decision to perform a regional or neuraxial anesthetic technique in a previously transplanted patient must be made on an individual basis. We must carefully consider potential benefit and risks of these techniques as well as the anesthetic alternatives when constructing the anesthetic plan in this population. There may be several advantages to choosing a neuraxial or regional technique in this population. Superior analgesia over systemic opioids, especially in patients who may have narcotics tolerance as a result of long-term opioid use, reduced pulmonary complications, and decreased incidence of graft occlusion are just a few of the benefits of regional and neuraxial anesthesia [56]. Clinically relevant doses of bupivacaine and ropivacaine, which are commonly used local anesthetics for neuraxial anesthesia, do not seem to result in toxic levels or increased risk of toxic effects in renal and liver transplant recipients. However, it is important to be prepared for the risk of hypotension because of pre-existing autonomic neuropathy and cardiac denervation in this population. Cautious correction of hypovolemia before epidural or spinal anesthesia may help to attenuate the hypotension. Concurrent hemodynamic monitoring is imperative during the procedure. Direct and indirect-acting adrenergic agonists should be readily available along with emergency airway supplies.

The consideration of spinal or epidural anesthesia is appropriate in this population as long as there is no increased risk for bleeding complications. It is necessary to perform a total blood count to exclude bone marrow suppression, especially thrombocytopenia, and coagulation tests (PT, INR, APTT, and fibrinogen). Peripheral nerve blocks became popular anesthetic option due to hemodynamic stability and better postoperative analgesia. Some studies show no difference in duration of peripheral nerve blocks in patients after transplantation compared to the general surgical population [57, 58]. Nevertheless, large prospective randomized trials are still lacking.

Although the risk of infectious complications is very low, it is important to be highly vigilant when monitoring these patients after a neuraxial anesthesia as the attenuated inflammatory response may diminish the typical signs and symptoms of infection [59]. Again, aseptic technique and a mask should be considered essential when performing these procedures.

3.3.5. Postoperative care management

Regardless of the procedure performed, successful outcomes also depend on optimal postoperative care. Depending on the type of surgery, patients' comorbidities, and preoperative condition, patient is after surgery transferred either to intensive care unit (ICU) or postanesthesia care unit (PACU). Adequate monitoring is tailored accordingly [60]. We reduce the delirium incidence by minimizing sedation, speed up extubation, and facilitate early ambulation and physical rehabilitation. Appropriate analgesia is essential component of postoperative surgical care. Opioids are the mainstay of analgesia in the early postoperative phase after major surgery. Parenteral paracetamol is an effective analgesic agent and may spare narcotics. There is no evidence of an increased risk of hepatotoxicity [61]. Once extubated, patient-controlled analgesia (PCA) devices are effective and well received by patients and nurses. Non-steroidal anti-inflammatory drugs should be avoided because of the risk of adverse interactions (e.g., gastrointestinal hemorrhage, nephrotoxicity, hepatic dysfunction). They augment nephrotoxicity of cyclosporine, as both drugs affect the renal microcirculation [62, 63].

Immunosuppressive therapy should be continued during the perioperative period and daily monitoring of steady-state cyclosporine or tacrolimus blood levels is recommended [64]. The dose of other immunosuppressive drugs should not be altered perioperatively unless the route of administration needs to be changed from oral to intravenous. In addition to the routine care as those for non-transplant recipients, increased attention should be paid to the preload status, renal function, and prevention of infection.

4. Specific anesthetic considerations

4.1. Heart transplant recipients

Transplanted heart is completely denervated, meaning it lacks neural regulating mechanisms [65]. Even though, it has the ability to adjust with compensatory mechanisms to the increased demands in stress returning the recipients to an active life. Transplanted heart has no sensory sympathetic and parasympathetic innervation. Therefore, it has a higher resting heart rate of 90–110 bpm secondary to the loss of vagal tone. The resting ECG is commonly altered showing two P waves: one is from the recipients' own SA node and the other is the donors' SA node. Patients are at higher risk of developing atrial flutter or atrial fibrillation. The transplanted heart is "preload dependent." Cardiac output becomes dependent on venous return. Therefore, it is important to maintain a sufficient systolic pressure and prevent hypovolemia [66].

Although the cardiac index of the transplanted heart is lower than that of normally innervated control hearts, it remains in the normal range. The catecholamine response is different from

that of normal heart because intact sympathetic nerves are required for the normal uptake and metabolism of catecholamines. The receptor density, however, remains unchanged, and the transplanted heart can respond to direct-acting drugs (adrenaline and noradrenaline) [67]. Isoprenaline and dobutamine have similar effects in both transplanted and normal heart. Because atropine has no effect on a transplanted heart, isoprenaline and adrenaline should be readily available to manage bradycardia and hypotensive emergencies. In recent years, milrinone and levosimendan, inotropic vasodilators, have been included in the pharmacological arsenal. These drugs increase myocardial contractility without myocardial oxygen consumption (unlike catecholamines). In addition, they lead to arterial and venous vasodilatation and afterload decrease [68, 69]. **Table 6** summarizes the hemodynamic response of some commonly used drugs for resuscitation.

Heart transplant recipients may present with ongoing rejection with myocardial dysfunction, accelerated coronary atherosclerosis, or severe dysrhythmias, all of which must be diagnosed before surgery. Chronic allograft rejection usually presents as accelerated coronary artery disease. Therefore, heart transplant recipients may have significant myocardial ischemia without any clinical symptoms of pain and silent myocardial infarction on the ECG. Severe rejection can lead to significant systolic and diastolic dysfunction [70, 71].

General anesthesia is usually preferred, as there is a possibility of impaired response to hypotension after spinal or epidural anesthesia. A goal of anesthesia in this setting is the avoidance of significant vasodilation and acute decrease of the preload. Invasive hemodynamic monitoring is extremely useful during surgery that involves large volume shifts, due to the fact that these patients are preload-dependent and may be prone to myocardial dysfunction and/or

Drugs	SA node rate	AV conduction	SVR	BP	CO	Remark
Atropine	0	0	0	0	0	Cannot be used in bradycardia
Dopamine	↑	↑	0	E	↑	
Dobutamine	↑	↑	0	↑	↑	Effect on HR is more than normal heart
Adrenaline	↑	↑	0	↑	↑	
Noradrenaline	↑	↑	0	↑	E	
Isoprenaline	↑	↑	0	↓	↑	
Phenylephrine	0	0	0	↑	E	
Digoxin	0	↓				
Milrinone	0	0	↓	↑	↑	
Levosimendan	0	0	↓	↑	↑	

SA – sinoatrial; V – atrioventricular; SVR – systemic vascular resistance; BP – blood pressure; CO – cardiac output; HR – heart rate; 0 – no effect; and E – equivocal.

Table 6. Response of denervated heart to various cardiovascular drugs.

ischemia. Instead of invasive monitoring, intraoperative transesophageal echocardiography may be considered [72, 73]. All preoperative drug therapy should be continued during the perioperative period. If a pacemaker is in place, its proper function should be confirmed.

4.2. Lung transplant recipients

Denervation of the lungs after transplantation is associated with a lack of cough reflex below the tracheal anastomosis level and are unable to clear secretions unless they are awake. Therefore, they are prone to retention of secretions and silent aspiration [74]. These conditions may lead potential hazardous conditions especially in general anesthesia, such as bronchoconstriction and the increased risk of chest infection. In light of potential complications, it is preferable to perform a regional anesthesia whenever possible and if there is no contraindication [75].

Before elective surgery patients should undergo chest radiograph and spirometry to exclude chronic rejection and infection [76]. If allograft rejection or infection is suspected, elective surgery should be postponed and appropriate investigations should be performed.

Altered lymphatic drainage in the transplanted lung may cause interstitial fluid accumulation. It has been recommended that these patients be treated with diuretics and limited crystalloid infusion [77, 78]. Invasive hemodynamic monitoring is often required in heart-lung transplant recipients since there is a narrow line between the pulmonary edema occurrence and maintaining cardiac output with fluid load [79].

4.3. Kidney transplant recipients

Kidney transplant recipient have high incidence of cardiovascular diseases, especially elderly patients with diabetes and after years of dialysis procedures. Spectrum of disease can range from hypertension to severe coronary disease. It is wisely to approach them with caution. Cardiovascular complications are the leading cause of death in renal transplant recipients, accounting for 32% of all deaths [80].

After successful transplantation, renal function is restored, with no need for renal replacement therapy. Still, beware of prolonged drug action and excretion in kidney transplant recipients, due to the fact that their glomerular filtration rate and effective plasma flow can be significantly lower than in healthy subjects [81].

You should suspect at chronic graft rejection when azotemia, proteinuria, and hypertension are seen [82, 83]. One of the important parameters to consider in the prevention of graft failure is the maintenance of appropriate renal perfusion pressure. Perioperative fluid management must ensure restoration and maintenance of intravascular volume, in order to obtain good graft function. Diuretics should not be given without careful evaluation of the patient's volume status.

In anesthetic management, it is prudent to choose drugs that do not rely on the kidney for excretion. Nephrotoxic drugs should be avoided [84]. Cardiovascular instability can be present after a recipient has been recently hemodialysed due to hypovolemia and hypokalemia, causing arrhythmias and increased susceptibility to muscle relaxants [85].

4.4. Liver transplant recipients

Liver synthetic tests should normalize over time pointing out a good liver graft function. There is also gradual decrease of all liver enzyme levels, as graft function becomes normal. Recovery of drug metabolism capacity occurs immediately after reperfusion of the liver graft. Liver transplantation itself results in reversal of the hyperdynamic state that characterizes patients with end-stage liver disease and cardiac performance improves in the months after transplantation. Hypoxemia caused by ventilation/perfusion mismatch is reversed over the course of the first postoperative months. Patients with pre-existing true shunts may require more time to achieve reversal of hypoxemia, or hypoxemia may not resolve at all [86]. Hepatorenal syndrome gradually diminishes, renal function improves over time, and creatinine level may become normal. However, kidneys are still in danger of injury due to immunosuppression side effects [87].

The most severe complication of liver transplantation is hepatic artery thrombosis. It has often been associated with massive transfusion of blood products leading to hemoconcentration. Therefore, liver transplant recipients should have minimal blood viscosity (hematocrit approximately 28%) during the perioperative period [88].

No individual general anesthetic agents are contraindicated when hepatic and renal function is normal. If an epidural or spinal anesthesia is planned, clotting studies and platelet counts should be normal. Neither regional nor general anesthetic techniques were associated with deterioration of liver function assuming proper anesthetic and intensive care management [3, 25].

4.5. Pancreas transplant recipients

Pancreas transplantation provides the most effective method of glycemic and metabolic control. It can be done as a single organ transplant or simultaneously with kidney (predominantly in type 1 diabetes) (SPKT). SPKT is a treatment of choice for uremic diabetic patients when a living-related kidney donor is unavailable. After successful transplantation, pancreas transplant recipients do not require insulin to compensate for the stress response to surgery [89].

However, due to long-lasting diabetes effect, these patients are in high risk of developing cardiovascular diseases. It is prudent to manage these patients with the assumption that they have coronary artery disease [90]. Pancreas recipients still have persistent complications of diabetes such as gastropathy and neuropathy. Aspiration risk may be increased as a result of delayed gastric emptying. This population is also at increased risk for lymphoproliferative disorders secondary to immunosuppressant drugs and lymphoproliferative growth may compromise any part of the airway or mediastinum and cause life-threatening airway obstruction during sedation and anesthesia [91].

Amylase levels in serum and urine should be closely monitored. They can be our only window in the graft rejection recognition [92]. Glucose levels should also be monitored perioperatively. In normal functioning grafts, the suppression of endogenous insulin secretion during hypoglycemia is sufficient to enable a normal glucagon response from the transplanted pancreas, even in surgical stress [93]. In patients with failed pancreatic grafts, perioperative management of glucose levels and acid-base status is the same as that for any diabetic patient.

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4.6. Intestine transplant recipients

There are three types of intestine transplantation: isolated intestinal transplantation, transplantation of combined intestine, and liver graft or multivisceral transplantation. The biggest problem in intestinal transplantation is graft rejection, and it is the main reason for morbidity and mortality. The diagnosis of rejection is confirmed by clinical symptoms, endoscopic appearance, and pathological specimens taken by endoscopy [94].

Denervation and lymphatic dysfunction of the intestine affect intestinal permeability and absorption. If the intestinal mucosa barrier is damaged by ischemia, rejection, or enteritis, bacteria translocate into the bloodstream and infections are often observed [95]. Some of these patients develop diarrhea and lose weight in the early post-transplantation period. Any imbalance in the electrolyte and acid-base status should be timely corrected. Fluid administration should be closely monitored to assure sufficient splanchnic perfusion. Venous access is of major consideration for the anesthesiologist due to chronic use of total parenteral nutrition and its thrombotic complications [96].

5. Special cases

5.1. Trauma

It is generally assumed that immunosuppressed patients are more susceptible to the effects of soft tissue damage and poor bone healing. Bone loss associated with chronic immunosuppressive therapy is a serious problem for most transplant recipients. These patients are prone to fractures (i.e. hip or compressive vertebral fracture) [97].

Only a few studies of traumatized transplant recipients have been reported. This is likely because of the infrequent presentation of these patients to trauma centers. The most common causes of trauma are car accidents and falls. The latest study by Scalea *et al.* determined that outcomes for traumatic injury in patients with organ transplants are not worse than that for non-transplant patients, despite common presumptions among physicians [98]. Transplant recipients sustaining trauma should receive the same initial resuscitation as any trauma victim. Patients should be assessed by a transplant surgeon as soon as possible and graft function should be closely assessed by a transplant team during hospitalization and after discharge from the trauma center [3]. Acute organ rejection within 6 months of admission for trauma is reported among 17% of solid organ recipients [99].

Transplant recipients, whose immune systems are already suppressed to prevent organ rejection, are presumed to be at greater risk of infection from traumatic injury. However, this was not observed in two latest studies [100]. Therefore, similar protocols of antimicrobial therapy should apply to both transplanted and non-transplanted patients to avoid the overuse of antimicrobial agents and ensure maintenance of the susceptibility patterns of pathogens.

Studies also show that transplanted organs are rarely injured in traumatic events. This is most likely related to careful selection of transplant recipients who are committed to self-care and continue to pursue healthy life style after transplantation.

5.2. Pregnancy

With the advances in transplantation medicine, female organ transplant recipients are able to conceive and carry pregnancies successfully to term. This state presents a unique challenge to attending physician, obstetrician, and anesthesiologist. These women are at an increased risk of comorbidities and obstetric complications. Therefore, all post-transplant pregnancies should be considered as high risk, and close monitoring is mandatory. Anesthesiologists are involved in the care of these patients for both labor analgesia and operative procedure. Anesthetic considerations include the effects of the physiologic changes of pregnancy on the transplanted organ, graft function in the peripartum period, and the maternal side effects and drug interactions of immunosuppressive agents. Anesthetic management should consider the important task of protecting graft function [101, 102].

Data are lacking regarding the optimal transplant-conception interval. The 2005 American Society of Transplantation Consensus Conference suggested that pregnancy 1 year after transplant is safe as long as the patient has stable graft function. This means: no episodes of rejection in the past year, a low risk for opportunistic infections, stable renal function (including in those receiving organs other than a kidney), and a low stable dose of maintenance immunosuppression [103].

Pregnancy does not appear to cause excessive or irreversible problems with graft dysfunction if the function of the transplanted organ was stable prior to pregnancy [104]. Maternal side effects of immunosuppression therapy include nephrotoxicity, hepatotoxicity, diabetes, and arterial hypertension, which could lead to possible dangerous complications. In kidney, heart, or heart-lung transplant recipients, the rate of complications, such as preeclampsia, premature labor, and risk of acute allograft rejection postpartum, is higher than that in the non-transplant population [105].

Current immunosuppressant drugs are not thought to be teratogenic and their use cannot be discontinued during pregnancy. All immunosuppressants cross the placenta. They are not strongly associated with the increased risk of congenital anomalies in the first trimester. However, they affect the immune system of fetus during the second and third trimesters and may result in premature delivery and low birth weight in newborn [106].

The anesthetic technique for cesarean section depends on indication, functional status of transplanted organ, and cardiovascular and hematological status. Central neuraxial blocks are not contraindicated if coagulation status is normal. However, documentation of paresthesia is important if regional anesthesia is planned. In the case of general anesthesia, all intravenous anesthetics and inhalational agents are safe. Neuromuscular function should be monitored particularly if the patient is receiving magnesium. Postoperative pain relief is provided with narcotics by epidural or spinal route if regional anesthesia is used or by parenteral opioids. Non-steroidal anti-inflammatory drugs should be avoided. Thromboprophylaxis should be administered because of the high risk of thromboembolic complications in these patients, especially after cesarean delivery. The threshold for admission to an intensive care or high-dependency unit should be low [107].

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5.3. Laparoscopic surgery

Laparoscopic surgery is currently a widely accepted approach to several surgical fields because of its advantages in terms of postoperative pain reduction and easy patient recovery. The number of minimally invasive surgical procedures performed in transplant recipients is constantly increasing [108].

Lymphoceles can be successfully treated surgically after kidney transplantation by laparoscopy under general anesthesia [109, 110]. There are also reports of successful laparoscopic bariatric surgery after simultaneous pancreas and kidney transplantation [111]. Laparoscopic cholecystectomy is considered to be safe procedure in the transplant population. It has advantage of short hospital stay, low morbidity, maintenance of oral immunosuppression, and early return to preoperative routines. There is however a slightly higher rate of conversion to an open cholecystectomy among transplant patients compared to general population (27% vs. 11%, respectively) [112, 113]. Generally, laparoscopic approach may be useful even in solid-organ transplantation surgery as a diagnostic or treatment procedure in some surgical complications.

5.4. Outpatient and esthetic surgery

With shorter medical procedure duration and fewer complications, there is growth in popularity of outpatient or ambulatory surgery. Procedures performed are broad in scope: knee, shoulder, spine and eye surgery (cataract, laser surgery), plastic surgery, some types of esthetic surgery, and upper gastrointestinal endoscopy and colonoscopy [114].

Improved immunosuppression and lifespans have afforded solid organ transplant recipients the opportunity to seek outpatient and esthetic surgery. Most commonly performed procedures are soft tissue excisions with local flap coverage, facelifts, breast augmentation, and abdominoplasty. Among solid organ transplant recipients, kidney transplant recipients most often underwent plastic surgery, accounting for over 68% [115]. The complication rate is very low and ranges from 4 to 8% [116]. Delayed wound healing or wound disruption is reported as the most common complication and is associated with immunosuppression therapy, such as steroids [117].

It is extraordinarily important to manage these patients with a multidisciplinary approach. They should obtain clearance from the transplant surgeon and from the organ-specific specialist. The anesthesiologist should be familiar with the organ-specific needs in the perioperative period (i.e. maintaining preload for heart transplant patients, judicious fluid management in the renal patient, and avoidance of volatile anesthetics in liver transplants) to avert unintended consequences. It is more reasonable to use of general anesthesia over regional in the heart transplant patients. Perioperative antibiotic prophylaxis and stress-dose steroids should be administered prior to surgery. NSAIDs should be avoided in postoperative pain regimen [118].

Elective esthetic surgery can be performed safely in patients with a history of solid organ transplantation after a careful patient selection and multidisciplinary approach. These patients can potentially experience significant improvements in their quality of life with low morbidity.

6. Conclusion

The increasing prevalence of previously transplanted patients makes it likely that every anesthesiologist will care for patients with end-organ failure or a transplanted organ, either for accidental or transplant-related surgery in the future. Local, regional, or general anesthesia can be safely delivered to transplant recipients and a successful anesthetic and perioperative management can be provided. However, for the safe management of solid organ recipients, it is essential to have an appropriate knowledge of the physiology of the transplanted organ, the pharmacology of the immunosuppressive drugs, and the presence of associated organ dysfunction.

Many of the perioperative problems in the transplant population have not been specifically studied, and there are no formal recommendations for their management. Additional research should be performed in order to identify perioperative issues and facilitate the formulation of guidelines for anesthesia in this particular transplanted population.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 13

Pancreas Transplantation

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Additional information is available at the end of the chapter

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Abstract

Pancreas transplantation is a treatment alternative to patients with type 1 diabetes, particularly to those with associated end-stage renal disease (ESRD). Recently, pancreas transplant centers have widened their criteria for pancreas transplantation to selected type 2 diabetic patients. This chapter reviews the most important topics on pancreas transplantation, including epidemiology and natural history of type I and type II diabetes, indications for pancreas transplantation, different alternatives for pancreas transplant recipients (simultaneous kidney-pancreas transplantation, pancreas after kidney, or pancreas transplant alone—PTA), and their outcomes. This chapter gives a detailed description of the surgical procedure for pancreas procurement and engraftment, as well as the most frequent surgical complications. An approach to the management of the recipient following pancreas transplantation (immunosuppression and infection prophylaxis) is also discussed. Finally, outcomes and complications following the pancreas transplantation are reviewed.

Keywords: pancreas transplantation, surgical complications, acute rejection, diabetes, end-stage renal disease, immunossuppression, kidney transplantation

1. Introduction

Diabetes is a disease characterized by an imbalance in glucose metabolism homeostasis, highly prevalent worldwide, and presents a significant morbidity and mobility due to vascular and neurological complications. It is the single most frequent cause of end-stage renal disease in incident patients on hemodialysis. The clinical presentation and epidemiology of diabetes varies according to the etiology. For selected patients, pancreas transplantation is the best treatment alternative to achieve glycemic control. In this chapter, we describe the most

important topics regarding pancreas transplantation, including indication and patient evaluation, surgical techniques, immunosuppression protocols, and outcomes.

2. Epidemiology and pathophysiology of diabetes

Diabetes is a spectrum of diseases characterized by a disorder in glucose metabolism leading to persistent hyperglycemia, with clinical manifestations varying according to disease etiology (**Table 1**).

Type 1 diabetes mellitus (DM) is an autoimmune disorder characterized by the generation of autoantibodies against β cells and the development of a localized inflammatory response with consequent islet destruction. Current models suggest that the disease progresses as a relapsing-remitting disease, with a nonlinear β cell mass loss at each relapse as a result of the imbalance between β cell proliferation and destruction, eventually leading to persistent hyperglycemia. At diagnosis, these patients may present with nearly normal serum concentrations of insulin and C-peptide but with a rapid decrease in the following 8–12 weeks.

Several autoantibodies have been described in patients with type 1 diabetes—antibodies to insulin (IAA), glutamic acid decarboxylase (GAD), Zinc transporter 8 antibodies (ZnT8A), and protein tyrosine phosphatase-like protein IA2 (IA2 or ICA512). The risk for overt diabetes better correlates with the number of autoantibodies present rather than the titter of a single antibody [1].

Type 1 diabetes usually presents at a young age (6 months to 25 years old), and there is a geographical variation, with a tendency toward an increased incidence in developed countries—, as

Diabetes	Age presentation	Clinical presentation	Etiology	Prevalence (of total diabetes)	Obesity
Type 1	6 months to young adulthood	Most often acute, rapid	Autoimmune (some genetic associations); β cell mass loss; Insulin deficiency	5–10%	Uncommon
Type 2	Adulthood	Variable; from slow, mild (often insidious) to severe	Insulin resistence; β cell exaustion	80–90%	Common
Monogenic	Often post pubertal except glucokinase and neonatal diabetes	Variable (may be incidental in glucokinase)	Reduced insulin production or secretion; higher glucose sensor threashold	2%	Incidence similar to general population

Table 1. Etiology and clinical presentation of diabetes.

recognized by the World Health Organization (WHO), this may be due to differences in registry data, since few data that exist from sub-Saharian Africa, South America, and Asia [2].

A genetic predisposition has been identified with an increased risk for the disease in siblings and offspring of diabetics. There is an increased incidence in patients with human leukocyte antigen–antigen D related (HLA DR)*03 and DR*04.

Type 2 diabetes is characterized by peripheral cells insulin resistance with a consequent persistent hyperglycemia. Insulin production and secretion is often maintained with normal or high serum insulin and C-peptide. It usually presents in adulthood and is often associated with obesity and a sedentary lifestyle.

According to the WHO, diabetes incidence and prevalence is increasing [3] at a particularly alarming rate among children. This has led to a shift in the paradigm of age of presentation of type 2 diabetes with an ever-increasing incidence in young adults, and consequent presentation of diabetes complications at younger ages [4].

The first line of treatment for type 2 diabetes is lifestyle modification, followed by oral antidiabetic agents. The mechanism of action of oral agents is diverse, from reduction in glucose absorption, to increase in insulin secretion, stimulation of gluconeogenesis, or reduction of tubular reabsorption with an increase in urinary glucose excretion. Despite these alternatives, several patients still need exogenous insulin to achieve glycemic control. Chronic insulin resistance may lead to β cell exhaustion, and insulin and C-peptide levels may decrease below normal range in patients with long-standing type 2 diabetes.

3. Indications for pancreas transplantation

Transplant of β cells is a treatment alternative to insulin-dependent diabetic patients with the objective of reestablishing glucose homeostasis without the need for exogenous insulin.

Both pancreas and islet transplantation are currently used in clinical practice as β cell mass transplant techniques. Islet transplantation is still limited to nearly experimental protocols, though a few centers have introduced them into their clinical practice. According to the Collaborative Islet Transplant Registry (CITR), 1927 procedures have been performed worldwide from 2004 to 2013 [5]. Though conceptually attractive, its high cost of isolation and the sub-optimal insulin-independency results (when compared to whole-organ transplantation) have halted its clinical application.

Pancreas transplantation is indicated in patients with insulin deficiency and end-stage renal disease (ESRD) in those or with brittle diabetes and normal renal function. Indications should take into account disease-related characteristics:

a. Type of DM: pancreas transplantation is indicated in patients with type 1 DM, selected patients with type 2 DM, as well as to those with diabetes secondary other etiologies(acute

and chronic pancreatitis, cystic fibrosis, and trauma). According to data obtained from the last US Pancreas Transplant Registry (OPTN/SRTR), pancreas transplantation in type 2 diabetes is increasing worldwide [6], representing up to 8% of all transplants performed in the US [7]. The most recent report demonstrates a 3-year graft survival of 83.3%. Indication in these patients is not consensual. At our center, we indicate in patients <50 years, body mass index (BMI) <30, at least 5 years of insulin therapy, C-Peptide <3.0 ng/mL, and daily insulin at <0.5 U/kg/day. Larger cohorts, standardized inclusion criteria, and long-term results are warranted.

b. Age: an age limit is not established. Though it is usual to accept candidates up to the age of 50 and assessing individually those aged between 50 and 55 years, some groups accept patients who are >60 years old [8].

c. Diabetic complications: the presence and severity of these complications, at the time the patient is studied for transplantation, is another parameter to assess. Successful pancreas transplantation requires suitable vascular permeability for arterial and venous anastomosis. Presence of severe calcifications in the iliac vessels, where the vascular anastomoses of the organs are usually performed, as well as the existence of a severe peripheral vascular disease, can technically allow the implantation of a graft, but it is inadvisable to implant both organs. In these cases, priority is given to kidney transplantation. Coronary heart disease is also a frequent contraindication for pancreas transplantation. The implantation of two organs requires a major surgery with longer anesthesia time and a greater probability of presenting some type of complication or surgical re-intervention. Other secondary diabetic complications, such as retinopathy and neuropathy, rarely represent by themselves as a contraindication for the transplant, but due consideration should be given to the patient during pre-transplant evaluation.

3.1. Indications according to transplant modality

Pancreas transplantation can be performed individually or simultaneously with kidney transplantation in patients with ESRD. Each type of transplant has certain characteristics that must be highlighted.

3.1.1. Simultaneous transplantation of pancreas and kidney (SPK)

Simultaneous kidney-pancreas transplantation (SPK) is the most common type of pancreas transplant, representing over 98% of all pancreas transplants performed in the US [7]. It is currently the best treatment alternative to patients with ESRD who are candidates to a kidney transplant. In addition to the demographic and clinical parameters previously described, immunological and waiting-list vintage should be considered when proposing a patient to an SPK. The presence of HLA alloantibodies reduces the probability of finding a suitable donor and increases waiting-list vintage. In moderate (cPRA > 50%) and highly sensitized (cPRA > 90%) patients, an individual approach is advised

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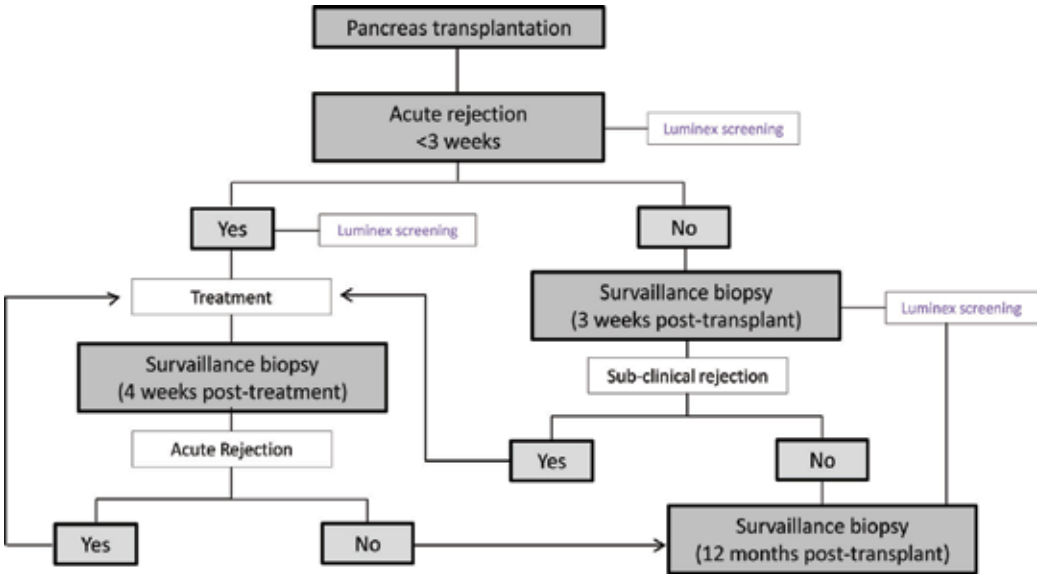

Figure 1. Hospital Clinic's flowchart for performing pancreas graft biopsy. Ultrasound-guided survaillance biopsies are performed at 3 weeks and 12 months post-transplant. Biopsies are also performed if clinicaly indicated, and are followed by a survaillance biopsy 4 weeks after the treatment of rejection.

and a kidney transplant followed by a pancreas transplant alone should be considered. Similarly, centers with long pancreas waiting lists (>2 years) should evaluate the risk of maintaining the patient on dialysis or use a pancrease after kidney transplantation (PAK) approach, since mortality on the waiting list can be up to 42% at 4 years in this population (**Figure 1**) [9].

3.1.2. Pancreas after kidney transplantation (PAK)

This type of transplant is considered for those patients who are candidates for a kidney and pancreas transplant and who have an available living kidney donor or where waiting-list vintage is significantly shorter to kidney when compared to pancreas transplant. The major benefit of PAK is reducing, or avoiding, time on dialysis while waiting for pancreas transplantation. Restoration of kidney function may reduce uremia-induced anticoagulation and possibly reduce bleeding during surgical complications. This approach implies, nonetheless, two different surgical procedures. Moreover, up-to-date results are somewhat poorer for patients who have undergone PAK transplant, with an inferior pancreas graft survival and higher acute rejection incidence [10]. The decision to perform a PAK (live donor kidney) or an SPK will depend fundamentally on the characteristics of the living donor (age, HLA compatibility), the possibility of performing a preventive transplant, the expected time on the waiting list, as well as the patient's expectations (**Table 2**).

	SPK	PAK
Advantages	**a.** Single surgical procedure **b.** Single cycle of induction immunosuppression **c.** Better graft survival	**a.** Minimizes or avoids the need for dialysis (in LDKT) **b.** Shorter surgical procedure **c.** Avoids uremia-asociated complications **d.** Time to pancreas transplantation usually shorter than for SPK
Disadvantages	**a.** Longer waiting-list time **b.** Lower probability of receiving kidney transplant preemptively	**a.** Two surgical procedures **b.** Two cycles of induction immunosuppression **c.** Higher incidence of acute rejection **d.** Inferior pancreas graft survival

Table 2. Pancreas after kidney transplantation: pros and cons compared to SPK.

This alternative has been gaining increasing interest in recent years due to the current shortage of cadaveric organs from young donors and the consequent increase in waiting-list time for kidney-pancreas transplantation. In some centers, PAK represents up to 50% of pancreas transplants, most of them having received a kidney transplant from a previously living donor.

The indications regarding age, type of DM, and vascular status of the recipient would be the same as in the case of SPK. However, a functioning kidney graft with good and stable renal function (glomerular filtration rate—GFR > 40 ml/min) is recommended prior to inclusion on the waiting list, due to the risk of acute kidney injury following the pancreas transplantation surgery and the increase in doses of immunosuppressors.

One of the issues raised in this type of transplant has been on when to perform pancreas transplantation after the kidney transplantation. There is no established time limit, and it depends on the progression of each patient following the kidney transplant. However, it seems that a better survival of the pancreatic graft has been observed when the interval between both transplants is less than 12 months. For some authors, the optimal interval between both procedures should be less than 4 months.

3.1.3. Pancreas transplant alone

Isolated pancreas transplantation in diabetics without documented kidney disease, and with little or no other secondary complication, would theoretically be the ideal transplant. These would be the ones who could benefit the most from the positive effects of this transplant, by being able to prevent the appearance of secondary complications, thanks to an early metabolic control. However, it is considered that the risk of the intervention, as well as the risk of

immunosuppression to which the patient must be subjected for life does not always justify the hypothetical advantages of the transplant.

The results obtained with the PTA are somewhat worse than those of the PAK and SPK. The incidence of technical complications (mainly graft thrombosis) and acute rejection is somewhat higher to the other types of transplant.

It is performed to patients with brittle diabetes and normal renal function, who require repeated hospital admissions due to metabolic decompensation and/or severe hypoglycemic unawareness. These should be confirmed during a hospital stay following treatment optimization using insulin pump and/or continuous glycemic control monitors. It may also be performed to patients with the brittle diabetes and incipient diabetic complications, in order to reduce progression of secondary complications.

The main indications, as well as the absolute and relative contraindications for pancreas transplantation, are presented in **Table 3**.

Indications
SPK: diabetes mellitus and end-stage renal disease (GFR <20 ml/min or dialysis)
PAK: diabetes mellitus and functioning kidney transplant
PTA: Type 1 diabetes with normal renal function (GFR >60 ml/min; proteinuria <1 g/day), and:
• Not aware of severe hypoglycemia (life threatening)
• Frequent hospital admission due to metabolic complications
• Failure to achieve glycemic control using other alternatives—such as insulin pump and/or continuous glycaemic control monitors—during a hospital admission
Absolute contraindications
Severe untreatable coronary heart disease; severe left ventricular disfunction
Chronic liver or pulmonary disease
Active infection
Active or past cancer without adequate remission period (excluded in situ and skin epitheliums)
Severe psycologic or psychiatric disease; drug and alchool abuse
Morbid obesity (BMI >35 kg/m^2)
Active gastrointestinal bleeding
Relative contraindications
Age: <18 and >55 years old
Obesity (BMI < 30 kg/m^2)
Recent acute coronary heart disease
Recent retinal hemorhage
Symptomatic cerebrovascular or peripheral vascular diseases
Severe autonomic neuropathy or diabetic gastropathy
Active smoking

Table 3. Indications and contraindications to pancreas transplantation.

4. Waiting list for pancreas transplantation

4.1. Evaluation of candidates

Evaluation of the candidates for pancreas transplantation should be performed as early as possible, in order to identify those who would benefit the most from the procedure. In patients with chronic kidney disease, we recommend referral to a pancreas transplant center as soon as glomerular filtration rate (GFR) falls below 25–30 ml/min. This early referral offers precious time for patient evaluation and possible inclusion on the waiting-list pre-dialysis. Additionally, and depending on transplant center policies, this allows the study of a possible living kidney donor and a preemptive kidney transplant.

Patient evaluation and clinical workup is similar to that performed for kidney transplantation, such as complete medical history, immunological study, uremic state, liver disease, cancer and infection screening, with some additional particularities related to diabetic disease: hormonal study, β cell autoantibodies, as well as study of the main diabetic complications.

- Hormonal assessment: the main purpose is to determine whether or not the patient has endogenous insulin secretion. For this, it is sufficient to determine the fasting plasma levels of C-Peptide. Its negativity indicates the absence of insulin secretion.
- Autoantibodies: the main objective of pre-transplant quantification of β cell autoantibodies (IAA, GAD, ZnT8A, IA2) is to establish a baseline. These tend to be negative after years of evolution of DM, and therefore negative at the time of transplantation. Their presence does not represent a contraindication for pancreas transplantation. During follow-up, nonetheless, its reappearance is associated with an increased risk for disease relapse.
- Diabetic retinopathy is present in up to 90% of of all transplant candidates, with varying degrees of severity. It is not considered an exclusion criteria for transplantation.
- Diabetic polyneuropathy is also present in majority of patients but rarely contraindicates the transplant. However, it is advisable to take into account the severe dysfunction of the autonomic nervous system, due to post-transplant complications and the negative impact on patient survival. Diabetic neuropathy can often affect the urinary bladder leading to incontinence or incomplete bladder emptying. If urinary exocrine drainage is used, a urinary urethrocystography is recommended to rule out pathology of the bladder and urethra, as well as a cystomanometry to study and evaluate bladder function.
- Cardiovascular evaluation is the most important due to the impact of cardiovascular disease on post-transplant mortality and morbidity. Previous history of myocardial infarction, angioplasty, or coronary bypass should not necessarily be a contraindication to transplantation. Workup should be exhaustive before including the patient on the waiting list. It is advisable to perform an electrocardiogram (EKG) and a pharmacological stress test with MIBI-dipyridamole, as well as an echocardiography to evaluate ventricular ejection fraction and exclude motility disorders. If any of these tests are pathological, coronary angiography should be performed to identify more accurately the existing lesions and

perform the appropriate treatment prior to transplantation (either angioplasty or coronary artery bypass graft—CABG). A recent acute miocardial infarction, untreatable significant coronary angiography lesions, or severe ventricular dysfunction are contraindications to transplantation (**Table 3**).

- Vascular evaluation: an angio-computed tomography (CT) should be performed to rule out vascular lesions, mainly at the level of the iliac vessels and the celiac trunk that could hinder the implantation of the grafts. In pre-dialysis patients, a magnetic resonance imaging (MRI) with no or low dose of low-risk gadolinum contrast can be used.
- Assessment by the transplant team: once the study of potential candidates has been completed, and before being included on the waiting list, it is advisable to carry out a joint assessment by all the members of the transplant team (nephrologist, endocrinologist, anesthesiologist, and surgeons).

4.2. Inclusion on the waiting list

At the time of inclusion on the waiting list, a checklist should be performed to ensure all pre-transplant studies have been completed, and revised by the medical team. It is important to ensure that the patient has received a clear and comprehensible information regarding the advantages, as well as of the possible complications of the transplant, so that he can decide to freely choose this form of treatment.

Logistical issues should also be discussed in advance with the patient, in order to minimize the time from patient contact to the surgical procedure (hence cold ischemia time). The patient and his closest relatives should be aware of the expected duration of the intervention, median hospital stay, and most important post-transplant cares and outpatient visits.

Also, and for as long as they remain on the waiting list, the patient should be made aware of the importance of maintaining regular communication with the transplant center. The high incidence of complications that may occur in these diabetic patients, especially if they are affected by chronic renal failure and are also waiting for a simultaneous kidney transplant, requires strict monitoring and follow-up as long as they are not transplanted. Ideally, they should be visited by a member or collaborating doctor of the transplant team every 3–4 months. Only in this way, it is possible to detect possible events that may represent a temporary contraindication for the intervention.

4.3. Pancreas allocation

Solid organ allocation protocols are the major influence on patients' waiting-list vintage. In the UK, the introduction of a new nationwide allocation system in December 2010 significantly reduced the number of long-lasting patients and increased the number of islet transplants [11]. These protocols must comply with national legislations and logistic constraints. Several factors must be taken into account, such as donor demographics, donor center, geographical proximity to transplant center, and recipient priority indexes. In the UK protocol, weighting factors included expected organ travel time, recipient sensitization, dialysis vintage, waiting-list time,

HLA mismatching, and donor BMI (differential weighting for islet or whole-organ transplantation). Other allocation protocols, such as the US and the Eurtotransplant, also include in their weighting factors donor, recipient and center characteristics.

5. Donor selection

Pancreas blood supply is performed under low-flux conditions. This low blood flow rate increases the risk of surgical complications, such as thrombosis and ischemia. In addition, exocrine pancreas produces a large amount of protein cleavage enzymes, making it very susceptible to ischemia-reperfusion injury during transplantation. Pancreas present the highest donor discard rate among abdominal solid organ transplantations, with up to 33% of all pancreas being discarded by surgical teams prior to pancreas extraction, and an additional 50% being discarded following the extraction due to macroscopic appearance.

In an attempt to standardize donor acceptance criteria and predict short-term pancreas graft function, several scoring systems have been developed. P-PASS was one of the first to be described and was used in the Eurotransplant area to increase sensitivity in allocation. It categorized donors in low (<17) or high (≥17) risk donors [12]. The initial enthusiasm was halted by the reports of its inability to predict short- and long-term graft survival [13].

In 2010, Axelrod et al. published a complex scoring system, including donor and recipient variables, which enabled to predict 1-year graft survival [14]. Despite the promising results, it lacked several key factors, which are thought to influence outcomes, such as previous cardiac arrest in donors after brain death and perfusion solution. In 2013, Finger et al. demonstrated that the presence of at least two factors such as BMI ≥30 kg/m^2, donor creatinine ≥2.5 mg/dL, donor age > 50 years, and preservation time > 20 h were associated with technical failure [15].

Donors after brain death (DBD) have been the most widely used deceased donors since late 1980s; donors after cardiocirulatory death (DCD) were the first deceased donors used for organ transplantation in many countries until a brain death diagnosis and its acceptance for organ donation was legislated. Since the mid-2000s, DCDs regained protagonism as a potential source to increase donor pool, with an increasing number of transplanted organs ever since. DCDs should be evaluated carefully, since the definition includes donors with different backgrounds. According to the Maastricht classification, DCD donors can be classified from type I–V (**Table 4**) [16]. For pancreas transplantation, both type II (uncontrolled) and type III (controlled) have been used. Results from single center and registry analysis suggest

Type of donor	Management of cardiac arrest
Type I—brought in dead	Uncontrolled
Type II—unsuccessful resuscitation	Uncontrolled
Type III—awaiting cardiac arrest	Controlled
Type V—cardiac arrest after brain-stem death	Uncontrolled
Type V—cardiac arrest in a hospital inpatient (added in 2000)	Uncontrolled

Table 4. Maastricht classification [16].

that DCD donors are a suitable source of organs for pancreas and islet transplantation in selected donors [17]. Age limit acceptance is usually lower for DBD donors (<45 years), and both warm and cold ischemia times should be strictly respected, at the risk of increased surgical complications.

Table 5 describes the acceptance criteria for both DBD and DCD donors at our center. In summary, all donors under 45 years without other risk factors, with BMI ≤30 kg/m^2, and transaminases and pancreatic enzymes <3× normal values are accepted for transplantation, regardless of being DBD or DCD. Beyond those criteria, individual evaluations are performed.

Characteristic	DBD donors	DCD donors
Donor age (years)	≤45 (46–55 evaluate individually)	≤45
Donor BMI (kg/m^2)	≤30	≤30
Expected cold ischaemia time (h)	≤12 h (>12 h evaluate individually)	≤8 h (>8 h evaluate individually)
Hepatic transaminases (times above normal value)	<3×s	<3×s
Pancreatic enzymes (times above normal value)	<3×s	<3×s
Warm ischaemia (minutes)	—	• Total (TWIT): <60 • Functional (FWIT): <30 • Hemodynamic instability (SBP <60 mmHg) prior to donation: <60
Clinical risk factors	• Arterial HT • Smoking • Alcoholism • History of pancreatitis	• Arterial HT • Smoking • Alcoholism • History of pancreatitis

Table 5. Hospital clinic pancreas donor acceptance criteria.

6. Surgical techniques

William Kelly and Richard Lillehei performed the first pancreas transplant at the University of Minnesota on December 17, 1966 [18]. In the last decades, the progress in immunosuppressive treatment has been parallel to a decrease in postoperative complications, to an improvement in the surgical technique, and ultimately to a better survival of both the graft and the patient.

The correct evaluation of the viability of the pancreas at the time of extraction in the donor is one of the basic pillars to obtain good results in the recipient. This must invariably be accompanied by a correct surgical technique during the extraction and implantation of the organ.

6.1. Pancreas extraction

Adequate donor selection is crucial in pancreas transplantation, as described in the previous section. The extraction technique is of well-documented importance for a successful outcome [19]. Whether it is advocated for an enteric or bladder drainage, it requires the extraction of the entire pancreas and a segment of the duodenum with its vascularization—perfused by the celiac trunk and superior mesenteric artery—and drained by the portal vein. As this vascularization is shared with the liver, surgical techniques have been developed to allow the simultaneous extraction of both organs. In specific cases of hemodynamic instability, rapid or block extraction must be performed in order to perfuse the preservation solution as quickly as possible.

The surgery begins with a xipho-pubic incision, with sternotomy and opening of the pericardium. The first step is to carry out a thorough examination of all the organs to identify any pathology that contraindicates the donation. It is important to have vascular control to allow rapid cannulation in case of instability, performing the dissection and individualization with ligatures of the aorta above the iliac bifurcation and the infrarenal cava, as well as the inferior mesenteric vein, in the case of portal vein being cannulated through it. The superior mesenteric artery is then dissected, located above and to the left of the confluence of the left renal vein with the cava, and a vessel loop is passed around it.

A first visual evaluation of the organ is performed, after the opening of the smaller sac, sectioning the gastrocolic ligament, to expose the entire anterior surface of the body and tail of the pancreas, together with palpation of the pancreatic head. The next phase comprises the dissection of the hepatic hilum to identify the possible anatomical variants of the hepatic artery. The most frequent are the right hepatic artery from the superior mesenteric artery and the left hepatic artery that derives from the stomatologic coronary artery. The common bile duct is dissected and sectioned at its most distal part. An incision is made in the gallbladder fundus and physiological serum injected into the fundus from the bile duct. The gastroduodenal artery and the hepatic artery are identified and dissected at the celiac trunk. In addition, the left gastric artery and the coronary vein are also identified, as well as all the lymphatic vessels in the upper border of the pancreas. The splenic artery is individualized and referenced with 6/0 prolene suture to prevent its retraction in the pancreas. A silk ligature must be passed through the abdominal aorta above the celiac trunk, following the blunt dissection of the esophageal hiatus. Finally, the dissection of the portal vein is carried out after identifying the stomachatric coronary vein. It is important to perform the Kocher maneuver in order to access the entire duodenum and the posterior aspect of the pancreatic head. The dissection of the pancreas must be done through the "no touch technique." For the release of the pancreatic inferior aspect, mobilization of the entire transverse colon to the splenic angle is required. Subsequently, all the ligaments that fix the spleen to the retroperitoneum are sectioned for its separation from the kidney and the left adrenal gland, as well as the fixation of the body and tail to the retroperitoneum. Likewise, the section of the short gastrosplenic vessels and the dissection of the duodenum below the pylorus and at the level of the fourth portion is completed, for its subsequent sectioning to these two levels by means of a self-suture device.

Once the dissection is completed intrathoracically and abdominally, and prior to cannulation, intravenous sodium heparin (3 mg/kg) is administered. The aorta is then cannulated above the bifurcation, together with the cannulation of the portal system (through the inferior, superior, or portal mesenteric vein) and the supraceliac aorta is clamped to initiate perfusion of the aorta with preservation solution, at which time the vena cava is drained after opening it intrathoracically or through a drainage cannula placed in the inferior vena cava. At this time, crushed ice is placed on the organs to keep them at a suitable temperature. After completing the infusion, pancreas and liver are separated in situ. It is generally accepted that the celiac trunk must go with the liver. The splenic artery divides right after its origin from the celiac trunk. The aorta, at the level of the superior mesenteric artery, is sectioned laterally to visualize the origin of the renal arteries. The superior mesenteric artery must be ligated after the origin of the inferior pancreaticoduodenal artery. In short, the aortic patch is divided into two—the liver with the celiac trunk, and the pancreas—with the superior mesenteric artery. The infrahepatic vena cava is sectioned above the origin of the renal veins. The suprahepatic vena cava is divided along with the diaphragm that surrounds it. Finally, the portal is divided halfway between the liver and the pancreas. Finally, the pancreas is removed once the liver is removed. Some surgeons prefer to perform the extraction of both organs en block and perform their separation on the bench.

The iliac vessels (common iliac arteries/veins together with their bifurcations) are extracted and sent along with pancreas and liver grafts if they are needed for vascular reconstructions during the implant.

The organ is introduced in a sterile bag with preservation solution at 4°C. This bag is protected by inserting it into two other bags and transported to the recipient hospital. Bench surgery can be performed at the extraction site or later at reference hospital.

6.2. Bench surgery

During the bench surgery, the duodenum-pancreatic graft is prepared. This must remain in conditions of hypothermia at 4°C until its implantation.

After ligation of the splenic vessels, splenectomy is performed. If a fatty pancreas is found to be present, it should be removed carefully, making the necessary sutures to minimize the hemorrhage during reperfusion. It is advisable to invaginate the line of staples of the duodenal ends (with continuous 3/0 silk suture, although it is variable depending on the group), to ensure maximum suture tightness and avoid further fistulas.

In case of absence of celiac trunk (usual in simultaneous liver and pancreas extractions), it will be necessary to carry out reconstructions of the arterial vascularization of the pancreas that allow a good anastomosis with the iliac vessels of the recipient.

There are different techniques of vascular reconstruction of the pancreatic graft:

1. Anastomosis of the arteries of the pancreas with a segment of the iliac bifurcation of the donor. It is the most used modality in the USA and Europe.

2. Spleno-mesenteric termino-terminal anastomosis between the splenic artery and the distal end of the superior mesenteric artery of the graft. For some groups, it constitutes the technique of choice for its simplicity.
3. Spleno-mesenteric termino-lateral anastomosis between the splenic artery and the superior mesenteric artery of the graft.

Once the bech surgery is performed, graft is perfused with about 100 cc of preservation solution and is ready to be implanted in the recipient.

6.3. Pancreas implantation

The simultaneous kidney and pancreas transplantation is the most frequent transplant modality performed worldwide. The surgical technique used for the implantation of the renal graft does not differ from that used for kidney transplant alone. For pancreas transplantation, although the surgical technique is not standard among centers, there is unanimous agreement in implanting the complete organ, including the second portion of the duodenum.

Traditionally, the intraperitoneal position has been preferred by most groups. In the last decade, different authors have suggested the implant of the graft in a retroperitoneal location, advocating a more physiological position [20].

The pancreas shloud be implanted prior to the kidney, given its worse tolerance to cold ischemia. The best way to perform the transplant is with a supra-infraumbilical midline laparotomy, from a point midway between the xiphoid and the umbilicus up to 2–3 cm of the pubis. The complete pancreas with a small portion of the donor's duodenum, which contains the Vater's ampulla, is located laterally in the right iliac fossa of the recipient. The cranial or caudal position of the head of the pancreas depends on each group. Placing the pancreas on the left side increases the risk of graft thrombosis.

The intervention begins with the dissection of the ureter and the right iliac vessels. These should be dissected and mobilized widely to facilitate subsequent vascular anastomoses. Hemostasis must be carefully performed, and the major lymphatic vessels must be ligated. To facilitate the venous anastomosis of the portal, it is advisable to mobilize the distal vena cava and the right iliac vein.

Once the iliac vessels are dissected, the venous anastomosis is performed first, between the portal vein of the graft and the most proximal part of the right primitive iliac vein or on the cava before the iliac bifurcation. Before starting the anastomosis, the vena cava is perfused with heparin (1 mg in 100 cc). The termino-terminal venous anastomosis is performed with two continuous sutures of Prolene 5/0.

The arterial anastomosis is then carried out between the right primitive iliac artery of the recipient and the superior mesenteric artery or the segment of the iliac artery of the graft, depending on the bench surgery performed. From the beginning of the anastomosis, the graft should be kept refrigerated by compresses of crushed ice. Once the arterial anastomosis is completed, the vessels are sequentially declamped, first the vein and then the artery. Pancreas should recover a normal coloration immediately.

Systemic venous drainage is most widely used. Some groups advocate the use of portal venous drainage for the hypothetical benefit of maintaining a more physiological insulin level and thus avoiding the hyperinsulinemia attributed to the systemic drainage. However, technically, it is more complex and its potential metabolic advantages are still controversial.

Pancreatic exocrine secretion can be drained to the urinary tract or to the intestinal tract.

The urinary drainage (duodenocistostomy) contributed extraordinarily to consolidate the pancreas transplant, since it allows to monitor the rejection by the determination of pancreatic enzymes in the urine. However, the high incidence of complications associated with it require the conversion to enteric drainage in 15–30% of cases [20].

Therefore, today, enteric drainage is the technique of election. The most common method of enteric drainage is the one in which the anastomosis is performed between the duodenum of the graft and the jejunum of the recipient, with the pancreatic graft positioned intraperitoneally. The enteric anastomosis can be performed to the proximal jejunum or to the distal ileum, in a termino-terminal, termino-lateral, or a latero-lateral anastomosis. The use of direct anastomosis is currently more widely used than Roux-en-Y anastomosis. However, Boggi et al. [21] have shown excellent results with the use of a Roux-shaped "Y" latero-lateral duodenojejunostomy (DY), with the retroperitoneal position of the pancreatic graft, and portal venous drainage. Exocrine drainage techniques to the stomach have also been described [22]. Duodenoduodenostomy (DD) is an interesting option for the drainage of digestive secretions when the pancreas is placed behind the right colon and is oriented in the cranial direction [23]. For the placement of the graft in the retroperitoneal position, the right colon is released medially together with the Kocher maneuver, so that the native duodenum is widely exposed. After the correct mobilization of the duodenum of the recipient, the latero-lateral anastomosis (2.5–3 cm) is performed between the duodenum of the graft and the second and third duodenal portion of the recipient with a doble suture, one internal for the mucosa with resorbable material (Vicryl 3/0) and a seromuscular external one, with nonabsorbable suture (3/0 silk). After this, the right colon is repositioned to its usual position so that the pancreas remains immobile [24].

After the end of the intestinal anastomosis, the peritoneal cavity is washed with povidone-iodine serum. Some groups perform the wash with antibiotic solution to minimize the risk of peripancreatic infection and mycotic aneurysms. The peritoneum is then closed and the surgical field is prepared for the kidney implant on the left side.

6.4. Surgical complications

The absence of complications after pancreas transplantation depends largely on the detailed knowledge of both the donor and the recipient. Therefore, to minimize morbidity, postoperative care begins at the pre-operative and intraoperative periods.

The first 24–48 h is the most crucial for the graft and the recipient due to (a) the surgical trauma to which the patient has been subjected, (b) the ischemia-reperfusion phenomena of the transplanted organ, and (c) immunosuppression. As expected, the combination of these three insults, especially in a diabetic patient with vascular complications, constitutes a challenge for the entire medical and surgical team.

Surgical complications are relevant since they can lead to graft loss. From 1983 to 1987, 25% of the pancreas transplants performed in the world were lost due to technical reasons [25]. However, in the last decade, the percentage of surgical morbidity has decreased drastically [6].

In general, the main complications of pancreas transplantation, in addition to the general complications of solid organ transplants, include those that are more specific as a consequence of certain organ characteristics: low vascular flow and exocrine component.

There are a number of factors that significantly increase the risk of developing surgical complications such as a donor and recipient body mass index >30 kg/m^2, organ preservation time >20 h, cause of death of the non-traumatic donor, and to a lesser extent the intestinal drainage of pancreatic exocrine secretion.

Following are the main surgical complications:

- Vascular complications: arterial or venous thrombosis represents one of the most frequent causes of early graft loss (5–10%). The incidence of thrombosis ranges from 5 to 10% in the simultaneous transplantation of pancreas-kidney and 10–20% in isolated pancreas transplantation. It usually happens to be a venous thrombosis (60%) and appears in the first few days of transplant evolution [26].

  The causes are still not fully understood: technical mistakes when performing vascular anastomoses, prothrombotic disorders and hypercoagulability, microvascular injuries produced during the period of extraction and preservation of the graft, as well as hemodynamic instability that reduce the intrinsic flow. It has also been associated with factors related to the donor, as the age and the cause of death, or a prolonged cold ischemia time.

  Doppler ultrasound in expert hands is the most available image technique to diagnose thrombosis. Computed tomography is used for the evaluation of vascular anastomoses as well as to rule out the presence of other abdominal complications. Arteriography may be used to confirm the diagnosis in cases of partial or total pancreatic vessel thrombosis and even interventional radiology may be necessary.

  In cases of total thrombosis, thrombolysis or thrombectomy should be attempted urgently by performing interventional radiology, and in cases where this is not possible or fails, surgical thrombectomy or transplantectomy should be performed. In partial venous thrombosis, if the thrombus occupies more than two-thrids of the lumen of the vessel, endovascular treatment may be attempted, and in the rest, heparinization. This has made it possible to reduce graft loss due to venous thrombosis to less than 1% [27].

  Other vascular complications of the pancreatic graft include hemorrhage, arteriovenous fistulas, pseudoaneurysms, and stenoses of the anastomoses. **Table 6** summarizes the most important complications observed in pancreas transplant recipients for each period.

- Intestinal complications: they usually present at the anastomosis of the duodenal segment. Its incidence has decreased considerably in recent years, and currently less than 1% of the grafts are lost due to this cause [28]. Its incidence ranges at 5–20% in bladder drainage and between 5 and 8% in the intestinal drainage. Early fistulas are usually attributed to ischemia or technical failure, while later fistulas are usually caused by infections or acute rejection.

They represent the second cause of relaparotomy after hemorrhage. The treatment depends on the type of derivation of the exocrine secretion and the importance of the leak.

- Graft pancreatitis: increase in serum amylase and lypase is common after pancreas transplantation, due to both factors inherent to the donor and lesions that the pancreas can suffer during extraction, preservation, implantation, and reperfusion. They are usually self-limited and do not tend to have an impact on the graft outcome. However, hyperamylasemia may be indicative of true graft pancreatitis, with symptoms that may include fever, abdominal pain, ileus, and abdominal distension. Pancreatitis that appears after the first weeks following transplantation is usually secondary to an acute rejection or infections (such as cytomegalovirus—CMV). In patients with bladder drainage, they can also be attributed to reflux of urine through the pancreatic duct. As a consequence of graft pancreatitis, fistulas, peripancreatic collections or abscesses, and pancreatic pseudocysts may occur.

Post-transplant period	**Complications**
Pre-trasplant	Graft damage during organ procurement:
	• *Vascular lesions (splenic artery, SMA, portal vein)*
	• *Duodenal lesion*
	• *Damage to pancreatic capsule or parenquima*
Peri-transplant	Acute surgical or post-surgical complications:
	• *Vascular lesion (recipient severe atheromatosis)*
	• *Hemorrhage*
	• *Pancreatitis*
	• *Inadequate graft perfusion*
	• *Cardiovascular morbidity*
Post-trasplant	Vascular complications
	• *Graft thrombosis (60% venous, 40% arterial)*
	• *Late vascular complications (anastomosisstenosis, pseudoaneurisms, arteriovenous fistulas)*
	• *Vascular complications of kidney graft (in SPK)*
	Infection of surgical wound
	Incomplete healing of surgical wound
	Intra-abdominal infection
	Fistulas due to duodenal leaks
	Graft pancreatitis
	Pancreatic pseudocysts
	Panreatic leak
	Hemorrhage (intra-abdominal, bladder, gastrointestinal)
	Urological complications
	Infections (bacteria, viral, fungal)

Table 6. Complications according to the time of appearance.

- Infections: they are frequent in this group of transplant recipients (80% throughout the first year), and they play an important role in the patient and graft survival. Diabetes, surgery, and immunosuppression are factors that predispose these patients to suffer infections of all types. Pancreas transplantation presents a risk of infection by CMV of 13–17%, largely due to the use of potent induction immnunosuppression. CMV infection is associated with increases in mortality, the rate of rejection, and the presentation of other types of infections. The incidence of intra-abdominal infections is 10–30%, most of them polymicrobial, with fungi present in less than 10% [29]. The current prophylaxis schemes (against bacterial, viral, and fungal infections), established from the moment of intervention, have managed to reduce its incidence in the short term. However, they still need to be monitored in the longer term.

7. Perioperative management

Recipients of pancreas transplantation are diabetic patients most often with a disease vintage over 10 years and frequently with secondary macro- and microvascular complications. The cardiovascular risk is superior to those of general population or recipients of kidney transplant alone. The perioperative management is of crucial importance not only to avoid the risk of hemodynamic instability and periods of low perfusion of the graft, but is also vital for organs such as brain and heart.

7.1. Volume, acid-base and electrolyte, and hemodynamic stability

Volume and electrolytes should be monitored closely during the first 48 h and fluids administered accordingly to avoid hypovolemia or acid-base and electrolyte inbalances. Although an individualized assessment should be performed in each case, it is considered appropriate to maintain a central venous pressure between 5 and 10 mmHG. The administration of fluids with dextrose should be avoided, as it may prolong the need for insulin.

Since most patients are also recipients of a kidney transplant, close monitoring of urinary output must be performed simultaneously. In the event of polyuria (urinary output >150 ml/h), aggressive volume reposition should be perfomed, usually at a rate of 1:1 during the first 24 h, and thereafter at a rate of 0.7:1 to avoind prolonging the polyuria. Fluid solution should be selected according to acid–base and electrolyte homeostasis, with 0.9% or 0.45% sodium chloride often being the first line of treatment.

In the event of delayed graft function and oliguria (urinary output <50 ml/day), fluids should be restricted to those needed for the minimum daily calories and electrolyte intake to avoid hastening the need for dialysis intended for volume management. When needed, dialysis modality (continuous vs. intermittent) should be discussed with the nephrologist and risk benefits must be weighed—intermittent dialysis may be performed with the need for anticoagulation, and with low ultrafiltration volumes, reducing the risk of surgical complications, while continous dialysis reduces hemodynamic instability and therefore decreases the risk of reducing organ perfusion.

Anemia is frequent among patients with ESRD. It is important to maintain adequate levels of hemoglobin (Hgb >10 mg/dl), especially in the case of postoperative bleeding. Controversy exists regarding the need for immediate anticoagulation (vide Section 8—prophylaxis).

Both hypotension and hypertension should be avoided. A systolic blood pressure < 100 mmHG increases the risk of arterial and venous thrombosis of the graft, especially in the immediate postoperative period. On the other hand, prolonged severe hypertension can lead to a stroke or myocardial infarction and may increase the risk of intra-abdominal hemorrhage. It is advisable to maintain the systolic pressure between 120 and 160 mmHg during the first 24 h post-transplant to ensure adequate perfusion of the graft and minimize the risk of adverse effects.

7.2. Graft function

The immediate evaluation of the graft (both pancreatic and renal, in the case of SPK) can be monitored in various ways. The protocol accepted by most centers combines the use of laboratory parameters together with image tests. The decrease in blood levels of blood urea nitrogen (BUN), creatinine, amylase and lipase is required, together with blood glucose levels within normality, to consider that the grafts function correctly (in case of SPK). Blood levels of amylases and lipases provide additional information regarding pancreatic injury. In the immediate postoperative period, blood levels of pancreatic enzymes may be elevated, with normal blood glucose levels, which translates into an ischemia-reperfusion injury, and usually resolves spontaneously. In cases of exocrine drainage to urinary bladder, the level of amylases in urine can be monitored. A decrease of 50% or more is suggestive of rejection or pancreatitis.

7.3. Image diagnosis

In the post-transplant period, radiological examinations should be perfomed to evaluate graft perfusion and exclude surgical complications, such as collections or thrombosis. Most centers rely on ultrasound as their preferred method, since it is easy to use, nontoxic, and may be pefomed as often as needed. When available, computerized tomography may provide further information, such as contrast-enhanced evaluation of arterial perfusion and venous drainage, as well as exclude possible hemorrhages. Herein, we describe in detail advantages of each option.

7.3.1. Color Doppler Ultrasound

It is the initial imaging technique for control and monitoring of pancreas transplantation. The study with electronic data capture (EDC) allows to assess the size and the structure of the graft, the presence of liquid collections (study in B mode), and the perfusion of the parenchyma (resistance index), as well as the permeability of the vascular anastomoses (Doppler study). An extension of the study can also be done with the ultrasound signal enhancer, if it is considered appropriate by the sonographer who performs the study. It is advisable to make a basal study, between 24 and 48 h post-transplant, and a follow-up study, every 3–4 days until the patient's discharge.

7.3.2. Abdominal CT scan

In some cases, Doppler ultrasound may be technically limited (abdominal distension, obesity) or the study might need to be extended, such as in the patient with abdominal pain, fever, and/or graft dysfunction, intra-abdominal collection not accessible to ultrasound drainage, intra-abdominal collection drained by ultrasound, but without adequate clinical response. If a vascular pathology or bleeding is suspected, a contrast-enhanced CT scan is advised.

7.3.3. Interventional radiology

Interventional radiology may be used for diagnostic confirmation and/or treatment (thrombectomy) of a partial arterial and/or venous thrombosis of the graft.

7.4. Monitoring of vascular thrombosis

Thrombosis is the most common vascular complication in the initial post-transplant (8–10 days post-transplant) period. Therefore, early diagnosis is important to establish adequate treatment.

If a first post-transplant imaging study to confirm the vascular permeability of the graft (splenic and mesenteric artery/vein) is not achieved due to bowel distention, the decision whether or not to extend the radiological procedure will be based on pancreas functionality: (1) in normal functioning graft the study is repeated in 24–48 h, (2) if dysfunctional the study is extended to a non-invasive imaging technique, such as angio-CT. Some groups advocate performing angio-CT as standard monitoring image technique due to its ability to establish a grading score for venous thrombosis [30]. In our experience, in the presence of an experienced radiologist, Doppler ultrasound, with or without contrast-enhanced ultrasound (CEUS), is a reliable screening technique.

During the first 48-h post-transplant, the patient usually stays in the intensive care unit, to be transferred later to the conventional hospital ward if there have been no adverse effects. Progressively, the oral intake is introduced and the abdominal drainage is removed. Before hospital discharge, it is important to to give a detailed description of medication and home care to the patient.

8. Immunosuppression and prophylaxis

8.1. Immunosuppression

Advances in immunosuppression protocols during the last two decades significantely increased short- and long-term pancreas graft survival. The purpose of immunosuppression is control alloimmune response, and protocols often include a combination of different drugs to minimize the damage to the graft and the risks to the patient. They are similar in all solid organ transplants. Nonetheless, it is of particular relevance in pancreas transplant recipients due to the increased risk of acute rejection, especially in recipients of pancreas transplant alone (PTA). The greatest burden of immunosuppression is then usually administered in the recipients of a PTA.

Immunosuppression is usually dived into two major categories—induction and maintenance. The former represents treatments used in the peri-transplant period alone, with the objective of inhibiting both the innate and adaptative immune response when the graft is first exposed to recipient immune system. The drugs used are the same as for other solid organ transplantations and have been described in other chapters. The next section describes how and what drugs are used in pancreas transplantation:

8.1.1. Induction therapy

This consists of the administration of a polyclonal or monoclonal antibodies and is currently assumed as standard treatment for pancreas transplantation. These decrease the incidence of acute rejection or delay its onset, and reduce the number of steroid-resistant rejections. Depleting T-cell antibodies may be polyclonal, most widely used, such as rabbit anti-thymocyte globulin (Thymoglobulin®/ATG-Fresenius®) or monoclonal, such as the anti-CD52 alemtuzumab (Campath®); among non-depleting monoclonal antibodies, anti-IL-2 receptor (anti-CD-25; basiliximab) is the most frequently used.

There is no consensus on which is the best protocol. Depleting antibodies appear to increase graft survival by reducing acute rejection risk [8] and are the most widely used. Nonetheless, due to financial constraints and also due to an increased infection and cancer risk associated with T-cell depleting agents, some groups use monoclonal anti-IL-2 antibodies in low-immunological risk simultaneous pancreas-kidney tranplantation.

8.1.2. Maintenance treatment

As an adjunctive treatment to induction therapy, and as long-term maintenance immunosuppression, a combination of three drugs is most often used: a calcineurin inhibitor (CNI), an anti-prolipherative, and steroids.

The discovery of cyclosporine 35 years ago marked a new era in solid organ transplantation. The incidence of acute rejection was drasticly reduced, and despite an increased risk for renal calcineurin toxicity and subsequent renal failure, the patient and graft survivals observed a spetacular improvement. Tacrolimus (or FK-506), also a CNI, exhibits a better and more potent immunsoppression profile and is currently considered the drug of choice in pancreas transplantation. CNI's act by inhibiting the transduction of the first signal between antigen-presenting cells and T-cells. Several comparative studies have shown a lower incidence of acute rejection, as well as a lower severity of rejection and a better survival of the pancreatic graft in the short and long term, in those patients treated with tacrolimus.

CNI is often associated with an anti-prolipherative agent. Their action focuses on a different pathway of the T- and B-cell activation and prolipheration. Azathioprine, the first to be used, arrests cell cycle in the G2 phase, inhibiting the progress to the M phase and subsequent clonal expansion. On the other hand, antimetabolite agents (mycophenolate-mofetil or mycophenolate sodium) inhibit nucleotide syntisis, removing the substrate to DNA replication, finally achieving the same result as azathioprine—prevents cell prolipheration. Finally, the latest agent to be introduced to solid organ transplantation were mammalian target of rapamycin

(mTOR)-inhibitors (sirolimus and everolimus). Both act as anti-prolipherative drugs by inactivating the mTOR pathway following the receptor CD25 activation by antigen-presenting cells.

Steroids are perhaps the most widely used immunosuppressive durgs for organ transplantation. Steroids present a pleotropic effect, with an action on both innate and adaptive immune responses. Steroids reduce antigen-presenting cells' cytokine transcription and secretion, reducing the ability of innate immune system to further recruit polynuclear cells. It also inhibits activation of mononuclear cells, such as T- and B-cells. Despite the great immunosuppressive profile, side effects mandate that these are reduced or withdrawn from maintenance treatment.

Triple therapy using one agent from each category has achieved excellent results. The most widely used combination is steroid, tacrolimus, and mycophenolate. An mTOR-inhibitor may be used instead of mycophenolate, but careful management of side effects should be undertaken. Although the results obtained with this association seem to be superimposable, as far as patient and graft survival is concerned [31], the incidence of complications attributable to rapamycin in the immediate post-transplant is greater, so this combination is not as widely used in the initial period of the transplant. However, it is a good option for long-term use.

Several studies have suggested that steroids can be suppressed as maintenance therapy, especially in patients receiving a calcineurin inhibitor associated with an antimetabolite or an mTOR-inhibitor, without affecting the survival of the grafts. However, there is no consensus regarding this topic [32], due to some reported increased risk of rejection following withdrawal. It seems reasonable that the decision to suppress steroids is focused for the moment on those patients with low-immunological risk, and it should be attempted during the first year of transplantation.

8.2. Prophylactic treatments

In pancreas transplantation, prophylactic treatments are usualy wider than those used in kidney transplantation. As previously stated, pancreas low blood flow, complex vascular anastomosis, the duodenal enteric anastomosis, and the increased infection risk due to persistent hyperglycemia prior to transplantation increase the need for thrombotic and infectious prophylaxis.

Antithrombotic prophylaxis: graft thrombosis is one of the most frequent early complications in pancreas transplantation. Therefore, most transplant centers perform prophylaxis. There is no standard protocol among different centers, but the most frequent is the use of heparin and/or aspirin. Some centers use low doses of intravenous heparin, unlike others who use subcutaneous low-molecular-weight heparin. In both cases, it is important to monitor coagulation parameters and adjust dose to renal function due to uremia-induced anticoagulation and/or anticoagulation used during dialysis sessions. Heparin is often associated with low-dose aspirin, which could be continued in the long term to reduce global cardiovascular risk.

Antimicrobial prophylaxis: infection remains one of the main causes of morbidity and mortality after pancreas transplantation. That is why it is usual to use a wider prophylaxis in these patients. At transplantation and during a variable period of time, broad-spectrum antibiotics to cover Gram negative, Gram positive, and anaerobic are recommended. They are used for 3–5 days and several associations are possible, usually cephalosporin + ampicillin or vancomycin or carbapenem + vancomycin depending on local post-transplant epidemiology. Antifungal prophylaxis with fluconazole is also often performed. Currently, some prophylactic guidelines have replaced fluconazole with a new drug, micafungin, with the advantage of avoiding interaction with tacrolimus. Since most patients receive induction treatment with polyclonal antibodies, which is well known to increase the risk of infections, especially viral infections, antiviral prophylaxis with valgancyclovir for CMV is also advisable. Finally, prophylaxis to pneumocystis jirovecii with trimetropin-sulfamethoxazole for 6 months is the treatment method, as used in kidney transplantation.

9. Long-term outcomes and complications

Pancreas transplant outcomes have increased in the last decades, with a median graft survival using current protocols up to 15 years. In order to achieve these outcomes, close ambulatory controls must be perfomed during the first year, with increasing the time between outpatient visits if follow-up is unremarkable. It is usual to perform a weekly control during the first 3 months post-Tx, biweekly until 6 months, and between 6 and 12 months on a monthly basis. They focus primarily on functional graft monitoring, immunosuppression, and complications secondary to diabetes.

To assess pancreatic graft functionalism, baseline glycemia, glycosylated hemoglobin (HbA1c), as well as serum amylases and lipases is determined in each outpatient follow-up. In the post-transplant period, after hospital discharge and again 1 year after transplant, it is convenient to perform an oral glucose tolerance test (OGTT). Subsequently, and as a follow-up guideline, the intervals between these analyses varies according to the teams. It is also advisable to perform a C-peptide determination to monitor insulin secretion throughout the follow-up, as well as the determination of anti-glutamic acid decarboxylase (GAD), in order to detect a possible recurrence of diabetic disease. Both should be checked at least once a year.

During patient follow-up, it is important to control secondary complications of diabetes. Despite having a functioning pancreas graft, recipients should continue to monitor secondary complications present prior to pancreas transplantation, such as diabetic retinopathy or macrovascular complications. Some patients experience an improvement of complications present prior to transplantation, particularly in neuropathic symptoms. As to macro- and microvascular complications, most lesions tend to stabilize [33]. Therefore, it is advisable to perform an annual ophthalmological examination, regularly assess neuropathy of both peripheral and autonomic nervous system, as well as having a special surveillance to the complications related to the vasculopathy, appearance of precordial pain, or peripheral ischemic lesions.

Not least important, patients should be advised to maintain a proper diet to avoid weight gain, promote physical activity, avoid sun exposure, prohibit or limit the consumption of alcohol and smoking, recommend suitable footwear to avoid chafing and periodic podiatric check-up, and, in women of childbearing age, recommend measures of contraception to avoid pregnancy during the first 1–2 years of the transplant.

9.1. Acute and chronic rejection

The incidence of acute rejection is higher in pancreas transplant than those reported in kidney transplantation. The actual incidence must be individualized according to transplant modality, recipient immunological risk, induction and maintenance immunosuppression, and transplant follow-up period.

Overall 1-year rejection incidence varies between 14.7% [34] and 21% [10]. PTA and PAK are associated with significantly higher incidence of acute rejection than SPK recipients [34]. Other risk factors include pancreas re-transplantation [10], absence of induction therapy or basiliximab and induction agent [8], donor age, number of HLA donor-recipient mismatch [10], and the presence of donor-specific antibodies (DSA) [35, 36].

In SPK recipients, monitoring of renal function and/or the performance of a renal biopsy had been advocated as an indirect method to establish the diagnosis and treatment of pancreas' acute rejection, since for many years it has been considered that acute rejection was present in the majority of the cases simultaneously in both grafts. However, it is now well documented that isolated rejection of one of the two organs occurs in up to 36% of cases [37].

To diagnose pancreatic graft rejection, the only biochemical markers available are pancreatic enzymes (amylases and lipases), which are elevated in most of these episodes. Lipase increase is more specific than amylase [10]. On the other hand, in acute rejection, it is possible to observe changes in graft size and eco-structure, with an increase in the resistance index when performing a Doppler ultrasound. Both parameters allow to establish the diagnosis of suspected acute rejection, but they are not specific enough to establish confirmation. This sometimes leads to unnecessary or incomplete empirical treatments, with the consequent repercussion for the patient and the graft. In addition, pancreatic rejection, as recently observed, can also be mediated by antibodies, which requires a different and specific treatment.

Currently, pancreatic biopsy is considered the gold standard for etiologic diagnosis of graft dysfunction. In our center, pancreatic graft biopsy is performed per protocol at 3 weeks and 12months post-transplant, or indicated in the following circumstances (**Figure 1**): (1) patients in whom the existence of an acute rejection of the pancreatic graft is suspected due to biochemical parameters (increase in serum glycemia, amylases, and lipases), and/or ultrasound (increase in size, changes in the graft structure, and involvement of the graft), (2) in whom the existence of a chronic rejection is suspected due to a persistent increase in amylases or serum lipases, progressive increase in glycemia and glycosylated hemoglobin, and/or progressive decrease in C-peptide secretion, and (3) in whom the recurrence of diabetic disease is suspected due to detection or progressive increase of anti-GAD antibodies, and pathologic oral glucose tolerance test. To establish the severity of the histological lesion of acute rejection, the Banff scheme should be taken as reference [38].

Pancreas' acute rejection can be successfully treated using steroids, polyclonal antibodies, and/or plasma exchange and immunoglobulins. Nonetheless, up tp 20% of grafts may be lost during the first year following an acute rejection [10].

9.2. Diabetes relapse

Type 1 diabetes is the most frequent indication for pancreas transplantation. As described in the first section of this chapter, DM1 is an autoimmune disease, characterized by autoantibodies directed to β cells. Disease relapse in pancreas graft is a well-known risk for graft failure. It has been reported in up to 7% of all transplant recipients [39]. Induction and maintenance immunosuppression are likely the reason for such a low incidence. Some factors have been associated with an increased risk for disease relapse, such as donor-recipient sharing of HLA-DR alleles, particularly HLA-DR3 [39], and the increase in autoantibodies, particularly ZnT8A, predicts the risk of disease relapse [40]. As with primary disease, no treatment is established for the management of disease relapse. Increase in baseline immunosuppression maybe attempted. If a pancreas re-transplantation is indicated, recipients must be advised of the risk of relapse in the new graft.

10. Islet transplantation

In addition to pancreas transplantation, β cell mass transfer may be performed through islet transplantation. This procedure consists of islet cell isolation from pancreas as potential donors using both mechanical and enzymatic digestion protocol, following which islets are separated using a Ricordi chamber [41]. Isolated islets are infused into recipient's portal vein, without the need for vascular and/or enteric anastomosis, reducing the surgical risks of the procedure.

The attractiveness of a minimally invasive procedure and the possibility of multiple infusions without the surgical risks associated with whole-organ pancreas transplantation have positioned islet cell transplantation as a promising treatment for patients with diabetes. Despite these advantages, islet transplantation presents two critical drawbacks when compared to whole-organ transplant. Islets obtained from a minimum of 2–3 pancreasas are often needed in order to achieve euglycemia [41], increasing organ demand, and risk of recipients' sensitization. Additionally, islets direct engraftment into a vascularized bed exposes β cells to platelets and lymphocytes. Instant blood-mediated inflammatory reaction (IBMIR) is a well characterized event associated with innate immune reaction and coagulation and complement activation following islet transplantation, leading up to 25% of total islet cell mass loss following vena cava infusion [42]. Moreover, it may increase the risk of rejection and reduces overall graft survival.

In early 2000, the Edmonton group published some promising results using an induction immunosuppression protocol [41]. By the end of 2015, over 15,000 procedures had been performed worldwide [5]. Reported 1- and 5-year insulin independence is up to 80 and 50%, respectively [5].

Novel biocompatible implantation devices and gene editing tools foresee a bright future for islet transplantation. 3D-bioprinting is being used to build an islet-blood interface which enables β cell survival and insulin production, while avoiding immune activation and graft rejection [43]. On the other hand, generation of human-induced pluripotent stem cells (hiPSCs) has opened the door for personalized medicine and cell-based therapy. iPSCs can proliferate unlimitedly in culture and harbor the potential to generate all cell types in the adult body. Generation of hiIPSC-derived β cells has been published by several groups, using different protocol, with varying degrees of success [44, 45]. The advantage is the possibility to generate tailor-made islet cells, particularly β cells, from recipient-derived hiPSC, evading the risk of alo-rejection. One can envision in the near future hiPSC-derived cells implanted in a biocompatible device for the treatment of type 1 diabetic patient.

For the time being, islet cell transplantation remains at an almost investigation level due to smaller insulin independence when compared to pancreas transplantation. Nonetheless, it is a suitable option for selected type 1 diabetic patients, and to those with a surgical contraindication for whole-organ transplantation. Finally, islet transplantation presents a promising future as the techinique of election for β cell mass transfer.

11. Conclusion

Pancreas transplantation is the best treatment alternative for patients with end-stage renal disease and type 1 diabetes and may be found in the selected group of type 2 diabetic patients. Patient and graft survival have greatly increased in the last decades, particularly due to improvements in surgical procedures and immunosuppression protocols, with graft half-life of 15 years. Following transplantation, patients should be carefully monitored due to the risk of acute rejection, disease relapse, or diabetic secondary complications present prior to the transplant.

Conflict of interest

The authors decline any conflict of interests.

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Chapter 14

Intestinal Transplantation

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Additional information is available at the end of the chapter

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Abstract

Intestinal transplantation (ITx) has evolved in the past few decades moving from an experimental procedure to a life-changing modality for patients suffering from intestinal failure (IF). It is particularly for those with complications as a consequence of parenteral nutrition and/or who have a high risk of dying due to their underlying disease. In addition to this, intestinal transplantation is also increasingly considered for the treatment of conventionally unresectable abdominal tumors. With advancements in immunosuppressive drugs, induction regimens, standardization of surgical techniques and improved postoperative care, survival is increasing. The ultimate goal for intestinal transplantation would be to become as good and safe as total parenteral nutrition (TPN) and as such, it could become a viable first-line option of intestinal failure.

Keywords: intestinal failure, parenteral nutrition, multivisceral, intestine, liver, immunosuppression, intestinal transplant

1. History

Lillehei was the first to report an experimental isolated intestinal canine model in 1959 and Starzl reported the first multivisceral experimental canine model in 1960 [1, 2]. The first attempt in humans has been attributed to Deterling in Boston in 1964 [unpublished], whereas the first official report of human intestinal transplant was made by Lillehei in 1967 [1]. It should be noted that the first successful series were reported in the 1990s, coinciding with the introduction of more effective immunosuppression. The first attempts of intestinal transplantation (ITx) in the 1970s were largely disappointing because of high incidence of rejection of small bowel allografts, sepsis and technical complications [3]. The introduction of tacrolimus [4] revolutionized interest in ITx. The superior clinical outcomes from tacrolimus across a

variety of organ transplantation compared with cyclosporine set the momentum for ITx as a life-changing therapy for patients with irreversible intestinal failure (IF) [5, 6].

Over the past 30 years, there has been a gradual increase in ITx cases, with nearly 2900 ITx cases performed worldwide, although there has been a decline in recent years [7]. This change could be attributed to the formation of specialized IF units to prevent and manage intestinal failure-associated liver disease (IFALD) [8]. Other possible factors include: inadequate reimbursement rates below the cost of performing the transplant; the extensive infrastructure demands required to address the frequent social problems of IF patients; concern over the narrow risk-benefit ratio for ITx in an era of improving outcomes with long-term total parenteral nutrition (TPN) for selected diseases [9] and/or the limited availability of experienced personnel to fill key positions. Finally, some transplant centers may be more willing to judiciously offer isolated liver transplants to patients with the short bowel syndrome and IFALD who have the potential for further intestinal adaptation [10, 11].

2. Indications

IF is characterized by the inability to maintain protein energy, fluid, electrolyte or micronutrient balance due to gastrointestinal disease. If the patient does not receive parenteral nutrition or become a recipient of an intestinal transplant, IF ultimately leads to malnutrition and even death.

The leading causes of IF differ between pediatric and adult populations (**Table 1**).

TPN is the current standard of care for patients with IF. Nevertheless, as survival following ITx improves, it is anticipated that ITx will become a valid alternative to total parental nutrition. However, because of significant complications that can arise from surgery and long-term use of immunosuppressive therapy, strict eligibility criteria exist to ensure appropriate patient selection.

Paediatric	Adult
Intestinal atresia	Crohn's disease
Gastroschisis	Superior mesenteric artery thrombosis
Crohn's disease	Superior mesenteric vein thrombosis
Microvillus involution disease	Trauma
Necrotizing enterocolitis	Desmoid tumour
Midgut volvulus	Volvulus
Chronic intestinal pseudo-obstruction	Pseudo-obstruction
Massive resection secondary to tumour	Massive resection secondary to tumour
Hirschsprung disease	Radiation enteritis

Table 1. Leading causes for intestinal transplantation in paediatric and adult populations.

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Short bowel syndrome caused by surgical removal is the leading cause of IF (68%). As an early alternative to transplantation or total parenteral nutrition (TPN) for patients with short bowel syndrome, surgical bowel lengthening without transplant may be attempted. This requires the serial transverse enteroplasty (STEP) or longitudinal intestinal lengthening and tailoring (LILT) procedures. STEP and LILT are particularly successful in patients with decreased transit times and dilated bowel. These procedures lengthen the small bowel while keeping the total surface area the same. Bowel is either split lengthwise or cut obliquely at multiple points. This will lengthen the bowel and shrink the luminal diameter [12]. If successful, this may reduce the amount of TPN required, or negate its use altogether. If patients are not acceptable candidates for STEP or LILT, sometimes a reversal of small bowel direction may effectively increase transit times. If none of these operations are successful, the standard of care is TPN.

Currently, ITx has been mainly performed in patients who developed life-threatening complications attributed to IF and/or long-term TPN. In 2000, Medicare defined failure of TPN as impending or overt liver failure (elevated serum bilirubin and/or liver enzymes, splenomegaly, thrombocytopenia, gastroesophageal varices, coagulopathy, stomal bleeding, hepatic fibrosis or cirrhosis), thrombosis of two or more central veins, frequent and severe central venous catheter (CVC)-related sepsis and frequent severe dehydration [13]. In addition, other conditions associated with early death despite optimal TPN, such as congenital mucosal disorders and ultra-short bowel syndrome, are also included as per American Society of Transplantation guidelines [14].

The European guidelines recommend TPN as primary treatment for patients with IF, with early referral to specialist centers for optimal rehabilitative therapy and timely assessment of suitability for ITx. It recommends assessment for candidacy for transplantation for the presence of one or more indications parallel to American guidelines (failure of TPN, high risk of death attributable to underlying disease and IF with high morbidity or low acceptance of TPN) resulting in rates of transplant of 62, 26 and 12%, respectively [15]. Because of a statistically significant increased risk of death on TPN from liver failure due to IFALD and invasive intra-abdominal desmoids, direct referral for ITx should be considered [16]. IFALD is partly caused by omega-6 fatty acids in TPN formulas, which can be synthesized into inflammatory molecules. IFALD can range from steatohepatitis, cholestasis or hepatic fibrosis to end-stage liver disease. Children are more likely to have cholestatic liver disease than steatohepatitis [17]. Severe liver injury has been reported in as many as 50% of patients with IF who receive TPN for longer than 5 years; this is typically fatal. If patients have life-threatening infections, IFALD, or lose their venous access, 1-year mortality is 70% without ITx [18]. Conversely, patients with CVC-related complications or ultra-short bowel syndrome did not have an increased risk of death on TPN and no patients considered to be an ITx candidate with poor quality of life (QoL) or chronic dehydration actually died while remaining on TPN. This notable finding forms the basis of non-indications in previous European guidelines [19]. Despite very limited evidence exploring the role of quality of life (QoL) as an indicator for ITx, this holistic aspect may also be factored in the decision-making process [20] (**Table 2**).

North America	Europe
Failure of TPN	Impending or overt liver failure due to IFALD-related liver failure
Impending or overt liver failure	
Central venous thrombosis of 2 central veins	
Frequent and severe central venous catheter-related sepsis	
Frequent episodes of severe dehydration despite intravenous fluids in addition to TPN	
High risk of death attributable to underlying disease	CVC-related multiple venous thrombosis (in appropriately selected patients)
Intra-abdominal invasive desmoids tumour	Intra-abdominal desmoids
Intestinal failure with high morbidity and low acceptance of TPN	Individual case by case decision for patients with IF with high morbidity or low acceptance of TPN
Congenital mucosal disorders	
Ultra-short bowel syndrome (<10 cm in infants, <20 cm in adults)	
Need for frequent hospitalization, narcotic addiction or inability to function	
Patient's unwillingness to accept long-term TPN	

Table 2. Intestinal transplantation guidelines.

3. Contraindications

The contraindications of intestinal transplantation are the same as for all other transplants and are frequently reassessed. These include:

1. significant comorbidities;
2. active uncontrolled infections or malignancies that are not totally resectable during the transplant process;
3. psychosocial factors (e.g. lack of post-operative support network);
4. anatomical challenges that can prove the operation high risk such as inferior vena cava (IVC) and portal vein (PV) thrombosis. Previous laparotomies can also complicate the operation significantly; and
5. opiate dependence is very common and rehabilitation should be considered early.

4. Types of intestinal transplant

The choice of transplant type depends on the underlying cause of IF, quality of native organs, state of liver disease (if present) and history of previous abdominal surgeries. The main types of ITx are:

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1. Small bowel transplant only (SBTx) (**Figure 1**): recommended for people with IF who have not developed liver disease. The arterial supply to the allograft is secured with anastomosis between the donor's superior mesenteric artery (SMA) and the recipient's infrarenal aorta; venous drainage is either onto the IVC or the recipient's portal vein (PV) or superior mesenteric vein (SMV) at the root of the mesentery.
2. Liver and small bowel transplant (SBLTx) (**Figure 2**): recommended for people with IF who also have advanced liver disease and extensive portomesenteric venous thrombosis precluding liver transplantation alone. The inclusion of a liver graft in combined liver-small bowel transplant has been associated with improved survival rates [21].
3. Multivisceral transplant (MVTx) (**Figure 3**): recommended for people with multiple organ failure and involves transplanting the stomach, pancreaticoduodenal complex, liver and small bowel.
4. Modified multivisceral transplant (MMVTx) (**Figure 4**): recommended for people with multiple organ failure and involves transplanting the stomach, pancreaticoduodenal complex and small bowel. The difference with the previous type is the exclusion of the liver. It is usually performed in patients with preserved liver function and coexisting pancreatic insufficiency such as patients with chronic pancreatitis, type I diabetes mellitus or cystic fibrosis, patients with intestinal dysmotility with concomitant severe gastroparesis and in cases with tumor involvement of the mesentery or the duodenum (e.g. in Gardner's syndrome) [22].

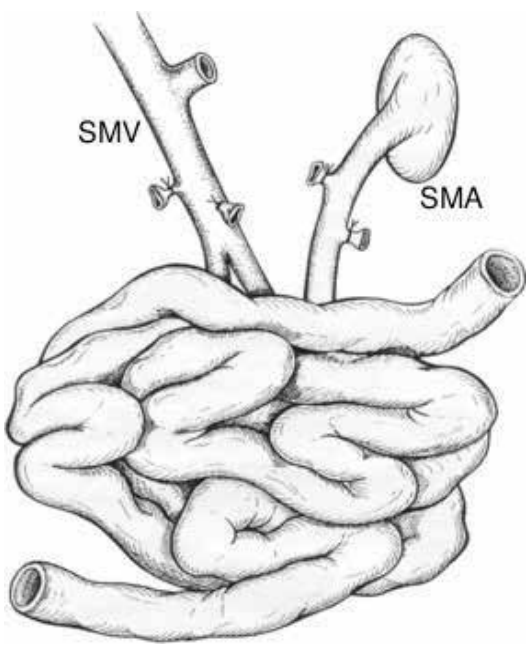

Figure 1. Small bowel transplant (SBTx).

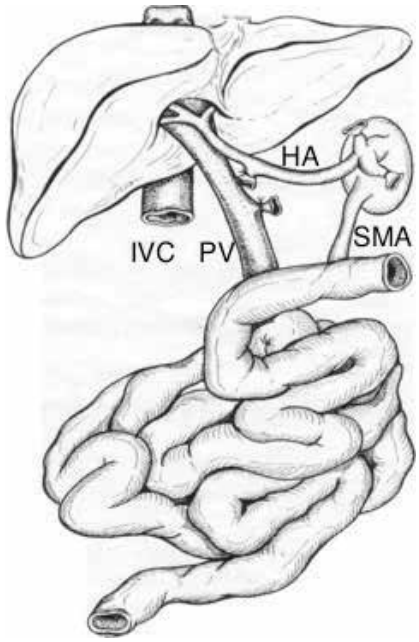

Figure 2. Liver and small bowel transplant (SBLTx).

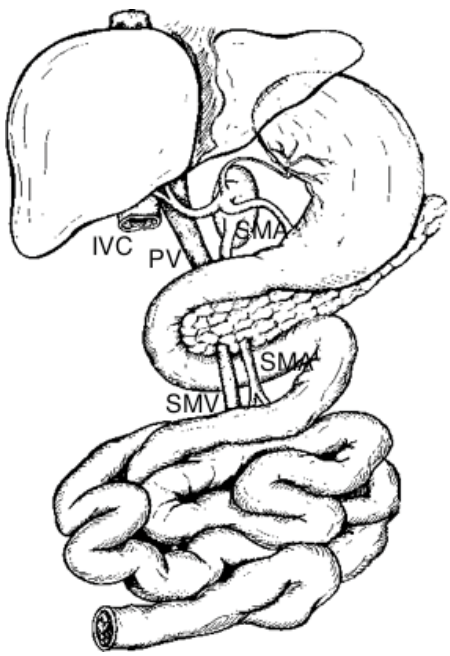

Figure 3. Multivisceral transplant (MVTx).

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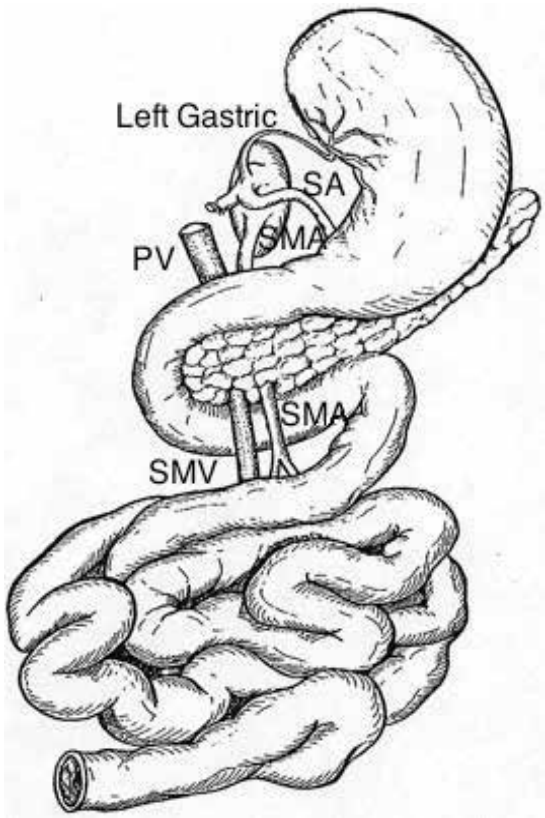

Figure 4. Modified multivisceral transplant (MMVTx).

In all the above ITx types, the right hemicolon can be included depending on the patient's native anatomy. Since 2000, there has been a sixfold increase in the inclusion of a colon segment resulting in a current inclusion rate of 30% [7]. The registry analysis has shown that inclusion of the colon did not adversely affect survival and recipients with a colon segment had a 5% higher rate of independence from supplemental parenteral nutrition (PN), as the retention of the ileocecal valve and the right colon enhance gut function through better fluid absorption and uptake of free fatty acids [23].

It's sometimes possible to carry out a small bowel transplant using a section of bowel donated by a living family member and the first standardized technique was reported by Gruessner and Sharp [24].

Because of previous surgery resulting in loss of abdominal wall domain and integrity, patients undergoing ITx face a problem with primary abdominal wall closure [25]. Surgical techniques such as reduction of the liver portion (left or right lobe) within the composite allograft [26], transplantation of composite abdominal wall tissue graft (**Figure 5**) [27, 28], the use of vascularized rectus sheath [29] and non-vascularized abdominal rectus fascia [30] have revolutionized abdominal wall reconstruction.

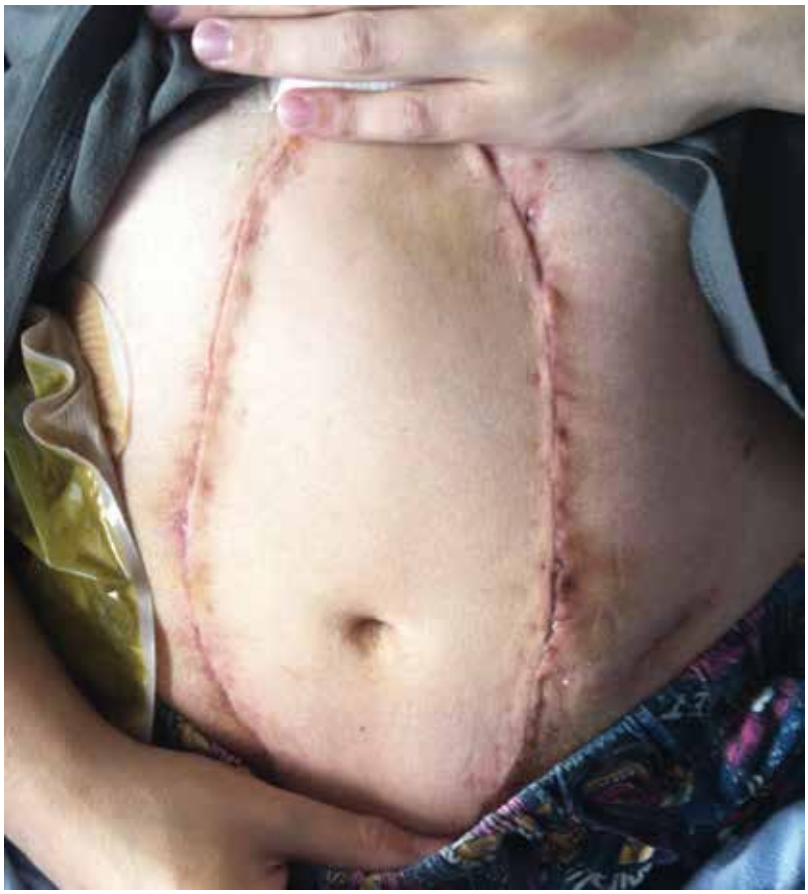

Figure 5. Abdominal wall transplant.

5. Recipient assessment

The assessment of a potential intestinal transplant recipient is robust and rigorous and needs to be done by a multidisciplinary team. This involves transplant surgery, gastroenterology, nutritional services, anesthesia, psychiatry and social work. However, due to the frequently pre-existing multiple comorbidities, consultation with other specialties may be required. Every assessment is 'tailor-made'.

Laboratory studies always include: full blood count (FBC), electrolytes and renal function, coagulation profile, ABO blood group, human leukocyte antigen (HLA) typing, panel-reactive antibody status, HIV and hepatitis B and C virus screening, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) screening.

Liver biopsy is indicated, if liver disease is suspected. The native intestine should be assessed both by imaging and endoscopy.

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Vascular access is of utmost importance and is assessed by magnetic resonance or computed tomography venogram. Securing upper-body vascular access is mandatory in cases where IVC occlusion is anticipated. Many patients will have central venous stenosis or obstruction and will mandate interventional radiology and/or vascular reconstruction before listing.

Manometry of the esophagus, stomach and rectum should be considered in patients with dysmotility disorders.

While on the waiting list, patients should be frequently reassessed, with specific attention given to any change in medical status, deterioration in liver function or vascular access.

6. Surgical technique

6.1. Intestinal retrieval

- Wide access to the abdominal cavity is needed and can be achieved via a midline incision from the suprasternal notch down to the symphysis pubis.
- The ascending colon and hepatic flexure are mobilized by using right-sided medial visceral rotation (Cattel-Braasch maneuver) that will expose the third and fourth portions of the duodenum.
- First, achieve control of the right common iliac artery or the distal abdominal aorta, which need to be mobilized for subsequent insertion of the infusion cannula.
- The structures of the hepatoduodenal ligament will have to be identified and slung for small bowel alone or modified multivisceral transplants.
- Depending on the type of transplant, sling the esophagus, the antrum or proximal jejunum.
- In case of MMVTx, the celiac axis has to be retrieved along with the left gastric and splenic arteries. This should be discussed with the liver implanting team in case of a left aberrant artery.
- Transect the gastrocolic ligament and, in case of large bowel retrieval, identify the middle colic vessels. Mark the transverse colon just distal to the vessels for the insertion of the gastrointestinal anastomosis (GIA) stapler. For small bowel, sling the ileum near the ileocecal valve.
- Expose the mesenteric root, abdominal aorta and infrahepatic IVC, including entry of the renal veins.
- If the pancreas is to be retrieved, the splenic flexure, spleen, and body and tail of pancreas are mobilized to allow adequate subsequent cooling of the pancreas.
- Perform proximal control for supraceliac cross-clamping, either above or below the diaphragm, depending on the presence of a cardiothoracic team.

- Cross-clamping of the supraceliac aorta is performed simultaneously with or immediately following venting of the IVC or atrium and cold perfusion is commenced.
- In situ cooling of abdominal organs, and exsanguination before removing the organs to the back table for preparation.
- University of Wisconsin (UW) Universal Organ Preservation solution for both in situ flushing and cold storage is most frequently used.
- Retrieve iliac, brachiocephalic and/or carotid arteries and veins as potential vascular grafts.

6.2. Back-table preparation of organs

Intestinal grafts require minimal back table; however, this depends on the retrieval technique and the extension of the allograft. Most commonly, back table involves to identify and tie the lymphatics. If the pancreas is retrieved along with a small bowel only graft, then it has to be removed/sacrificed on the back table.

6.3. Recipient operation

- Implantation begins commonly with adhesiolysis, as adhesions are usually abundant secondary to previous surgeries.
- Depending on the type of transplant, the aorta and IVC or SMA and SMV/PV are dissected and mobilized for the vascular anastomosis.
- The proximal and distal ends of the native digestive track are identified and dissected.
- Venous anastomosis to the graft is frequently performed to the recipient cava. However, when possible, venous anastomosis to the portal system is preferred.
- Arterial anastomosis is performed to the abdominal aorta via arterial jump graft.
- After reperfusion of the graft and careful hemostasis, the proximal and distal ends of the intestinal graft are anastomosed to the proximal and distal ends of the native digestive track. In some cases, the distal end is brought out as a permanent stoma.
- Most centers bring out a temporary stoma by utilizing various techniques (e.g. Bishop Koop), for post-operative endoscopic surveillance. This stoma is usually reversed in 6–12 months.
- Closure of the abdominal wall can be very challenging and should not be attempted under tension; if this is the case, keeping the abdominal wall open and planning for a sequential closing is preferable. Some centers are routinely performing abdominal wall transplantation from the same deceased donor in order to achieve closure.

7. Postoperative considerations

Patients are monitored in intensive therapy unit (ITU) post-operatively. It is common practice to administer broad-spectrum intravenous antibiotics and antifungals for 5–7 days post transplantation. Blood tests are sent daily and as well as arterial/venous blood gases to check bleeding and homeostasis.

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Stoma output is monitored daily and will indicate the appropriate timing to resume enteral feeding via nasogastric tube or jejunostomy/gastrostomy. Some centers start elemental enteral feeding very early and gradually increase volumes depending on nasogastric tube aspirates. TPN is maintained for at least 2 weeks and can be discontinued once enteral nutrition is sufficient. Chyle leak can often be seen post-operatively due to the severed intestinal graft lymphatics. A no-fat or low-fat diet (<10 g/day) can be initiated as a first measure. Absorption of long-chain triglycerides, depends on lymphatic drainage, whereas medium-chain triglycerides are directly absorbed into the portal circulation.

Antiviral prophylaxis with intravenous ganciclovir (5 mg/kg OD) is common practice and regular CMV polymerase chain reaction (PCR) DNA tests are sent for monitoring. Oral valganciclovir is usually prescribed for 1-year post transplantation (900 mg OD). Epstein-Barr virus (EBV) is also monitored regularly by PCR. Trimethoprim-sulfamethoxazole is commonly used to prevent pneumocystis pneumonia for 1-year post operatively. Routine cultures are sent from all lines and most centers perform regular intestinal transplant endoscopies and biopsies via the stoma.

Oral medication is generally avoided in the early phase due to the unpredictable absorption and thus, bioavailability. Tacrolimus can be given sublingually and regular trough levels are sent for confirmation.

Plasma citrulline levels have emerged as a measure for overall for intestinal health as it is an indicator of enterocyte mass. However, compromised renal function is an important factor when considering plasma citrulline levels as a marker of intestinal failure as this potentially can increase circulating citrulline values [31]. Reduced citrulline levels can indicate the need for urgent investigations and also, commencement of TPN.

8. Immunosuppression

The intestine is the largest lymphoid organ in the body and hence, appropriate immunosuppression has been a real challenge. The lack of effective immunosuppressive agents hampered the first attempts of ITx in the 1960s. Over the years, advances in immunosuppression have transformed ITx with the intent of attenuating the intestinal allograft immunity and shifting it to a tolerogenic status [32].

Induction strategies to minimize rejection by reducing the recipient's T-cell load were implemented, initially with cyclophosphamide induction therapy, which was later replaced by daclizumab, an interleukin-2 receptor antagonist (IL2RA) [33]. Basiliximab, another IL2RA, in addition to tacrolimus and prednisone immunosuppression has also been utilized and shown to decrease the incidence of acute rejection [34, 35].

Alemtuzumab induction is becoming increasingly popular and Lauro et al. [36] reported significantly less early rejection episodes, with no sepsis implications. The use of Basiliximab monthly as part of maintenance immunosuppression has been associated with a decrease in acute rejection in liver-excluding transplants [37].

Immunosuppression regimen varies, with several protocols having been reported: Tacrolimus and steroids (35.8%) followed by tacrolimus, mycophenolate and steroids (18.7%), tacrolimus and mycophenolate without steroids in 15.4% of cases and tacrolimus alone in 13.8% of cases [38].

Target trough levels of tacrolimus vary between centers. Pittsburgh has reported target levels between 10 and 15 ng/ml in the first 3 months and thereafter 5–10 ng/ml [39]. Tacrolimus with low-dose steroids remains the most effective and durable long-term combination therapy [21] and is the most common maintenance immunosuppressive regimen [7].

Sirolimus, a rapamycin inhibitor, has been shown as a useful adjunct to tacrolimus in the presence of nephrotoxicity or rejection [40]. However, it carries the disadvantage of severe debilitating oromucosal ulceration. Azathioprine and mycophenolate mofetil have also been used as adjunctive immunosuppressive therapies [33]. Mycophenolate mofetil, however, causes symptomatic diarrhea (increased stoma output) and microscopically evident apoptosis in approximately 40% of solid organ transplant recipients, which could regrettably be mistaken for rejection [41].

9. Complications

Complications following ITx may result in graft failure and invariably death. Patients undergoing ITx have a higher incidence of life-threatening infectious complications than other transplant recipients. This is due to the high bacterial load of the transplanted graft [42]. Therefore, any breach to the intestinal transplant mucosal barrier can lead to bacterial translocation.

Graft loss would need TPN resuming and consideration of re-transplantation, which has a lower rate of success compared with the initial transplantation [43]. Common causes of graft loss include allograft rejection, infection, GVHD and post-transplant lymphoproliferative disorder (PTLD) [33].

9.1. Acute rejection

Allograft rejection has always been one of the most significant challenges to long-term graft and patient survival and can occur either as acute (commonly in the early phase, though can occur late) or chronic (typically taking months to years). Rejection can occur at any time but is most common in the first year, particularly the first 6 months. It affects 45% of ITx patients within the first post-transplant year, with implications on graft survival [7] and is mostly characterized by T-cell response to donor antigens.

Acute rejection should be suspected in all cases of bowel dysfunction (increased stoma output) and symptoms include fever, vomiting, abdominal pain and distension. Diagnosing acute rejection is always challenging and requires a combination of clinical, endoscopic and histopathological investigations. Intestinal allograft endoscopy and biopsies is the gold standard. However, diagnosis can be difficult to establish because of the patchy nature of rejection. Not to mention, that it is not always easy to differentiate between rejection and infection.

Most centers will endeavor to perform early, frequent endoscopies because of high prevalence of acute rejection in the early post transplantation phase [21] and then continue with regular endoscopies as part of their surveillance protocol [44].

The Oxford group, who is utilizing vascularized composite allograft (VCA) transplants from the same donor [45], is now mostly relying on the VCA as a surrogate marker for rejection and is not strictly adherent to early, intensive intestinal biopsy protocols [46]. They have reported that the VCA can provide lead time (about 7 days) before the onset of bowel dysfunction and this could be proven as a unique prognostic tool [45].

Rejection episodes are usually treated with pulses of methylprednisolone, and in resistant cases, thymoglobulin [32] or alemtuzumab [46].

A recent case series reported good outcomes in ITx of positive cross-match patients with only one patient developing acute rejection, with the use of intravenous immunoglobulin (IVIG) and rituximab [47]. Bortezomib, a proteasome inhibitor, has also been recently shown to be effective against donor-specific antibody (DSA)-related rejection [48]. Recently, Eculizumab, a monoclonal antibody that inhibits complement factor 5a, was shown to be effective in maintaining a 2-year rejection-free period in a highly sensitized patient [49]; however, high costs make this approach prohibitive for general use.

As mentioned before, biomarkers such as citrulline have gained interest in recent years. The prospective cohort study by Hibi et al. [50] reported excellent negative predictive value (range 93–99%) for citrulline levels as exclusionary marker for all types of acute rejection (cut-off point, 20 mmol/l) and moderate or severe acute cellular rejection (cut-off point, 10 mmol/l). Another study by the Bologna group [51] showed that citrulline sensitivity and specificity in detecting acute rejection, when adjusted for chronic renal failure, almost doubled the sensitivity of citrulline as a non-invasive marker of acute rejection in ITx. In general, citrulline can act as an exclusionary tool, as high levels are unlikely to be found in intestinal allograft rejection.

Recently, a large series evaluating video capsule endoscopy has shown to be potentially useful in the diagnostic management of patients with ITx [52]. Other non-invasive predictors of rejection include a recent retrospective study that revealed low insulin-like growth factor-1 and high calprotectin levels during malnutrition states post-ITx, and these findings should prompt the clinicians to investigate for acute rejection or infection [53]. Circulating apoptotic T cells following Caspase 3 activation may be a non-invasive marker for patients who are less likely to have rejection episodes than those who have lower levels [54].

9.2. Chronic rejection

Chronic rejection is diagnosed histologically with the identification of an obliterative arteriopathy in medium-sized vessels in the serosal layer with diffuse concentric intimal thickening [55]. This necessitates full-thickness biopsy and makes diagnosis challenging. Chronic rejection is clinically associated with diarrhea, ulceration, focal loss of mucosal folds, mural thickening and pruning of mesenteric artery arcades [55]. Surgery in such a hostile environment may lead to unwanted enterotomies and fistulae. Re-transplantation should therefore

be considered. Evisceration is a potential life-saving option for ITx patients who developed severe rejection, and has similar survival rates with patients who underwent simultaneous enterectomy with re-transplantation; however, high sensitization may prevent re-transplantation [56].

9.3. Donor-specific antibodies

Preformed and de-novo DSAs have been associated with acute rejection and may be implicated in chronic rejection and graft loss [46, 57]. Five-year graft survival of less than 30% was noted in ITx patients who developed de-novo DSA, whereas survival rates of more than 80% were observed in recipients without DSA [44]. Yet, others have not found a statistically significant trend towards worsening outcomes [58] between those with or without de-novo DSA formation.

9.4. Infection

The use of immunosuppression in ITx poses a significant risk of infection and, historically, high levels of immunosuppressants have been utilized in ITx. Sepsis remains the most common cause of death and graft failure, accounting for over 50% of cases [7]. Bacterial infections are common in the early phase post-ITx and have a significant impact on patient survival. Invasive candidiasis has been reported as the commonest fungal infection [59].

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) viraemias are common and can be implicated in acute and chronic rejection as well as PTLD [60, 61]. CMV status is vital to anticipate the risk of developing de-novo infection in a non-infected recipient from the donor and through reactivation of a latent infection. CMV prophylaxis with oral valganciclovir is often continued for 1 year following ITx [62]. Most programs would not accept CMV-positive donors for CMV-negative recipients as this is high risk and should be avoided [63]. Last but not least, it has been demonstrated that isolated graft CMV disease without overt CMV viraemia can indeed occur [64].

9.5. Post-transplant lymphoproliferative disorder

In the updated 2016 WHO lymphoma classification, PTLD has been sub-classified as plasmacytic hyperplasia PTLD, infectious mononucleosis PTLD, florid follicular hyperplasia PTLD, polymorphic PTLD, monomorphic PTLD and classical Hodgkin lymphoma PTLD [65]. PTLD following SOT occurs in up to 20% with the highest incidence in intestinal and multi-organ transplants (5–20%), followed by lung and heart transplants (2–10%) and then by renal and liver transplants [66].

About 70% of cases of PTLD are associated with Epstein-Barr virus (EBV), especially in cases which occur early after transplantation. Pathogenesis of the disease is linked to EBV proliferation in the setting of chronic immunosuppression leading to an inhibition of T-cells immune function. However, in 30% of EBV-negative PTLD patients, pathogenesis is not clearly understood [67].

It is believed that in ITx, the lymphatic-rich intestinal allografts in combination with splenectomy and immunosuppression pose an increased risk for PTLD [68]. It has been reported that the presence of PTLD has significantly reduced patient survival within the first post-transplant year [69]. PTLD is treated with immunosuppression reduction and rituximab containing chemotherapy regimes in the presence of CD20 positive cases [70].

9.6. Graft versus host disease

The small intestine has abundant lymphoid cells that can mount an immunologic response to the host (i.e. a graft versus host disease [GVHD]) reaction. GVHD occurs in 5–7% of intestinal transplants, compared to 1% for solid organ transplants, and risk factors include younger age, MVTx recipients and intra-operative splenectomy [71]. It is more common in intestinal transplants due to the large volume of lymphatic tissue that is transplanted along with the graft. GVHD diagnosis is allegedly difficult to establish and patients usually present 1–8 weeks after transplantation with skin rash, ulceration of oral mucosa, diarrhea, lymphadenopathy or native liver dysfunction. Diagnosis is confirmed by skin or bowel biopsy.

Treatment strategies vary and most frequently involve tacrolimus, high-dose steroids or anti-thymocyte globulin (ATG) [71].

9.7. Renal dysfunction

Renal dysfunction is invariably seen post ITx due to a combination of high-dose tacrolimus therapy [72] and dehydration episodes, especially in the presence of stomas. The incidence of stage 4 or 5 CKD is 21.3% in patients with ITx [73] and approximately 9% of ITx patients will receive renal replacement therapy by a median of 7.4 years [74].

10. Graft and patient survival

Patient survival has been steadily improving because of improved first-year graft survival [7]. Graft survival has been reported for 1, 5 and 10 years at 74, 42 and 26%, respectively (SBTx); 70, 50 and 40% (MVTx); 61, 46 and 40% (SBLTx); overall patient survival was 76, 56 and 43%, respectively. Studies evaluating 1-year and 5-year patient survival rates at various transplant centers revealed comparable results [75, 76].

Patients on TPN have 1-year, 5-year, 10-year and 20-year survival of 91–93, 70–71, 55–59 and 28%, respectively, following IF commencement [77]. It should be noted, that 11–15% of deaths while on TPN were attributed to TPN complications (5–6% from sepsis or central-line sepsis and 6–9% from IFALD) [77]. A three-year prospective study reported 94 vs. 87% three-year survival in TPN non-transplant candidates vs. TPN transplant candidates who did not undergo transplantation. In addition, the three-year survival was 89 vs. 85 vs. 70% for those having first SBTx vs. transplant candidates with central venous catheter complications vs. candidates with parenteral nutrition-related liver failure [78].

11. Quality of life

Quality of life plays an important role in the decision-making for ITx candidates. ITx patients have reported better fatigue, gastrointestinal symptoms, stoma management/bowel movements, ability to holiday/travel and global health/QoL and probably better eating ability but worse sleeping patterns [79]. Others have found that ITx recipients have similar QoL to those who are stable on TPN, but both are better than those with complicated intestinal failure on TPN [80].

12. Future perspectives

ITx and its secrets have not been deciphered yet. The main issue remains the challenging balance between appropriate immunosuppression and over-immunosuppression. Therefore, ITx teams have been trying to develop markers that will provide adequate warning in case of rejection [45].

Multivisceral organ transplantation may solidify its role in the treatment of slow-growing abdominal cancers that are deemed "non-resectable" [81–83].

Another challenge will be to understand the physiology of the transplanted small bowel, such as its altered microflora and altered motility. New research has suggested that the flora composition within the graft may be a risk factor for acute rejection when more immunogenic species predominate [84].

Tolerance remains the 'Holy Grail' of transplantation and is characterized by increased allograft survival in the absence of immunosuppression and absent or reduced donor-specific response. Groups have used donor-specific blood transfusion in order to induce tolerance by upregulating graft protective memory Tregs [85]. Also, centers have introduced experimental models to induce microchimerism and tolerance by transplanting bone marrow along with the intestinal allograft [86]. These protocols could allow for sufficient immunosuppression with lower doses of immunosuppressants. This ongoing research may change the future of ITx.

13. Conclusion(s)

ITx continues to evolve and graft survival rates are nowadays more comparable with the results of other solid organ transplants. The main challenge is to develop immunosuppression protocols that can ensure long-term intestine graft function and less infectious complications. When this is accomplished ITx could potentially change from being a life-saving treatment to becoming a realistic first-line therapy for IF.

Conflict of interest

The author declares no conflict of interest.

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Chapter 15

Cardiovascular Diseases in Patients with Renal Transplantation

Daniela Loncar and Selma Hodzic

Additional information is available at the end of the chapter

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Abstract

Kidney transplantation has become the primary method of treating severe chronic renal failure. The first successful kidney transplant was performed in 1954 in Boston, the graft was in function for 7 years, and patient died because of the heart disease. Cardiovascular disease is the leading cause of death in patients with a transplanted kidney. Despite the fact that patients with a transplanted kidney are highly susceptible to infections and have an increased tendency to develop malignant diseases, these patients die mainly of cardiovascular disease. Patients with a transplanted kidney are exposed to atherogenic risk which is associated with previous dialysis treatment and the use of immunosuppressive drugs. An excessive risk of developing cardiovascular disease in patients with a transplanted kidney is due to the high frequency and accumulation of atherogenic risk factors before and after transplantation. Pre-transplant cardiovascular disease is a major risk factor for the development of post-transplant cardiovascular disease. Risk factors for the development of cardiovascular diseases in patients with a transplanted kidney are divided into traditional and nontraditional. Traditional risk factors such as immutable (age, gender, and inheritance) and variable (smoking, hyperlipidemia, hypertension, obesity, diabetes mellitus, physical activity, stress). Nontraditional risk factors such as risk factors related to the status of transplantation and its treatment and risk factors associated with chronic regression in allograft function. The most common cardiovascular diseases in patients after kidney transplantation are as follows: ischemic heart disease, congestive heart failure and left ventricular hypertrophy. Of all cardiovascular complications, ischemic heart disease is by far the most common cause of mortality (more than 50%) in patients with a transplanted kidney. Frequency of left ventricular hypertrophy ranges from 50 to 70% in patients with a transplanted kidney. Early detection of high-risk patients for the development of cardiovascular diseases allows timely application of an appropriate therapeutic strategy that ensures high survival rates for patients with a transplanted kidney.

Keywords: kidney transplantation, risk factors, traditional risk factors, nontraditional risk factors, cardiovascular disease

1. Introduction

1.1. History of transplantation

Organ transplantation is one of the greatest achievements in history of medicine in the twentieth century. Kidney transplantation has become the primary method of treating severe chronic renal failure. The first successful kidney transplant was performed in 1954 in Boston, the graft was in function for 7 years, and patient died because of the heart disease. Over the past decades, organ transplantation has achieved incredible proportions through the development of surgical techniques, organ preservation methods, new diagnostic procedures, but, in particular, through discovery of powerful immunosuppressants.

1.2. Kidney transplant recipients

1.2.1. Becoming a kidney recipient

Kidney transplantation has become the primary method of treating severe chronic renal failure. After kidney transplantation, life is better, more quality and longer than during dialysis. It is recommended that all patients in end-stage kidney disease should be considered as potential kidney transplant recipients no matter of absolute contraindications. Because of the lack of available organs for transplant and all possible risks of immunosupresive drugs, careful consideration and adequate preparation of patients are indispensable. All studies related to the preparation of the transplant patient are covered by a protocol that includes detailed medical history, physical examination, system review including an examination by a psychologist. To be considered as transplant recipient, patient should be put on the transplant program's waiting list. To select recipients from a deceased donor in our country, we use the prescribed criteria. Among these criteria, an important place includes a blood type, match between the recipient and the donor, tissue typing, length of time on the dialysis and sensibility of the recipient [1].

1.2.2. Contraindications for kidney transplantation in the recipient

Contraindications for kidney transplantation in the recipient may be absolute, relative and temporary. Absolute contraindications: contraindications for general anesthesia or surgery, metastatic malignancy, refractory cardial decompensation, chronic respiratory insufficiency, advanced coronary or cerebrovascular disease, coagulopathy, chronic infection, mental retardation, psychosis, alcoholism, drug addiction. Relative contraindications: frequent respiratory infections, heart failure with frequent exacerbations, frequent digestive bleeding, previous malignancy, primary renal disease with a high degree of postoperative recurrence.

Temporary contraindications: tuberculosis and other chronic infections, bleeding, unexplained focal infection, unresolved bladder anomalies, arterial hypertension with complications, pronounced secondary hyperparathyroidism.

1.3. Kidney transplant donors

The kidney can be transplanted from deceased-donor (cadaveric transplantation) or from living-donors: genetically related (living-related) or nonrelated (living-unrelated) transplants, depending on whether a biological relationship exists between the donor and recipient. The basic prerequisite is for the donor to be mentally and physically healthy and willing to give the kidney. Numerous studies have shown that there are no proteinuria, hypertension or renal insufficiency in the carefully selected donors at 10 and 20 years after the kidneys in relation to the healthy population.

1.4. Pre-emptive kidney transplant

A pre-emptive kidney transplant (before kidney function deteriorates to the point of needing dialysis) should be offered to all candidates for transplantation who have a potential live donor. Earlier kidney transplants from a cadaveric donor can be offered to all potential recipients, but of particular importance are for children and patients with diabetes mellitus. To do the preemptive kidney transplantation, two conditions must be fulfilled: the patient must have irreversible and progressive renal impairment and the creatinine clearance must be less than 15 ml/min. Numerous studies have shown that survival of patients and calves after pre-emptive kidney transplantation was the same or even better than the transplantation done after the dialysis began [2].

1.5. Surgical procedures in kidney transplantation

Transplantation of the kidneys is a surgical procedure where the donor's kidney is placed in the lower part of the abdominal cavity of the recipient. The renal artery and veins connect to the large pelvic artery and vein of the recipient. The urinary tract of the transplanted kidney is attached to the recipient's bladder.

1.6. Immunosuppressive therapy

Renal transplantation is an important form of treatment for patients with terminal renal insufficiency. However, an obstacle to the success of the greatest number of transplants represents the immune response of a recipient directed against the transplanted kidney.

Due to the constant immunological response to renal allograft, permanent immunosuppressive therapy is carried out to prevent graft rejection. Theoretically, it should not be used only on identical twins. The basis of immunosuppression is a calcineurin inhibitor that is combined with corticosteroids and mycophenolate mofetil (Cellcept). Immunosuppressive drugs have enabled the success of organ transplantation, but they do not only act to prevent rejection of the transplanted organism but also they have numerous side effects on the body. Important

side effects of immunosuppressants are the increased risk of infection and the atherogenic effect of immunosuppressive therapy.

1.7. Transplantation complications

The early period after kidney transplantation relates to the first 2 months after the surgery [3]. Acute surgical complications (bleeding, thrombosis of the transplanted kidney) are common in first few days after surgery, other clinical and immunological complications occur later.

Reactions of graft rejections are classified into four types based on the clinical picture, but differ in pathogenesis, histomorphological picture, and reactions own flow [4]. Those are hyperactivity, acceleration, acute and chronic rejection. Hyperacute rejection occurs in the first minutes or hours after kidney transplantation. Accelerating rejection begins 5–7 days after kidney transplantation and is demonstrated by rapid deterioration of the graft function. Acute rejection usually occurs from the fifth day to the end of the third month after kidney transplantation, but it can occur later on. Chronic graft rejection signifies the process in which transplanted kidney progressively deteriorates for several months and years, although the transplantation was successful in the first place. Other complications after kidney transplant are: urological complications, cardiovascular diseases, infections, gastrointestinal tract and liver diseases, malignant tumors, skin, bone and muscle diseases [4].

2. Risk factors for cardiovascular disease after kidney transplantation

Risk factors for the development cardiovascular diseases in patients with a transplanted kidney are more common than the risk factors in the general population.

They include traditional risk factors, such as arterial hypertension, diabetes mellitus, hyperlipidemia, and nontraditional risk factors associated with reduced glomerular filtration, such as anemia, hyperhomocysteinemia, or factors typical for transplantation, including direct effects of immunosuppression or rejection [5].

Patients with a transplanted kidney are exposed to atherogenic risk which is associated with previous dialysis treatment and the use of immunosuppressive drugs. An excessive risk of developing cardiovascular disease in patients with a transplanted kidney is due to the high frequency and accumulation of atherogenic risk factors before and after transplantation. Pretransplant cardiovascular disease is a major risk factor for the development of post-transplant cardiovascular disease [6].

Cardiovascular diseases represent the most frequent cause of morbidity and mortality in patients at the end stage of renal diseases. Left ventricle hypertrophy occurs in 75% of patients treated by chronic dialysis. The prevalence of coronary heart disease in patients who are treated by chronic hemodialysis is 40%. The frequency of congestive heart failure in patients undergoing hemodialysis is 46%. Cardiac diseases represent the leading cause of death of dialyzed patients of which sudden cardiac death is the most frequent that is responsible for

around 25% of all deadly outcomes. The rate of cardiovascular mortality in patients undergoing chronic dialysis is nearly 9% per annum. In patients treated by chronic dialysis, the risk of development of CVD is 10–20 times higher than in general population. Uremic milieu contributes to occurrence of atherosclerosis and atherosclerotic cardiovascular complications and often the development of accelerated, galloping atherosclerosis is present. The patients undergoing chronic dialysis are exposed to traditional and nontraditional risk factors for the development of cardiovascular complications. Nontraditional risk factors are the consequences of the uremic milieu and are related with the dialysis technique itself, and they are divided into hemodynamic and metabolic risk factors. Hemodynamic risk factors are anemia, retention of sodium and water, arteriovenous (AV) fistula, while the metabolic risk factors are hyperhomocysteinemia, hypoalbuminemia, oxidative stress, microinflammation and secondary hyperparathyroidism.

Risk factors for cardiovascular diseases in patients with a transplanted kidney are divided into traditional and nontraditional [5]: traditional risk factors are: immutable (age, gender, and inheritance), variable (smoking, hyperlipidemia, hypertension, obesity, diabetes mellitus, physical activity, and stress); and nontraditional risk factors are: risk factors related to the status of transplantation and its treatment (immunosuppressive agents, graft rejection, and viral infection —cytomegalovirus) and risk factors associated with chronic regression in allograft function (anemia, volume load, hyperhomocysteinemia, oxidative stress, secondary hyperparathyroidism, and microinflammation).

2.1. Traditional risk factors

Smoking is not only a risk factor for the development of cardiovascular diseases, but is also associated with the risk of developing chronic kidney disease defined as a reduction in glomerular filtration to <45 ml/min/1.73 m^2. Long-term smoking of more than 20 cigarettes per day is associated with 1.52 times higher relative risk of chronic kidney disease [7]. For comparison, smoking and obesity are associated with a 1.77 times higher relative risk of chronic kidney disease. These risks are more conspicuous in men than in women. Smoking begins and improves the process of atherosclerosis in the blood vessels. In various studies, it has been shown that smoking leads to cardiovascular complications, reduces survival of the patient and graft, and its effect on patient survival is similar to the same one on patients with diabetes mellitus [8]. Smoking cigarette is an independent risk factor for the development of cardiovascular events in patients with a transplanted kidney. In the population of patients with transplanted kidney, the prevalence of smoking is 25%, and the smoking cessation includes pharmacological therapy—NRT (nicotine replacement therapy).

Risks of cardiovascular diseases increase with increasing body weight because it increases the blood pressure, serum lipids, and glucose intolerance. A particularly harmful factor is the central obesity characterized by an increase in intra-abdominal fat tissue. Central obesity (waistline >88 cm for women and >102 cm for men) is a risk factor for declining allograft function in patients with a transplanted kidney. Obesity fosters the development of the insulin resistance, diabetes mellitus, ischemic heart disease, and reduces survival of allograft [9].

A cohort study by Adabag of 14,941 men and women, aged 45–64 years, showed that obesity was associated with an increased risk of sudden cardiac death [10]. According to research by Chan, obesity is a risk factor for the development of cardiovascular disease following kidney transplantation [11]. Although the role of obesity in the pre-transplant period is uncertain, after kidney transplantation, obesity increases the risk of graft failure and mortality.

Weight loss reduces and changes other risk factors for the development of cardiovascular disease [12]. Metabolic syndrome is a risk factor for the development of cardiovascular disease in patients with a transplanted kidney. It is characterized by obesity (central type of obesity), physical inactivity, hypertension (arterial blood pressure greater than 130/80 mmHg), hyperlipidaemia (triglycerides greater than 1.7 mmol/l, HDL cholesterol less than 1.0 mmol/l in men and less than 1.3 mmol/l in women) and systemic insulin resistance (fasting blood sugar greater than 6.5 mmol/l). Six years after kidney transplantation, 63% of patients have criteria for metabolic syndrome, reduced survival of allograft and an increased number of cardiovascular incidents [9].

Diabetes mellitus is one of the most common causes of renal disease, this disease independent and in itself increases the risk of cardiovascular disease. The results of two large clinical studies, Framingham Study and Multiple Risk Factor Intervention Trial (MRFIT) show that diabetes mellitus doubles the possibility of coronary artery disease in men and triples the risk of coronary artery disease in women [13]. Diabetes mellitus is a relatively common complication after kidney transplantation and is defined as a fasting glucose greater than 7 mmol/l. The incidence of diabetes mellitus is between 3.6 and 18% and depends mainly on immunosuppressive therapy. Kidney transplantation can lead to the deterioration of existing diabetes mellitus or to the development of "de novo" diabetes mellitus. The most common cause of post-transplantation diabetes mellitus is corticosteroid therapy, and it depends on immunosuppressive therapy. The two main mechanisms by which corticosteroids cause diabetes mellitus are inducing insulin resistance and increasing body weight [14]. Immunosuppressive drugs can lead to the development of diabetes. The prevalence of post-transplant diabetes in patients treated with cyclosporin ranges from 2.5 to 20%. The treatment of diabetes in hospitalized transplant recipients requires attention to a multitude of factors that can impact glycemic control and influence the risk for adverse effects. Methods to manage hyperglycemia vary among transplant centers and also vary according to whether the patient is immediately post-transplant or is being admitted in the post-transplant setting for another issue. Immediately after transplantation in post-transplant we use frequently require IV insulin infusion protocol. Once iv requirements are established and stable, switch to insulin every 8 h plus fast-acting correction insulin every 4–6 h.

The prevalence of post-transplant hypertension is between 60 and 85%. Causes of hypertension in patients with a transplanted kidney are as follows: stenosis of graft renal artery, presence of native kidneys, immunosuppressive therapy, graft dysfunction, genetic predisposition of donors and recipients. Native kidneys and pre-transplant hypertension have been described as independent factors associated with post-transplant hypertension. Native kidney of recipient can cause systemic hypertension through the renin-angiotensin system. Immuno suppressive drugs are also responsible for the appearance of hypertension in patients with a

transplanted kidney. It is believed that corticosteroids aggravate hypertension through hemodynamic and hormonal disorders as well as retention of salt and water. The role of cyclosporin in the development of hypertension in the transplantation of the heart, liver and bone marrow, as well as in patients with uveitis and diabetes mellitus, has been demonstrated, while its role in kidney transplantation is controversial. On the one hand, cyclosporin can cause hypertension with its nephrotoxic effect, and on the other hand, if cyclosporin prevents chronic rejection, it may delay the occurrence of hypertension. In two large retrospective studies with a follow-up period of 2 and 3 years, the prevalence of hypertension remained stable over time, but in the group of patients treated with ciclosporin, the prevalence of hypertension increased by 25% compared to patients treated with azathioprine [15]. Tacrolimus therapy causes hypertension, whose mechanism is probably similar to cyclosporin-induced hypertension. It is a general opinion that the main cause of hypertension after renal transplantation is chronic graft dysfunction or chronic graft nephropathy. Hypertension is often the first clinical sign of chronic rejection. It is known that arterial hypertension is a risk factor for cardiovascular disease in the general population. In a retrospective analysis, Ponticelli and associates have found a higher number of cardiac infarcts after kidney transplantation in patients with hypertension than in normotensive patients [16]. Arterial hypertension correlates with cardiovascular disease after transplantation [17]. In most cases for treatment post-transplant hypertension, routine antihypertensive treatment is effective. We can use ACE inhibitors, beta blockers and calcium channel blockers.

Hyperlipidemia is known as the traditional risk factor for the development of cardiovascular diseases, both in the general population and in the population of patients with terminal stage of renal failure. Several observational studies have shown that total cholesterol and low-density lipoproteins (LDL) are one of the most important independent factors of cardiovascular morbidity and mortality [18]. Patients with renal function impairment have significant changes in the metabolism of lipoproteins, whose exact role in the pathogenesis of atherosclerosis in these patients is still controversial [19]. Hyperlipidemia can occur already after 3 months of transplantation and does not resolve spontaneously, and in most patients, it can persist for a very long, even 10 or more years after kidney transplantation [20]. Patients with a transplanted kidney usually have an increased total cholesterol, LDL cholesterol and triglycerides, while HDL cholesterol is usually normal or even high, although its composition may be pathological [21]. Immunosuppressive drugs such as corticosteroids, cyclosporin, tacrolimus and, in particular, sirolimus contribute to the development of post-transplant hyperlipidemia, usually depending on the dose of medicine [22]. Many epidemiological studies of patients with a transplanted kidney showed a correlation between elevated total cholesterol, triglyceride, LDL and incidence of cardiovascular diseases, the same as a low level of HDL is associated with an increase in cardiovascular risk [23]. Hyperlipidemia in patients with a transplanted kidney may affect the progression of chronic graft nephropathy. Based on data about the prevention of cardiovascular disease, it is known that the reduction of LDL-cholesterol by 1 mmol/l over 4–5 years reduces the risk of coronary and cerebrovascular incidents by 25%. Extrapolation from general population studies and some data in kidney transplant patients support the view that the assessment and treatment of dyslipidemias should be part of routine post-renal transplant care. For treating hyperlipidemia in kidney transplant patient, we use statins.

2.2. Risk factors related to the status of transplantation and its treatment

In patients who at the moment of transplantation have no signs of atherosclerosis, reduced graft function and immunosuppressive therapy may cause hypertension, dyslipidemia, diabetes mellitus, and proteinuria, which can lead to myocardial infarction, stroke, or peripheral vascular disease.

Allograft function disorder is a risk factor for the development of cardiovascular disease [24]. A year after renal transplantation, chronic allograft disease at stage 3 (glomerular filtration <60 ml/min/1.73 m^2) has 60%, and in stage 4 (glomerular filtration value <30 ml/min/1.73 m^2) has 15% of patients [25]. With the decline in the allograft function, nontraditional risk factors appear. They occur when the glomerular filtration value falls below 60 ml/min/1.73 m^2, and especially when it is below 45 ml/min/1.73 m^2.

Reduced function of transplanted kidney in transplant recipient is an independent and important risk factor for the development of cardiovascular diseases due to adverse effects on hypertension, anemia, dyslipidemia, hyperhomocysteinemia [26]. The American National Kidney Foundation (NKF) reported that the value of glomerular filtration (GFR) measured or estimated is a better parameter of renal function than serum creatinine only [27]. The prevalence of left ventricular hypertension is inversely proportional to the level of glomerular filtration. In one study, the frequency of left ventricular hypertension was 45, 31 and 27% in patients with creatinine clearance <25, 25–50, and >50 ml/min [28].

2.3. Risk factors relating to chronic decline in graft function

With an increased risk of cardiovascular complications, homocysteine, infection, pathological coagulation and fibrinolysis are associated.

Anemia is a risk factor for the development of cardiovascular disease in patients with a transplanted kidney. The American Society of Transplantation (AST) defines anemia as a hemoglobin concentration lower than 120 g/l in men and lower than 110 g/l in women [29]. Anemia occurs in 20–60% of patients with a transplanted kidney, and its prevalence is the highest in the early post-transplant period (6–12 months after kidney transplantation). The main causes of its development in the early post-transplant period are: blood loss due to surgery, abrupt termination of erythropoietin administration, iron deficiency, bone marrow suppression caused by induction therapy, increased erythropoietin resistance due to infection (viral infection) and/or inflammatory status caused by systemic immune response on the presence of alloantigens, the use of drugs (mycophenolate mofetil). Reduced production of endogenous erythropoietin due to loss of allograft function, erythropoietin resistance due to secondary hyperparathyroidism and chronic microinflammatory disease are the main causes of anemia in late post-transplantation [30].

Post-transplantation anemia (a hemoglobin less than 110 g/l 3 months after renal transplantation) was associated with the development of congestive heart failure, a lower survival rate of allograft and patient, and a higher rate of acute rejection [31]. The relative risk for the cardiovascular incident was 1.32 with a decrease in hemoglobin by 0.5 g/dl, which is just slightly less

than the relative risk of 1.37 related to an increase in systolic pressure of 15 mmHg. Anemia together with hypertension leads to left ventricular hypertrophy.

Treatment of patients with transplanted kidney and post-transplant anemia should be started with erythropoietin, when the hemoglobin concentration is less than 110 g/l and the target hemoglobin level should be 110–120 g/l.

Homocysteine concentration is an independent risk factor for cardiovascular disease after kidney transplantation [32]. In patients with a transplanted kidney, the concentration of homocysteine decreases compared to patients treated with dialysis but is higher than the concentration of homocysteine in healthy population, so hyperhomocysteinemia is common in patients after kidney transplantation [33]. For the treatment of hyperchomocysteinemia, we use folate and Vitamin B (12).

There is clear evidence that elevated levels of fibrinogen, factor VII, and von Willebrand factor in the general population are associated with an increased risk of acute insult or coronary disease [34]. These factors have also been elevated in patients following kidney transplantation, in more patients with cardiovascular disorders than in patients who do not have them.

Secondary hyperparathyroidism is a risk factor for the development of cardiovascular disease in patients with a transplanted kidney [35]. After transplantation of the kidney, hyperparathyroidism is maintained in 50% of patients, characterized by hypercalcaemia, hyperphosphatemia and increased parathormone concentration [36]. Secondary hyperparathyroidism in post-transplantation may also occur new, as a consequence of the decline in the function of the allograft and the lack of calcitriol. The disorder of metabolism of calcium and phosphate results in calcification of peripheral arteries, including coronary artery calcification. Cinacalcet may be useful in the treatment of persistent hyperparathyroidism after kidney transplant.

The effect of local inflammatory stimulus such as products of the oxidation process, the end products of glycosylation and chronic infectious processes alter the blood vessel in terms of the development of atherosclerosis. Microinflammation is a risk factor for the development of atherosclerotic cardiovascular diseases in patients with a transplanted kidney [24]. These patients have a low level of microinflammation as a consequence of a systemic immune response to the presence of all antigens but also because of chronic infections [25]. C-reactive protein >5 mg/l > 0.5 mg/dl) is associated with an increased risk of developing cardiovascular events in the population of patients with a transplanted kidney.

Proteinuria occurs in one-third of post-transplant patients and is a risk factor for the development of cardiovascular disease in patients with a transplanted kidney [37].

3. Cardiovascular disease in patients with transplanted kidney

Thanks to the progress in histocompatibility testing and better immunosuppressive therapy, graft survival has increased, so that the leading cause of graft loss after transplantation has been the death of a patient with functional graft. Cardiovascular disease is the leading cause of

death in patients with a transplanted kidney [38]. Despite the fact that patients with a transplanted kidney are highly susceptible to infections and have an increased tendency to develop malignant diseases, these patients die mainly of cardiovascular disease. Although kidney transplantation, in contrast to dialysis, reduces the risk of cardiovascular disease by restoring kidney function, it also brings new risk factors related to the status of transplantation and its treatment and risk factors related to the chronic decline in the function of the allograft. The increased incidence of cardiovascular diseases in patients with transplanted kidney is a consequence of the high prevalence of risk factors for the development of cardiovascular diseases in these patients. The incidence of cardiovascular disease in patients with a transplanted kidney is three to five times higher than in the general population [39]. A cardiovascular event with congestive heart failure or coronary heart disease was manifested in almost 40% of patients 36 months after kidney transplantation [9]. Cardiovascular disease is the most common cause of death in transplanted patients, accounting for 35–50% of all causes of death, and occurs at least two times more often than in the general population [40]. Most kidney recipients die with functional graft and half of these patients die due to ischemic heart disease or other vascular diseases [41].

The most common cardiovascular diseases in patients after kidney transplantation are as follows: ischemic heart disease, congestive heart failure and left ventricular hypertrophy [6]. Of all cardiovascular complications, ischemic heart disease is by far the most common cause of mortality (more than 50%) in patients with a transplanted kidney [41]. Frequency of left ventricular hypertrophy ranges from 50 to 70% in patients with a transplanted kidney [42]. Left ventricular hypertrophy is associated with an increased degree of ventricular arrhythmias. In Europe, cardiovascular disease account for 36% of the total mortality of patients with a transplanted kidney [43]. In the United States, the annual mortality rate of cardiovascular diseases in these patients is 0.54% and is approximately twice as high (0.28%) than in the general population [39].

Ischemic heart disease causes 53% of deaths in patients with transplanted kidney and preserved graft function. The risk of mortality from ischemic heart disease is 6.4 times higher in nondiabetic transplanted renal patients, 8.6 times higher in dialysis patients and 20.8 times higher in transplanted renal patients with diabetes than in the general population [41]. Diagnostic strategy for early detection of patients with an increased risk of developing asymptomatic disorders of the systolic and diastolic function of the left ventricle should include: echocardiographic examination, tests for coronary artery disease and tests for the determination of myocardial function (BNP, Tt-pro BNP). In a study of Aakhus and associates on cardiovascular diseases in patients with a transplanted kidney, involving 406 patients with a transplanted kidney, the mean annual mortality was 4.4, and 74% of them were cardiovascular causes of mortality [44].

3.1. Left ventricular hypertrophy

The most significant risk factors for the development of left ventricular hypertrophy are as follows: hypertension, arteriosclerosis, secondary aortic stenosis and anemia.

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Left ventricular hypertrophy is a risk factor for the unfavorable outcome of patients with a transplanted kidney. The selection of patients with an increased risk of left ventricular hypertrophy, the timely application of appropriate treatment, the achievement and maintenance of the target values of risk factors, lead to decrease of the development and regression of existing left ventricular hypertrophy, reduction in the rate of cardiovascular morbidity and mortality, and improve the quality of life of patients with a transplanted kidney.

Left ventricular hypertrophy is present in most patients who begin treatment by replacing renal function and are associated with poor outcome. Time spent on dialysis can also affect the development of left ventricular hypertrophy. Transplantation of the kidneys leads to the withdrawal of left ventricular hypertrophy together with the normalization of blood pressure. The regression of left ventricular hypertrophy continues during the first 2 years after transplantation. Older age and hypertension can slow down this process [45].

The presence of left ventricular hypertension as well as the weak systolic function of the left ventricle before transplantation are related to an increased mortality after renal transplantation during 7.5-year follow-up, while other traditional risk factors such as hypertension, hyperlipidemia and smoking did not show such a connection. On cardiography of the heart made 4 months after transplantation, only left ventricular hypertension had a strong association with poor prognosis during monitoring [46].

3.2. Heart failure

Heart failure is a clinical syndrome characterized by reduced tolerance of physical activity and overload volume. The incidence of congestive heart failure in patients with a transplanted kidney is two to five times higher than the incidence in the general population [47]. The incidence of congestive heart failure in patients with a transplanted kidney was 10.2% 12 months after transplantation and 18.3% 36 months after transplantation [48].

In a study of Higashi and associates, 11 out of 190 (5.8%) kidney recipients, with preserved left ventricular systolic function and the presence of diastolic left ventricular dysfunction, developed a postoperative edema of the lungs [49].

Diastolic left ventricular dysfunction is present in 45% of patients with a transplanted kidney.

The clinical presentation is the same as in all other patients with cardiac insufficiency. The following symptoms are reported: difficulty breathing, intolerance of physical activity, edema of the lower extremities and stomach.

Treatment: the first measure is to reduce physical activity and reduce salt intake. If this is not enough, medication therapy is started with a combination of diuretics, ACE inhibitors and digitalis.

3.3. Ischemic heart disease

Ischemic heart disease (IHD) is the most common disease in the large group of all cardiovascular diseases. The prevalence of ischemic heart disease in patients with a transplanted kidney

is five times higher than in the general population. The main cause of ischemic heart disease is atherosclerosis of the coronary arteries, and in patients with transplanted kidney there are many risk factors that contribute to atherosclerosis: elevated arterial blood pressure, lipid metabolism disorder, microinflammation, hyperhomocysteinemia, oxidative stress and secondary hyperparathyroidism.

In patients with a transplanted kidney, there is a high risk of sudden cardiac death. The main causes are coronary arterial heart disease, left ventricular hypertrophy, and reduced coronary perfusion. In patients with a transplanted kidney, the prevalence of coronary heart disease is five times higher than the general population [6].

In all patients with ischemic heart disease, the following should be done: detailed anamnesis and physical examination, ECG at rest, blood biochemical analysis (lipids, glucose, creatinine, urea, hepatogram, complete blood count, uric acid, fibrinogen, hsCRP, etc.) echocardiographic examination, ergometric testing. In patients with transplanted kidney, the risk factors for the development of atherosclerosis should be controlled in the primary prevention and prevention of coronary artery disease development. In clinical practice, patients with acute myocardial infarction with elevation of ST connectors are using modern reperfusion therapy (thrombolysis or percutaneous coronary intervention). Medical treatment implies the use of anti-aging therapy, statins and beta blockers.

3.4. Valvular heart disease, heart rhythm disorders, and pericardial disease

Valvular heart disease is common in patients with chronic dialysis. Current knowledge of heart valve disease (the evolution of pre-dialysis disease or the emergence of de novo diseases of the heart valve) in patients following kidney transplantation is scarce. In the literature, there are few data about heart rhythm disorders in patients with transplanted kidney. In a study by Sever and associates on the frequency of pericarditis in patients following kidney transplantation, an incidence of 2.4% was observed [50].

The development of pericarditis in patients with a transplanted kidney may contribute to the use of immunosuppressive therapy as it increases the risk of infection [51].

4. Assessment of the risk of cardiovascular disease in asymptomatic patients with a transplanted kidney

Cardiovascular diseases are the main cause of morbidity and mortality in post-transplant patients [52]. The risk assessment of cardiovascular disease in asymptomatic patients with a transplanted kidney is of great practical importance because it allows the identification of patients at high risk for cardiovascular events, which further allows timely intervention before the disease develops. To assess the risk of cardiovascular disease in asymptomatic patients with a transplanted kidney, we can apply the scores used for assessment of cardiovascular risk in general population. The scores for the general population are the Systematic Coronary Risk

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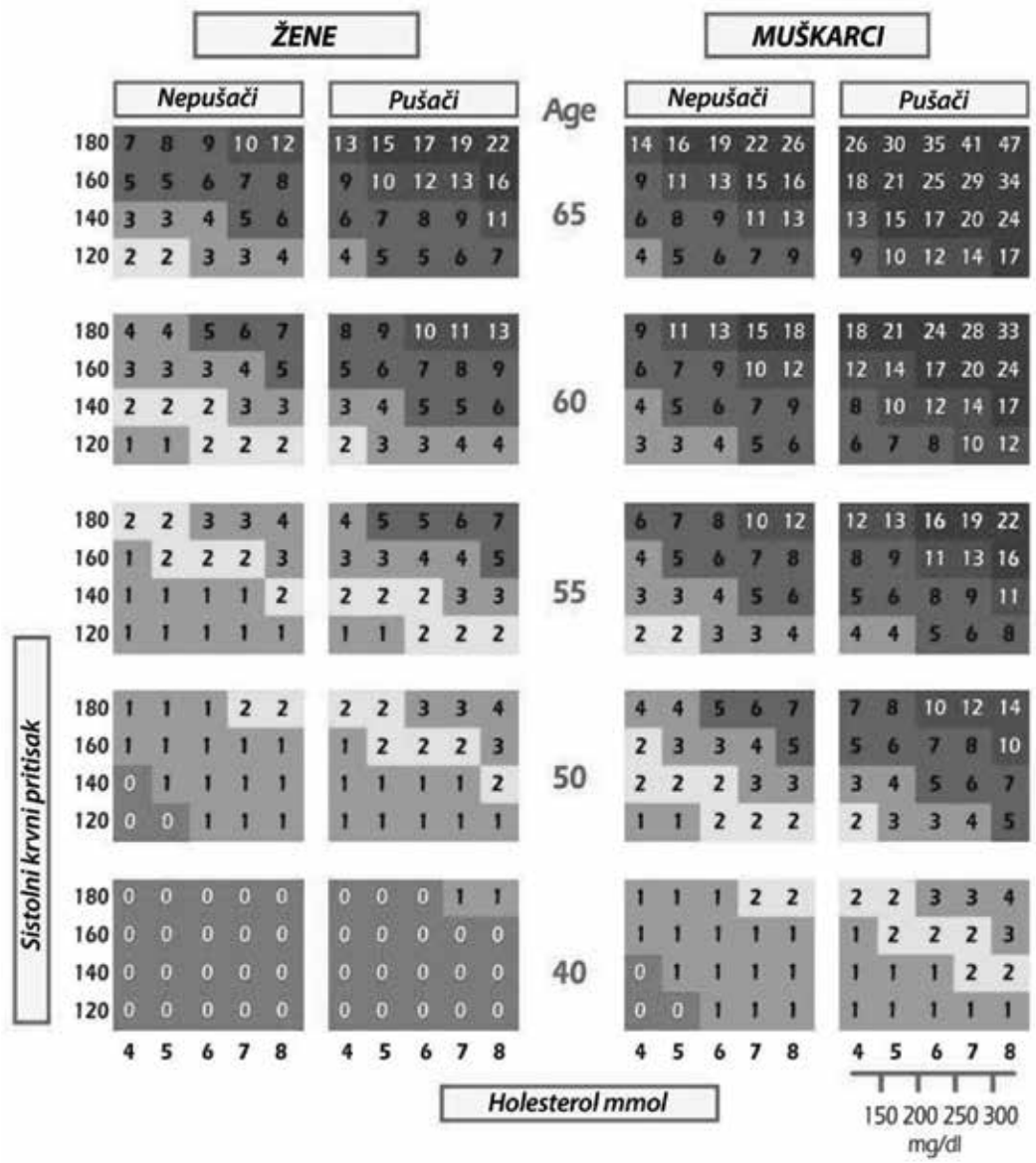

Table 1. Heart score high risk map.

Evaluation SCORE system, the Prospective Cardiovascular Munster PROAM score system, and the National Cholesterol Education Program Adult Treatment Panel III NTEP ATP III [53].

The new risk assessment model is based on the Heart Score system (Systematic Coronary Risk Evaluation). The Heart Score Risk Card has been developed on the basis of large prospective studies in Europe and predicts the fatal outcomes of cardiovascular disease for 10 years. The

assessment is based on the following risk factors: age, sex, smoking, systolic blood pressure, total cholesterol, or cholesterol/HDL ratio. A high risk threshold based on a fatal cardiovascular outcome is defined as more than or equal to 5%.

Early detection of high-risk patients for the development of cardiovascular diseases allows timely application of an appropriate therapeutic strategy that ensures high survival rates for patients with a transplanted kidney (**Table 1**).

Assessing the patient's risk of developing cardiovascular disease enables the identification of patients at high risk for the development of cardiovascular events, which allow the intervention before the disease develops. In asymptomatic patients, cardiovascular risk assessment should be carried out and preventive activates should be performed accordingly. Patients with established multifactorial risk should be subjected to preventive activities, and, if necessary to medication therapy.

Several studies have shown that the use of standard scores in the assessment of the degree of risk of cardiovascular disease in patients with renal disease is insufficient [54]. In these scores, we use the following data: age, gender, blood pressure, lipid level, without taking into account nontraditional risk factors for occurrence of cardiovascular diseases.

Nontraditional risk factors that are not included in these scores can play an important role in the insufficient assessment of the risk of cardiovascular disease in patients with a transplanted kidney. All these point should be consider by creating a new risk score that would include both traditional and nontraditional risk factors for cardiovascular disease. It is recommended that standard risk factors be enhanced with additional risk factors (e.g., homocystein, C-reactive protein). Additional risk factors are recommended for increasing the precision of risk assessment.

5. Conclusion

Cardiovascular disease is the leading cause of death in patients with a transplanted kidney. The incidence of cardiovascular disease in patients with a transplanted kidney is 3–5 times higher than in the general population. Risk factors for the development of cardiovascular diseases in patients with a transplanted kidney are divided into traditional and nontraditional. Traditional risk factors: immutable (age, gender, inheritance), variable (smoking, hyperlipidemia, hypertension, obesity, diabetes mellitus, physical activity, stress). Nontraditional risk factors: risk factors related to the status of transplantation and its treatment (immunosuppressive agents, graft rejection, viral infection - cytomegalovirus) and risk factors associated with chronic regression in allograft function (anemia, volume load, hyperhomocysteinemia, oxidative stress, secondary hyperparathyroidism, microinflammation). The most common cardiovascular diseases in patients after kidney transplantation are as follows: ischemic heart disease, congestive heart failure and left ventricular hypertrophy. Of all cardiovascular complications, ischemic heart disease is by far the most common cause of mortality (more than 50%) in patients with a transplanted kidney. Frequency of left ventricular hypertrophy ranges from 50 to 70% in patients with a transplanted kidney. The incidence of congestive heart failure in patients with a transplanted kidney is 2–5 times higher than the incidence in the general

population. The incidence of congestive heart failure in patients with a transplanted kidney was 10.2% 12 months after transplantation and 18.3% 36 months after transplantation. The risk assessment of cardiovascular disease in asymptomatic patients with a transplanted kidney is of great practical importance because it allows the identification of patients at high risk for cardiovascular events, which further allows timely intervention before the disease develops. To assess the risk of cardiovascular disease in asymptomatic patients with a transplanted kidney, we can apply the scores used for assessment of cardiovascular risk in general population. Several studies have shown that the use of standard scores in the assessment of the degree of risk of cardiovascular disease in patients with renal disease is insufficient. It is recommended that standard risk factors be enhanced with additional risk factors (e.g., homocystein, C-reactive protein). Additional risk factors are recommended for increasing the precision of risk assessment. Assessing the patient's risk of developing cardiovascular disease enables the identification of patients at high risk for the development of cardiovascular events, which allow the intervention before the disease develops.

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Chapter 16

Xenotransplantation

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Additional information is available at the end of the chapter

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Abstract

Xenotransplantation, defined as the transfer of cells, tissues or organs between species, has been a subject of significant interest for decades as a response to the increasing demand for biological materials to treat patients. In this review, the history and recent progress in xenotransplantation research will be discussed, including the immunological challenges that need to be overcome and the molecular biological methods which are required to allow the complex genome engineering to meet the critical need for organs.

Keywords: xenotransplantation, immune rejection, swine, transgene, gene editing, gene knockout, tolerance

1. Introduction

Over the course of the past 100 years, the rapid progress in drug development and surgical techniques has created a paradox for the area of transplantation medicine. Surgical protocols have become more successful and medicines have overcome many mechanisms of chronic rejection and allowed increased survival of transplant patients. However, the number of organs available for transplant has remained essentially constant. In addition, not all organs available through donation are viable for transplant. Organs such as lung, which are more prone to damage due to trauma, disease or deterioration, are available in drastically reduced numbers compared with heart or kidneys. Therefore, while there is an increasing number of patients who would survive and thrive long-term after organ transplantation, the limited number of organs available means a smaller percentage of eligible patients can actually undergo transplant surgery.

A hard truth about human organ donation is that even with exponential increases in donor numbers, it is unlikely that the organ shortage would be relieved. The diversity of the human species, paired with the efficiency of the immune system, significantly reduces the chances that a given organ will be compatible with the patients in greatest need. Although immunosuppressive drugs can enhance length of survival, chronic rejection remains a risk the greater the mismatch between organ and patient. Furthermore, the donor geographic proximity, organ size and timing of availability of a compatible organ with a matching patient may be limiting. Thus, even as human organ donation continues to be optimized, there remains an immense need for additional organs above and beyond the availability of human donors.

In order to address the above concerns and provide sufficient numbers of compatible organs, a number of approaches, both biological and mechanical, are being actively explored. Use of animal organs provides solutions to the challenges of availability and function. Multiple mammalian species possess organs which may substitute effectively for their human analogs and, in the case of agricultural species, can be rapidly bred in sufficient numbers to overcome organ shortages. Through use of controlled facilities, production of animals can be regulated and disease exposure eliminated. Additionally, careful breeding schedules can provide organs of appropriate size for any given patient on a predictable schedule for optimal timing of surgery. Finally, recent advancements in DNA sequencing and assembly and genome engineering technologies, paired with the advanced understanding of the cellular and molecular immunology responses in transplant rejection, allow the creation of animals which could provide an unlimited supply of rejection-free organs.

2. Early beginnings

Examples of xenotransplantation can be found recorded as early as the seventeenth century, in which the transfusion of blood from animals into human patients was described [1]. In the eighteenth century, more complex tissues such as skin were tested as grafts in human patients [2]. In 1905, Princeteau transferred rabbit kidney sections into a child with immediate positive results, however, after 16 days the child died of pulmonary complications [3]. Soon thereafter, two kidney xenotransplants were attempted, with one patient receiving an organ from goat, the other from pig. Unlike Princeteau's experiment, neither organs functioned and both apparently failed due to thrombosis [4]. Similarly, an attempt by Unger in 1910 to transplant kidneys from a chimpanzee into humans led to failure due to thrombosis in about a day [5]. In 1923, Neuhof transplanted a kidney from a lamb into a human patient, allowing the patient to survive 9 days [6].

In the early twentieth century, an odd offshoot of xenotransplantation was created due to interest in "rejuvenation" via transplant of animal testis in human males, as demonstrated by Voronoff in Russia [7] and Brinkley in the US [8] using chimpanzee or goat testis, respectively. So popular was the use of goat testis in the US, an entire radio empire was built around advertising the services, with many patients claiming enhanced fertility and sexual function [8].

The field of immunology developed in parallel with surgical approaches to xenotransplantation. As the mechanisms of immune rejection were better defined, the enormity of the challenges facing transplant of organs between members of the same species were recognized.

During and after WWII, pharmaceutical companies created a series of increasingly effective immunosuppressive drugs which could inhibit some rejection responses, renewing interest in xenotransplantation.

3. First attempts at human xenotransplantation with primate organs

During 1963–1964, Reemstma carried out a series of transplants into 13 human patients using chimpanzee kidneys, with one patient surviving 9 months after transplant surgery [9]. The need for these experiments was driven in part by the desperate human organ shortage and lack of alternatives. Cadaveric organs often proved insufficient in quality, and volunteer human kidney donation, high risk at the time, was untenable for ethical and legal reasons. Although chronic dialysis had been demonstrated by the early 1960s, it was not widely available for patient treatment [10]. Therefore, despite the risks, xenotransplantation was considered a potentially viable solution.

Reemstma was not alone in exploring xenotransplantation as a means to overcome critical organ shortages. Hume attempted transplanting a chimpanzee kidney into a human, but the organ failed to show renal function [11]. Hardy and team focused on heart, observing survival for only 2 hours after transplanting a chimpanzee heart into a human patient [12]. Starzl carried out a series of transplants in human patients with baboon kidney [13] and livers, with variable results [14]. These seminal attempts at xenotransplantation showed that although surgical techniques and immunosuppressive drug treatments had greatly improved, they were insufficient to address the multitude of challenges in overcoming the xenorejection response. Indeed, it was nearly a generation later before Bailey used a baboon heart for transplantation into an infant, who survived several weeks after receiving the organ [15].

4. A shift in species

Although the close evolutionary relationship between non-human primates and humans would suggest an advantage in using chimpanzee or baboon organs for xenotransplantation, clinical, practical and ethical considerations prevent them from being a viable option. Non-human primate organs do indeed function almost identically to human organs, but are subject to a variety of diseases which are readily transmissible to humans [16]. Given the relatively fragile state of patients receiving multiple immunosuppressive drugs, the risk of primate zoonoses is too great. In addition, chimpanzees, baboons and many other non-human primates are impractical for large scale breeding. The low numbers of progeny of non-human primates limits the production of large numbers of animals by natural breeding or *in vitro* fertilization compared with agricultural species. Finally, use of non-human primates as organ donors faces insurmountable ethical barriers.

A much more viable approach is the use of pig organs for xenotransplantation. Porcine organs are structurally and physiologically close to humans, and therefore can functionally substitute for analogous human organ functions. Unlike non-human primates, pigs are more evolutionarily distant from humans and thus have a greatly reduced risk of transmission of diseases to human patients, which can be essentially eliminated through genetic manipulation [17].

Husbandry techniques for pigs are extremely well-understood, with large litter sizes and rapid cycle times, allowing production of populations that could overcome organ shortages in a much shorter timeframe than possible with non-human primates. Furthermore, a suite of genome engineering technologies is available for use in pigs to make critical changes to enhance survival and function of the pig organ, while avoiding the human immune rejection response. In fact, the complex engineering approaches now available may actually provide organs with advantages over even closely-matched human organs.

5. Current status and challenges for xenotransplantation

A variety of academic, clinical and industrial institutions have made substantial progress in recent years in the understanding of the molecular mechanisms of the xenorejection response and the genetic modification of pigs to overcome these mechanisms [18]. Professional organizations are working with the FDA to develop guidelines for clinical use [19], with several groups indicating their intention to initiate clinical trials in the near term with porcine organs [20].

For xenotransplantation to become a viable routine human therapeutic option, there are a number of challenges that still need to be overcome. These challenges fall into two broad categories; the biology of the xenorejection responses and the engineering technologies needed to restructure the porcine genome to overcome these responses.

6. The immune system and rejection

The immune system is an evolutionarily ancient collection of structures, mechanisms and cells that detect and eliminate harmful organisms from the host. In older texts, the immune system is often described as distinguishing "self" from "non-self," but more recent research demonstrates that there are a variety of roles for the microbiome ("non-self" micro-organisms resident within, on or around the host) in maintaining the health of the host organism. Thus, the host immune system must be able to not only identify and eliminate harmful pathogens, it must also tolerate the presence of a variety of beneficial bacteria, fungi and yeast [21]. Because transplantation of cells, tissues and organs is an unnatural situation created through deliberate medical intervention, the human immune response uses incredible precision to identify even closely related human cells as "non-self" and efficiently removing them, a process referred to as "immune rejection." In general, the strength of the response is proportional to the degree of difference between the host and donor materials, therefore, when exposed to materials from an animal, the rejection response is much faster and stronger, increasing the challenge in controlling the immune response.

7. Xenotransplant rejection

Xenorejection is a much more exaggerated and rapid form of the allorejection response. Four overlapping and inter-related reactions occur temporally; hyperacute rejection (HAR), acute vascular rejection/acute humoral xenograft rejection (AVR/AHXR), acute cellular rejection (ACR) and chronic rejection (CR) (**Figure 1**) [22]. Although these processes can be

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characterized as distinct stages based on histological and clinical data, each is due to highly interconnected pathways and mechanisms that are challenging to separate. In fact, these responses were defined as pathologic observations prior to development of more detailed analyses of the molecular immunology mechanisms.

HAR is primarily due to immediate binding of pre-existing host natural antibodies specific for xenoantigens expressed by the donor tissues. Antibody binding can activate the endothelial cells, causing the release of immune activators, as well as inducing the complement-mediated destruction of the endothelial layer, reducing the barrier function and allowing host cells to infiltrate the organ. Cell debris released by the damage to the endothelium and products of the complement cascade also stimulate coagulation and the innate inflammatory response. These pathways synergize during rejection to create stronger responses that are more pathogenic and can be less amenable to control.

AVR/AHXR, like HAR, is also mediated by host antibodies. However, instead of pre-existing natural antibodies, AVR/AHXR is often the result of humoral responses which lead to production of antigen-specific antibodies. The AVR/AHXR is delayed due to the time it takes to induce

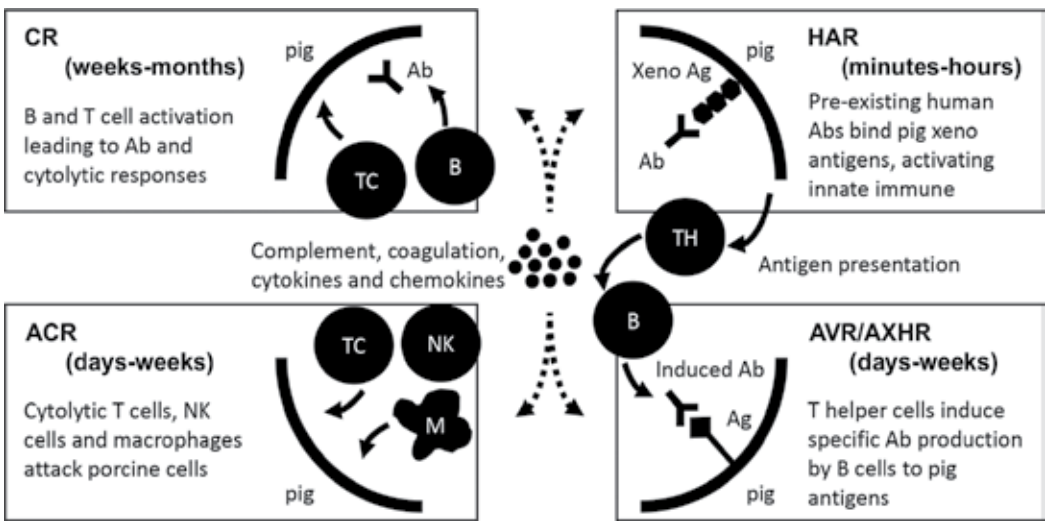

Figure 1. Overview of the xeno-rejection response. The human immune response to xeno-organs initiates within minutes to hours with hyperacute rejection (HAR, upper right), in which pre-existing antibodies (Ab) in human serum bind to xenoantigens (xeno Ag) on the surface of the pig cells. This results in cell destruction and presentation of porcine antigens to human helper (TH) and cytolytic (TC) T cells, as well as groups of pro-inflammatory and pro-immunity cytokines and other soluble mediators. Activation of human helper T cells (TH) stimulates human B cells (B) over the course of days to weeks, resulting in production of induced antibodies (induced Ab) as part of the acute vascular rejection/acute humoral xenograft rejection (AVR/AHXR, lower right) response. These secondary antibodies are often more specific and higher affinity than the pre-existing human serum antibodies, and also cause cellular destruction of the xenograft. in parallel with AVR/AHXR, the acute cellular rejection response (ACR, lower left) is carried out by human NK cells (NK), macrophages (M) and the xenograft-specific cytolytic T cells (TC) recruited to the xeno-organ within days to weeks. The activated cells express a variety of molecules to attack the porcine cells, as well as secrete additional cytokines to recruit more human immune cells. After weeks to months, the human immune response may be again induced to react to the xeno-organ during chronic rejection (CR, upper left), leading to specific antibody (Ab) responses from B cells (B) or cytolytic T cell (TC) destruction. A large collection of cytokines, chemokines, complement and coagulation factors (center) play a key role in regulating the complex set of reactions occurring in every aspect of rejection.

an adaptive immune response via germinal center reactions, typically days to weeks. Much like natural antibodies, the induced antibodies recognize components of the xeno-organ and, similar to HAR cause activation of the endothelial cells and their destruction via the complement system. The specific antibody binding also attracts multiple elements of the cellular immune system, such as NK cells and phagocytes, creating further damage of the target tissues and secreting soluble factors, such as cytokines and chemokines, which further enhance immune responses.

ACR includes predominantly cellular responses to the graft, such as T cell activation, which occur within days to weeks of organ transplant. Although ACR is well-established in allotransplant, the importance of ACR in the xenorejection response is not entirely clear. This may be due to either the more rapid activation of hematopoietic populations in HAR and AVR/AHXR compared with allotransplantation. However, some groups have proposed that reduction of the HAR and AVR/AHXR in earlier stages of xenorejection would unmask ACR which would otherwise be unnoticed amidst the earlier more pathogenic responses. In either case, ACR is expected to be substantially similar between allo- and xenotransplantation and thus more readily controlled by immunosuppressive drugs already in use for allotransplant.

CR is longer term, occurring within months or even years after transplantation. CR can be due to complications due to other immune activity, such as infection, or escape of humoral or cellular responses from immunosuppressive drug control. CR is well-understood in allotransplant and effective treatments are available for control and reversal of CR.

HAR and AVR/AHXR are the most unique and most critical to address in xenotransplantation. These earlier reactions can greatly enhance later reactions, with some of the mechanistic elements of the xenorejection response initiated even before the transplant surgery itself occurs. Therefore, it is essential to control the initiating events as early as possible in order to reduce the course of later responses. Much like the layers of an onion, removing one layer reveals the next, but as each layer is removed the overall size may be diminished.

The latter two responses, ACR and CR, are mechanistically similar between xeno- and allorejection responses [23]. Use of currently-available immunosuppressive drugs are believed to be able to control both responses as evidenced by the extensive data from allotransplants in humans. However, the speed and violence of the HAR and AVR reactions against xenoorgans can greatly accelerate and strengthen ACR and CR. Thus, even well-established treatments for allorejection may need to be reviewed as xenotransplantation proceeds toward clinical trials.

8. Innate and adaptive immunity in xenotransplantation

The immune system has two inter-related arms; the innate and the adaptive immune systems, both of which contribute to the rejection of xenotransplanted cells, tissues and organs. Although often described as separate, the systems have a large network of connections which are interdependent, and thus are not completely distinct. Both systems utilize multiple mechanisms to protect the host, creating a series of defense layers of increasing specificity. When functioning properly, a given layer may not be 100% efficient, but in aggregate will capture the overwhelming majority of pathogens. In addition, the ability to detect subtle differences between highly

related cells has the potential benefit of identifying and eliminating cells with oncogenic mutations, preventing tumors before they have a chance to establish themselves [21].

8.1. The innate immune system and xenorejection

The innate immune system is evolutionarily ancient, with related mechanisms found in both plants and animals. The innate immune system consists of relatively invariant mechanisms for the identification of pathogens, and, although less specific, is extremely rapid and strong in response. The rapidity of the innate immune system provides an immediate barrier to pathogen infiltration and infection of the host, limiting the pathogen burden and giving the adaptive immune system time to develop more specific responses [24].

The use of physical barriers is one of the most critical elements of innate immunity. Although organ transplant bypasses the skin as a protective layer, the endothelium of the blood vessels, which connect the organ to the host circulatory system, remains as the main interface between the human hematopoietic system and xeno-organ tissues. As such, many of the immediate mechanisms of the innate response are greatly influenced by the interactions between the human immune cells and the porcine endothelial cells. Once the human innate immune system attacks the porcine endothelium, the barrier function is quickly lost, followed by rapid influx of human immune cells, pro-inflammatory infiltrates and edema, and then necrosis and destruction of the xeno-organ. It is important to note that the endothelium is an extremely active part of the immune response, which responds to soluble factor and cellular interactions to induce a variety of immune and inflammatory responses. Therefore, any efforts to improve the engraftment of xeno-organs must take into account the functional role of the endothelium in regulating the rejection response [25].

8.2. Inflammation

Inflammation is one of the earliest innate responses, driven by pattern recognition receptors found on human immune cells which recognize damage-associated molecular patterns (DAMPs). The binding and signaling of DAMPs causes the immediate secretion of proinflammatory mediators, such as cytokines and chemokines, which attract additional innate immune cells and induce a variety of local responses which would be highly beneficial during an infection but destructive to xeno-organs. For example, vasodilation and increased vascular permeability, which would normally allow host immune cells greater access to tissue to rapidly eliminate pathogens, instead causes the xeno-tissue to be more quickly infiltrated by human innate immune cells, which in turn leads to more inflammation and destruction. Similarly, there are blood-borne proteinaceous biochemical cascades activated by inflammation, such as the coagulation and the complement systems, which further degrade xeno-organ function and survival [26].

8.3. Xenoantigens

The genes encoded by the porcine genome can encode proteins that are substantially different from their human counterparts or may carry post-translational modifications which are not present in humans. Interestingly, some of these molecules, referred to as “xenoantigens”,

are recognized by pre-existing natural antibodies found in human serum. One subset of these antigens is the swine leukocyte antigens (SLA), which are the physical and functional equivalent of the human leukocyte antigens (HLA). Much like the case for human allotransplant, the SLA genes are highly diverse and individual patients will have a variable level of cross-reactive antibodies in their serum for a given set of SLA genes [27]. A separate group of xeno-antigens are glycan molecules, such as Gal alpha (1,3) Gal and Neu5Gc, which are expressed in porcine, but not human, cells [28].

Although specific induced antibodies are produced by B cells as part of the adaptive response, the presence of pre-existing antibodies in human serum contributes to the innate response. The specific reasons for the existence of these human natural antibodies are not entirely clear. In the case of glycan structures, one hypothesis is that the molecules are related to those found in pathogens, and that the natural antibodies are cross-reactive to each. Alternately, consumption of porcine materials in the human diet may induce antibody formation. Regardless of the specific source in human serum, xenotransplantion of porcine cells and tissues in humans leads to binding of these pre-existing natural antibodies, activation of complement and eventual destruction of target cells carrying the xenoantigens.

Several approaches have been taken to address xenoantigens, including cross-matching donors and recipients for reduced immunoreactivity, removal or modification of the xenoantigen from the donor pig, or the reduction of the ability of the antibodies to induce the complement cascade. In the first case, typing of patients and porcine donors to find the best matches would be very similar to the current system used for determining allotranplant cross-reactivity [29]. Use of gene targeting or editing technologies can eliminate the genes encoding SLA or the enzymes required for expression of the relevant glycan. This has been proven to be highly effective for ablating the genes GGTA1 (the gene encoding alpha 1,3-galactosyltransferase essential for Gal alpha (1,3) Gal), CMAH (cytidine monophosphate-N-acetylneuraminic acid hydroxylase critical for Neu5Gc biosynthesis) and B4GALNT2 (beta 1,4 N-acetylgalactosam inyltransferase). In each case, the elimination of the glycan leads to greatly reduced recognition of porcine cells by natural antibodies in human serum, and reduction in complement-mediated destruction [28]. Unfortunately, as the number of antibody targets increases there is a risk that one or more of the xenoantigens alone or in combination may have essential functions which cannot be eliminated without damaging the development or function of the pig. Therefore, efforts to introduce more subtle mutation in SLA which remove immunogenic epitopes while leaving critical antigen-presentation functions intact, or even replacement of SLA with HLA, may be more effective.

The second approach, which is often used in combination with the first, is to reset the threshold at which the complement cascade is activated, making it more difficult for the binding of human natural antibodies to targets on porcine cells to induce the complement cascade. There are a series of "complement regulatory proteins" (CRPs), such as CD46, CD55 and CD59, expressed on the cell surface which prevent complement activation by the inadvertent non-specific binding of human antibody to human cells [30]. By overexpressing one or more of the CRP molecules on the porcine endothelium, the amount of antibody binding required for complement activation is increased, which reduces the amount of antibody-mediated cell destruction due to human natural antibodies [31].

8.4. Coagulation

Inflammation and vascular leakage, due to loss of endothelial barrier function, both induce coagulation, which normally is required to repair localized endothelial damage. In the case of xenotransplantion, the attack of the endothelium is rapidly occurring at multiple sites, therefore, coagulation spreads throughout the blood vessels in the xeno-organ and can overcome the normal control mechanism. The thrombosis produced by the procoagulant environment leads to occlusion of the vessels within the graft, known as thrombotic microangiopathy (TM). The lack of blood flow results in hypoxia and tissue damage and necrosis, further complicating transplant function. The relatively greater amount of endothelial injury and coagulation in xenotransplant therefore creates more frequent and extensive TM and contributes to the more rapid destruction of the graft [32].

In addition to physiological pathways induced by human innate immune responses, there are non-physiological activities caused by mismatches between porcine and human constituents of the coagulation cascade [33]. For example, porcine von Willebrand factor (vWF) has been shown to bind more avidly to the human GP1b receptor and activate human platelets, leading to coagulation and rapid loss of platelets from the circulation [34]. Ongoing efforts seek to engineer porcine vWF to eliminate the inappropriate interactions with GP1b, while maintaining normal coagulative phenotypes. In addition, porcine proteins which provide positive and negative feedback to control the coagulation cascade do not function as efficiently upon the human coagulation targets, leading to dysregulation of the cascade. The targeting the porcine genome to express human regulatory proteins in porcine cells has been shown to help control human coagulation in response to exposure to the modified porcine materials [35].

8.5. Innate immune cells

Macrophages and neutrophils are two of the earliest host cell types to infiltrate xeno-organs. Both cell types are instrumental in the phagocytosis and destruction of pathogens during infection. During a xenorejection response, the damaged porcine cells release a variety of DAMPs which are recognized by the human innate cells, inducing phagocytic functions which further damage the xeno-organ and increasing production of additional proinflammatory and other immune mediators which attract more innate immune cells [36, 37].

Similar to the molecular mismatch described above for vWF and coagulation, macrophages express the SIRPA receptor, which must interact with the surface receptor CD47 to prevent the target cell destruction by the macrophage. Thus, the CD47 receptor expressed on the cell surface binds to SIRPA to instruct the macrophage not to consume the target cell. In the case of porcine CD47, the interaction with human SIRPA appears to be unproductive and cannot inhibit the macrophage activity. Expression of the human form of CD47 in porcine cells has been shown to greatly reduce human macrophage activity directed against the porcine cells [38].

NK cells are functionally analogous to cytolytic T cells, and even share some mechanistic pathways for targeted cell destruction. NK cells express a collection of stimulatory and inhibitory receptors on the cell surface, which engage conserved targets on the surface of target cells. The balance of activation and inhibition via combinatorial signaling determines whether

the NK cells are stimulated to kill or ignore the target cell. The target cell receptors, such as HLA-E, may be perturbed by pathogens or tumorigenesis, which is detected by the NK cells and the target cells eliminated [39].

In the case of xenotransplantation, the porcine cell receptors, although expressed normally, are not sufficiently well-conserved with their human counterparts and thus cannot inhibit NK cell attack. By expressing on porcine cells the human versions of receptors which stimulate the inhibitory receptors on NK cells, the damage may be averted. With careful genetic modification, the normal mechanisms for detection of infection or other dysfunction may be maintained, allowing normal NK functions while eliminating the xeno-specific destruction [40, 41].

8.6. Resolution of innate immune responses

There are a variety of mechanisms used to resolve innate immune reactions. Many of the soluble mediators of innate immunity have extremely short half-lives which allows them to dissipate quickly. In addition, immune receptors become increasingly desensitized to further stimulation during the course of the innate response, reducing reactions. A variety of negative regulators are also produced to further inhibit the innate effectors. All of these mechanisms are in place to prevent over-reaction of the immune system and the destruction that it can cause once the pathogenic threat has been eliminated [42]. In the case of a xenorejection response, however, the "threat" that is recognized comes from every porcine cell and thus the innate response is never fully resolved without intervention. It may be possible to take advantage of these resolution mechanisms to create porcine cells with an enhanced ability to curtail or end a human inflammatory response through careful genetic modification.

8.7. The adaptive immune system and xenorejection

The adaptive immune system is comprised of cellular and antibody components which recognize pathogens and develop highly specific responses, which can increase in specificity and effectiveness over time and exposure. The adaptive immune response also creates immunological "memory" to allow more rapid reactions should similar pathogens be encountered in the future. Because of the time required to develop specific responses, the adaptive immune system generally becomes more critical after the initial innate immune response [21].

8.8. Antigen presentation and T cells

Antigen presentation is a crucial mechanistic part of the adaptive immune response and plays a major role in the decision between immunity and tolerance for a given target. There are two main routes for antigen presentation to the immune system, reflective of the different classes of pathogen antigens, intracellular or extracellular.

Intracellular antigens, either natural cellular proteins or those derived from viral or bacterial infection of cells, are enzymatically cleaved into peptides which bind to the ubiquitously expressed class I human lymphocyte antigens (HLA). The peptide-HLA class I complex is displayed on the cell surface where it can be surveyed by the binding of cytolytic T cells expressing T cell receptors (TCR) and CD8 co-receptors on the T cell surface. Similar to antibodies, TCRs are assembled combinatorially, creating a diversity of specificities for HLA-peptide

complexes, with only a small subset of TCRs binding to a given complex. Should a given CD8 T cell be activated by the HLA-peptide complex, it will express a series of cytolytic molecules which kill the target cell. This system works due to the efficient T cell selection mechanisms applied during T cell development. After initial production of a rearranged TCR, the nascent T cell is tested in the thymus for inappropriate reactivity against cellular antigens. If the T cell survives the selection process, it exits to the body and theoretically will only be activated when it encounters and antigen that does not naturally exist in body, such as a peptide from a pathogenic organism, or a mutant peptide from an oncogenic cell [43].

Extracellular antigens can be any molecule taken up by a cell from its environment and degraded in lysosomes intracellularly. The resulting peptides are then loaded onto HLA class II molecules which, unlike HLA class I, are expressed on only a subset of immune-related cells. The class II HLA-peptide complex is recognized by a different T cell subset expressing TCRs with the co-receptor CD4. The CD4 T cell subset also undergoes thymic selection as observed with CD8 T cells, to eliminate recognition of self-antigens [44]. However, CD4 T cells can be induced to create different phenotypes once they specifically recognize class II HLA-peptide complexes. A large variety of T cell subsets have been described, including production of helper T cells, which participate in the activation of B cells for the production of antigen-specific antibodies, or regulatory T cells, which act to inhibit the immune response [45]. The choice of outcomes is driven by the soluble mediators, such as cytokines, found in the local environment, and the collection of co-receptors expressed on the antigen presenting cells.

HLA itself is a significant direct contributor to rejection responses outside of its role in antigen presentation. As described above, T cells are selected for lack of recognition of self-antigens. This not only includes the recognition of self-peptides bound to HLA molecules, but of the HLA molecules themselves. Normally, T cells bearing TCRs with inappropriately high affinity for binding HLA molecules, even in the absence of peptide, are eliminated early in T cell development. Because the human T cells have not been exposed to, or selected by, the class I or II swine lymphocyte antigens (SLA), a subset of human TCRs will bind to SLA and induce strong T cell activation, regardless of the peptide presented in the SLA [46]. As porcine cells are attacked by the human immune system, donor peptides are efficiently presented by human cells via HLA to human T cells as part of the normal human adaptive response. Conversely, depending upon the organ transplanted, there can also be donor T cells and antigen presenting cells transferred which result in donor immune responses against the host tissues, referred to as graft versus host disease (GvHD) [47]. In all cases, the immune cells are responding normally, but in the setting of xenotransplantation can be extremely pathogenic due to the artificially high concentration of immunogenic targets present.

Because HLA matching is part of organ selection in allotransplantation, a frequent question is whether introduction of human HLA in place of porcine SLA would help overcome rejection. Although SLA ablation may be helpful in averting antibody-dependent damage, this approach does not resolve some of the challenges related to antigen presentation in xenorejection responses [29]. It is true that the human T cell binding directly to pig SLA could be eliminated by substitution of SLA with HLA, however, the HLA genes are highly polymorphic, hence the need to HLA match human patients. This means that for a given patient, a donor pig would need to be engineered to specifically express the HLA homologous to that patient, which would be limiting given the timelines necessary for production and validation

of genetically-modified pigs. In addition, in the normal situation human cells display human peptides in the HLA, the overwhelming majority of which will be conserved between a human donor and recipient, and thus much less likely to induce a response. If pigs were engineered to express human HLA which is perfectly matched to the patient, the donor porcine cells could now be significantly more efficient at displaying porcine peptides to the human immune system and more rapidly induce T cell activation. Therefore, introduction of human HLA in place of porcine SLA may not provide a benefit without additional engineering.

Humans possess a number of pre-existing antibodies specific for porcine antigens which can contribute to the xeno-organ damage during HAR. As the donor tissue is damaged, the antigens are released and presented to T cells as described above, causing the activation of helper T cells. These T cells interact with B cells in lymphoid organs, inducing the activation of any B cells which express antibodies specific for the xeno-antigens. This initiates the germinal center reaction, in which antigen-specific B cells rapidly proliferate and mutate their antibody sequences and are then progressively selected for improved antibody function. The resulting B cells expressing the affinity-matured antibodies exit the germinal center and can differentiate further to plasma cells, which act as factories that can produce extraordinarily high levels of serum antibody [48]. These induced antibodies, like natural antibodies, further amplify AVR/AHXR and contribute to the destruction of the xeno-organ.

The *de novo* production of antibodies can be quite rapid and are a risk for the lifetime of the transplant whether for allo- or xenotransplantation. There are a number of drugs available for the control of B cell reactions. One of the most effective approaches is the depletion of B cells using antibody therapeutics such as Rituxan, specific for the CD20 surface molecule [49]. However, the constitutive ablation of host B cells will create long term immunosuppression and could be prohibitively expensive. Although highly related to CR in allotransplant, the B cell responses in xenotransplant are stronger and more challenging and likely to require more stringent therapeutic control.

9. Immune suppression and tolerance in xenotransplantation

Advancements in understanding of immune mechanisms in immune rejection have elucidated a number of targets and pathways for intervention, and discovered a variety of small molecule and protein therapeutics for the suppression and manipulation of the immune system. However, the restraint of the immune system required to prevent xeno-organ rejection places the patient at significant risk of infections, tumors and other diseases which are preventable by an intact immune system. Therefore, there is a growing interest in the application of immune tolerance mechanisms in the transplant setting.

Immune tolerance is the natural unresponsiveness of the immune system to targets which may otherwise create an immune response. As mentioned previously, there are many mechanisms used by the immune system to identify non-self-antigens to prevent autoimmune diseases. As the body of literature regarding the molecular basis of immune tolerance has grown, interest in testing tolerance mechanisms in xenotransplantation has also increased [50].

Mixed chimerism is one route that has shown significant promise in both allo- and xenotransplant settings. This approach combines the transfer to the recipient of both the organ and hematopoietic cells from the donor. Typically, the patient is pre-treated with radiation or drugs to allow hematopoietic cell engraftment prior to the organ transplant. The combination of hematopoietic cells from host and donor allows cross-tolerance of host immune cells to donor tissue as well as donor immune cells to host tissue. Therefore, the resulting immune system is a combination of the donor and host, or a "mixed chimera," which recognizes the donor organ and host tissue as "self" despite the differences in genetic origin [51].

A further refinement of mixed chimerism includes transplant of donor thymus into the recipient, allowing selection of host T cells via donor antigen presentation [52], suggesting that tolerance is T cell dependent. A large body of evidence points to the role of regulatory T cells (Treg) as a driver of immune tolerance. Treg cells are antigen-specific but upon binding of the specific HLA-peptide complex on antigen-presenting cells will produce a variety of immune inhibiting and tolerogenic factors. The Treg cells may be derived from either thymus selection (central tolerance) or selection in tissues (peripheral tolerance), with central tolerance believed to be more durable, and the conceptual basis for donor thymus transplantation in mixed chimerism [53].

A critical factor in the maintenance of tolerance is the balance between Treg and effector T cells over time. Any imbalance that increases the number of effector T cells can rapidly lead to immune rejection. If indeed the Treg population is the main active component of immune tolerance, then it may be desirable to specifically bolster the numbers of Treg cells transferred to the recipient to more greatly ensure that the balance is biased firmly toward tolerance. A number of groups have established protocols for the generation of Treg cells that are specific for xeno-organs and tissues through *in vitro* selections and expansions [54]. While this has been shown to have positive effects in allograft tolerance, the durability is variable and, worse, some studies have described conversion of Treg to effector T cells which then contribute to rejection [55]. Despite these concerns, mixed chimerism, with or without Treg supplementation, remains a potentially valuable approach to immune tolerance.

10. Genome engineering to improve xenotransplantation

The progress of xenotransplantation research in recent times has closely paralleled the advancement in genome engineering technologies. As the complexity of the engineering toolsets has increased, so too has the complexity of porcine genomic manipulations increased to address the immunological challenges described in previous sections.

Complex mammalian genome engineering has advanced much more rapidly in mice than in virtually any other species, including pigs. The reason for the rapid progress in mice is the availability of embryonic stem (ES) cells which can be maintained in culture for extended periods time and undergo extensive transfection/transduction protocols and drug selection without losing the ability to produce large numbers of fertile progeny via blastocyst injection [56]. Although several labs have made strides in this area, similarly manipulable and viable ES cells are not currently available for routine use in the generation of cloned pigs [57].

The most common approach for production of genetically-modified pigs is very similar to the protocol described in the creation of "Dolly the sheep." Briefly, the nucleus from a pig cell carrying the desired genome changes is extracted and introduced into a pig oocyte, which has previously had its own nucleus removed, and then induced to initiate embryogenesis using electrical and chemical induction, a process referred to as somatic cell nuclear transfer (SCNT). The newly-created cells are implanted into surrogate female pigs and allowed to develop to birth. Compared to genetically-modified mouse production, this process is significantly less efficient and more costly, limiting the number of facilities capable of effectively carrying out this complex process [58].

A key factor in the success of SCNT is the source of the donor nucleus. These cells are typically primary cells derived from fetal sources. Extended culture, transfection, or drug selection of these donor cells can all cause a significant loss of viability for subsequent productive SCNT. Therefore, the approaches commonly used for mouse ES cell manipulation such as multigenic targeting and selections with various drugs over long periods in culture would not allow for production of modified pigs using SCNT. Similarly, any genome manipulations of pig cells must also maintain the viability of the cells for SCNT, which alters the approaches available compared with mice.

10.1. Gene knockouts

One of the earliest genome engineering approaches applied to pigs was introduction of gene knockouts (KO). For any given gene, mutations which remove or disrupt the coding sequence can eliminate the expression of the gene and, provided that the KO is not lethal, create an organism which is entirely missing the gene product. The introduction of gene KO technology has been a key factor in the rapid advancement of the field of xenotransplantation [59].

As discussed above, there are several glycan molecules present in pigs which are absent in humans. These glycans are recognized by antibodies present in human serum which leads to rapid and extensive antibody-mediated damage to the porcine cells. Therefore, the elimination of the specific carbohydrate structures should help prevent human antibody recognition of the pig tissues. Unlike protein antigens which are directly coded by the DNA, glycosylation is due to the action of enzymes which create post-translational modifications of a variety of proteins produced by the cell. Therefore, glycosylation pathways must be examined to identify the key enzyme that creates the immunogenic glycan while otherwise leaving cellular metabolism intact.

The GGTA1 gene is responsible for creating the Gal alpha (1,3) Gal epitope in pigs. Although the specific reasons for this are unclear, human patients can express high levels of antibody specific for the Gal alpha (1,3) Gal epitope, presenting a major challenge to xenotransplantation [60]. The KO of the GGTA1 gene is one of the earliest genetic modifications of pigs for application in xenotransplantation, and results in greatly reduced human antibody recognition of porcine cells [61]. However, elimination of the GGTA1 gene alone has been shown to be insufficient due to a variety of other xenoantigens present in pig cells which are recognized by antibodies present in human serum. Generation of KO of CMAH [62], B4GALNT2 [28] and other xenoantigen genes have further decreased the reactivity of porcine cells to human serum. However, it is important to keep in mind that the greater the number of gene KO, especially when made in combination, may lead to detrimental effects on pig health.

10.2. Gene insertions

The use of gene KO approaches is highly useful for eliminating xenoantigens but does not address the need for expression of human or synthetic versions of genes necessary for control or proper function of dysregulated pathways. This requires the ability to permanently introduce heterologous DNA into the genome in a manner which maintains gene function.

The initial approach to gene insertions was simply random integration of DNA into the target genome. These genes are introduced from elsewhere and thus termed "transgenes" (TG). Once the ability to introduce DNA into mammalian cells was established using a variety of technological approaches, it became clear that over long-term culture a subset of cells could be isolated which have permanently incorporated the heterologous DNA. Because many transgenes do not provide a straightforward means to identify cells which have incorporated foreign DNA from the population that have not, TGs often include genes encoding drug resistance markers. In order for cells to survive drug treatment they must incorporate the resistance gene, greatly reducing the population to be screened, and increasing the chance of identifying cells which incorporate the TG of interest along with the drug resistance gene [63].

The integration of transgenes is rapid but relatively uncontrolled. Although there may be some preferences for integration site based upon chromatin accessibility, these are hard to predict and may be related to DNA breakage sites at which repair mechanisms fortuitously insert the transgene DNA [64]. The random nature of the insertions can create risks. For example, the same transgene inserted at different sites can yield highly variable results in expression. Furthermore, some insertions may be deleterious to cell function, causing them to grow more slowly or die off, or, if these cells are used for generation of animals *in vivo*, there is a possibility of insertions creating mutations, instability or even lethality.

Due to the risks of random integration, significant effort has focused on protocols to create targeted integration, or gene knock-in (KI), of heterologous DNA into the genome. This is accomplished in mice by taking advantage of ES cells which undergo homologous recombination. In this approach, the transgene of interest is flanked by DNA sequences that are identical to regions of the genome to be targeted. After introduction of the heterologous DNA, the regions of DNA sequence identity are aligned with the target sequence and the homologous recombination machinery creates crossover events to switch the endogenous sequence with the heterologous sequence. This approach is much less efficient than random integration of TG, therefore drug selection schemes often need to be employed to identify the relatively rare targeting events [64].

Homologous recombination is well-established for targeting in mice but requires ES cells which express the enzymes necessary for the targeting event. Unfortunately, porcine ES cells are not available that both possess homologous recombination function and can reliably generate cloned animals. For reasons that are not entirely clear, generation of ES cells competent for homologous recombination and cloning seems to be challenging for most species other than mice [57]. Therefore, alternate approaches are required for targeted integration in the pig genome [65].

10.3. Tools for genome engineering

A number of novel enzymatic molecules have been created which help resolve the dilemma of targeted integration in porcine cells. Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector Nuclease (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are all synthetic molecules based on genuine proteins which allow the precise targeting of genomic DNA based upon sequence [66–68]. In each case there are two functional components, a targeting module which recognizes a specific genomic sequence, and an enzymatic module which introduces a double-stranded DNA break at the target site. In the case of ZFN and TALEN, the targeting module is a complex array of protein sequences which have previously been shown to recognize specific DNA sequences and can be mixed in a modular way to bind to any desired sequence. Although both technologies have shown great success, the effort and cost required to identify a single functional molecule can be significant. In contrast, the relatively more recently recognized CRISPR, and related prokaryotic systems, is much more easily applied in mammalian cells. The DNA binding module in this case is RNA base-pairing to provide sequence specificity. The enzymatic module cleaves DNA, creating a double strand break similar to ZFN and TALEN. When heterologous DNA is present, the cellular repair machinery may use the synthetic DNA to repair the break, inserting the TG at the desired genomic site. It is important to note that all of these systems, ZFN, TALEN or CRISPR, are essentially the same in that they introduce double strand DNA breaks at a selected site in the genome and do not directly affect the rate of DNA insertion. Therefore, it is often necessary to include selection schemes for identification of the modified cells. The greater efficiency and ease of use of these systems, CRISPR in particular, has allowed targeted insertion of DNA into genomes that were not previously able to be modified [69].

Due to the challenges of creating genomic modifications in porcine primary cells while maintaining their viability for SCNT, more efficient engineering methods are desirable. One approach to enhance efficiency is to target a specific region of DNA, called a landing pad, with multiple genes at once. By inserting a DNA vector bearing multiple therapeutic genes at once, a large amount of breeding and testing can be circumvented using a single event. This approach has the added advantage of avoiding inefficient crossbreeding necessary to bring loci from distinct chromosomes together in one lineage. When combined with the use of tools such as ZFN, TALEN and CRISPR more rapid progress in the genetic modification of animals has been greatly facilitated [70].

11. Conclusions and future prospects

The increasing sophistication and accessibility of genome engineering toolsets and deeper understanding of immunological rejection mechanisms has allowed greater advancement in xenotransplantation than ever before. A key question is just how many genetic changes are required in order to make a pig organ suitable for transplantation? While the critical experimental data needed for such an assessment is still accumulating, it is clear that the number of alterations required for one organ may be different from another. For example, xeno-hearts with relatively minimal genetic modifications have demonstrated months to years survival in transplantation studies with non-human primates, whereas xeno-lungs with more extensive modifications have

yet to survive more than a few weeks. This is due to the relative differences in structure and function of organs, the resilience to trauma, and susceptibility to rejection responses. Furthermore, tolerance mechanisms may be able to supplant the need for some genetic modifications, and thus the specific protocols and treatments will govern the ultimate complement of alterations.

The immediate need in xenotransplantation is to define the specific genetics required for xeno-organ survival, however, it is possible to project further enhancements such that porcine organs may be superior to human organs for human transplant. Synthetic biology approaches have created novel genetic circuits which can react in real time to human immune responses, inducing counter-reactions in the porcine cells to circumvent and tolerize the xeno-organ against human rejection. Furthermore, xeno-organs may be engineered to express protein therapeutics to further control human immunity while saving hundreds of thousands of dollars in expensive biotherapeutic treatments. Thus, the first version of pigs appropriate for xenotransplantation are likely to be further refined and improved to create increasingly useful rejection-free organs.

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Chapter 17

Immunosuppressive Minimization Strategies in Kidney Transplantation

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Abstract

The long-term graft survival in renal transplantation results is still controversial, the toxicity and adverse reactions of the immunosuppressive drugs are implicated, as well as cellular and humoral antigen-specific immune mechanisms; therefore, different strategies for adapting immunosuppression are used to reduce the complications associated with the use of these drugs. Calcineurin inhibitors (CNI) require an adequate dose-dependent concentration leading to the appearance of drug-related adverse reactions. The variability in the required dose of CNI leads to minimization strategies that do not result in a higher acute rejection (AR) incidence when compared to other immunosuppressive agents. Early steroid withdrawal is another strategy, although with an increase in AR, but without an impact on the function and survival of the renal graft. The reduction of mycophenolate mofetil to 1.5 g/day seems to be a therapeutic option, decreasing the infectious, hematological and gastrointestinal adverse reactions. Finally, alemtuzumab, bortezomib, belatacept and cellular therapies are in the search for the new treatments, whose premise is the induction of donor-specific nonresponse in the context of operational tolerance or mixed chimerism. The use of adapted and adequate immunosuppression has led to variable results and some are very encouraging; however, they must be validated with experimental studies.

Keywords: renal transplantation, immunosuppressive minimization, acute rejection

1. Introduction

Renal transplantation (RT) is currently considered the best therapeutic option for renal replacement therapy in patients with end-stage renal disease (ESRD), with controversial results related to long-term graft survival [1–3]. Several factors can contribute to loss of the renal graft over time, which may be nonimmunological in nature, such as chronic nephrotoxicity due to drugs used for transplantation maintenance [particularly calcineurin inhibitors (CNI) tacrolimus (TAC) and cyclosporin] or for the side effects of immunosuppression when corticosteroids are involved, such as: infections, neoplasms, dyslipidemia, hypertension, cardiovascular disease, and new-onset diabetes mellitus (NODAT) that can lead to high mortality in patients with a functional graft [4–6]. Other conditions that induce long-term graft loss are the antigen-specific humoral and cellular immune mechanisms that contribute to an increase in the number and severity of episodes of acute rejection (AR), inducing chronic alloimmune damage [5–14]. These damage mechanisms raise the awareness that there must be a balance in posttransplantation immunosuppression; however, the new and powerful immunosuppressive drugs used today, and the alarming loss of kidney grafts, particularly due to the side effects of immunosuppression, have motivated transplant centers globally to try to minimize, suspend, or change the immunosuppressive maintenance drugs to try and further reduce the complications associated to them [15–39].

2. Minimizing immunosuppression with calcineurin inhibitors in kidney transplantation

The introduction of CNI has achieved exceptional short-term results in recent years in the field of allograft transplants, especially by reducing the rate of AR episodes, reaching, in the last 20 years, an overall graft survival of more than 90% in the first year [39]. However, the attention now focuses on the search for better long-term outcomes with strategies that sustain a low AR rate along with a decrease in the side effects of immunosuppression. The immunosuppressants have three effects: the therapeutic effect (rejection of suppression), unwanted consequences related to immunosuppression (infections, neoplasms, metabolic and hemodynamic disorders), and the nonimmune toxicity to tissues [40]. The nonimmune toxicity is immunosuppressive agent-specific and is related to the mechanism of action of the drug, since they target-specific molecules with certain functions in nonimmune tissues, conditioning progressive tissue damage, and gradual kidney graft failure. This, coupled with the death of the patient with a functional graft, encourages the new concept of focusing on nonimmune factors that intervene in the long term, evoking enthusiasm for strategies to minimize the side effects of CNIs.

3. Pharmacodynamics and nonimmune toxicity of the calcineurin inhibitors

In the classification of immunosuppressants, small molecules are included (from which the immunophilin-binding drugs are derived, such as CNIs, mechanism target of rapamycin

(mTOR) inhibitor (imTOR), nucleotide blocking agents, and antimetabolites); the protein-depleting and nonlymphocyte-depleting agents (monoclonal and polyclonal antibodies), the intravenous immunoglobulin, and corticosteroids [40]. The effects of CNI are proportional to the serum concentration levels, since this depends on the saturation dose of its targets [40], which makes the dosage and the control of serum levels important in maintaining the balance between the desired immunosuppressant effect and the unwanted toxicity.

Cyclosporin A (CsA) is a fungal origin polypeptide (derived from *Tolypocladium inflatum),* composed of 11 amino acids, with a molecular weight of 1203 Da, which interacts by binding to its cytoplasmic receptor (cyclophilin); a protein from the family of immunophilins, forming a complex that binds to the calcineurin, inhibiting its normal phosphatase action on regulatory nuclear proteins (nuclear factor -KB and activator protein 1), preventing the cytokine production (IL-2), and eventually the T lymphocyte activation [41]. The adverse reactions to CsA, related to the serum concentration of the drug, include: nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, and tremor [42]; and, less frequently, hemolytic uremic syndrome, and NODAT [40].

In 2004, a longitudinal cohort where 888 renal biopsies were collected from 99 patients who were in immunosuppressive treatment with CsA for 10 years after renal transplantation, was evaluated; finding arteriolar hyalinosis as the most sensitive marker for nephrotoxicity due to CsA [43]. Another CNI introduced in the mid-1990s, that was initially called FK506 and is currently known as TAC, is a macrolide isolated from the fungi *Streptomyces tsukubaensis* that possesses suppressive effects similar to CsA (cell-mediated and humoral immune responses) [41]. The TAC binds to a protein called FKBP12 (binding protein of FK506–12) and a complex that inhibits the phosphatase activity of calcineurin, preventing the activation of the T cell, and selectively affecting the transcription of IL-2 and other cytokines. The adverse reactions are similar to those of CsA but with less incidence of hypertension, hyperlipidemia, hirsutism, and gingival hyperplasia; however, the incidence of NODAT and nephrotoxicity is higher [44].

The mechanism through which nephrotoxicity occurs is explained by the endothelial dysfunction associated with reduced production of local vasodilators (nitric oxide and prostaglandins) and increased production of vasoconstrictors (endothelin and thromboxane) [45].

The determination of the serum levels of the CNI is part of the management of immunosuppression in transplant recipients, due to the variability between patients (and the intra-patient variability). The inter-individual variability with TAC is explained by polymorphisms in genes that encode transporter proteins and enzymes that metabolize the drug. The TAC is metabolized in the intestine, liver, and kidney by cytochrome P450 (CYP) 3A4 and 3A5. Inter-individual differences in CYP3A activity are the most important determinants of variability in TAC metabolism. Polymorphisms in the CYP3A5 gene explain 40–50% of the variability in the TAC dose requirement to maintain adequate serum levels: the most studied one is the single nucleotide polymorphism CYP3A5*3. This allele causes a reduced enzymatic activity associating with the need to reduce the administered dose of TAC. On the other hand, when CYP3A5 is expressed, a dose of about 50% higher is required [46, 47]. To a lesser extent, the CYP3A4 genotype with impact on the determination of doses in transplant patients receiving TAC has also been identified. Individuals carrying the CYP3A4 * 1B allele reported up to a 35% dose

reduction in order to achieve a therapeutic concentration. Similarly, it has been identified that the CYP3A4 * 22 variant reduces the enzymatic activity of CYP3A4, associated with a lower dose requirement. On the other hand, there are ethnic considerations that participate in allelic variability since Caucasian patients are commonly carriers of the CYP3A5 * 3 allele [46].

4. Minimization strategies of immunosuppression with calcineurin inhibitors

Given the nonimmunological toxic effects of CNI, two general strategies to reduce CNI are proposed: *de novo* minimization, where maintenance immunosuppression with CNI is sought immediately after transplantation at low doses subsequent to a powerful induction; and the second strategy, selective minimization, in which a class of immunosuppressants is avoided, showing a reduction of the undesired effects related to the drug. The Symphony study evaluated 1645 patients divided into four groups: (1) Standard dose of CsA, mycophenolate mofetil (MMF), and prednisone (PDN); (2) Low dose of CsA with induction therapy with daclizumab; (3) Low dose of TAC with induction with daclizumab; (4) Low dose of sirolimus (SRL) with induction with daclizumab. The primary aim was to reduce the nephrotoxicity using a low dose of CNI and SRL and to secondarily reduce the side effects, at the same time as maintaining the efficacy in terms of avoiding acute rejection, improving the overall survival of the patient and the graft. Their results at 1 and 3 years showed a better glomerular filtration rate (GFR) and graft survival in the group with low dose of TAC, as well as a low AR rate, compared with the SRL group [42, 48].

On the other hand, avoiding CNIs is the complete omission of these drugs from the maintenance immunosuppression regimen, while minimization schemes use reduced doses of CNI in order to avoid their nephrotoxicity [49]. Larso et al., compared regimens without CNI (SRL, MMF and PDN) and with CNI (TAC, MMF and PDN), in RT recipients, with similar results at 12 months in patient survival (98% SRL, 96% TAC, $p = 0.42$) and graft survival (94% SRL, 92% TAC, $p = 0.95$), as well as in the incidence of AR between both groups [50]. The regimens without CNI were also evaluated in the ORION51 study, which compared the efficacy of three schemes; (1) SRL + TAC with discontinuation of CNI at 13 weeks; (2) SRL + MMF; and (3) TAC + MMF. The SRL + MMF group presented more AR events (32.8%) compared to SRL + TAC (17.4%) and TAC + MMF (12.3%); however, the graft and patient survival were similar and there was the presence of hyperlipidemia in the group treated with SRL and NODAT in the group with TAC (**Table 1**) [51].

The BENEFIT study [52] compared two regimens (an intensive and a less intensive dose) with betalacept (selective T cell co-stimulation blocker) *versus* CsA in patients with living donor RT with standard criteria; finding better renal function with belatacept regimens (GFR of 65, 63, and 50 ml/min, respectively) but with a lower AR rate with CsA (22, 17 and 7%, respectively).

On the other hand, Weir et al. [53], who evaluated the efficacy and safety of the combination of MMF and SRL *versus* MMF and a CNI (TAC or CsA) at 24 months, found that the GFR was higher in the MMF/SRL regimen, with a similar AR rate in both groups.

Finally, a meta-analysis and systematic reviews related to these strategies have recently been published, with the aim of preventing nephrotoxicity and graft loss by a nonimmune character.

Immunosuppressor	Used dose in renal transplant	Adverse reactions	Minimization strategy
Tacrolimus	0.1–0.15 mg/kg/day divided in two doses	Nephrotoxicity, tremor, headache, dizziness, gingival hyperplasia, hypertension, carbohydrate intolerance, increased risk of infections and neoplasms.	Dose reduction; with lower nephrotoxicity without a higher acute rejection rate.
Cyclosporine	5–8 mg/kg/day divided in two doses	Nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, lymphoproliferative disorders associated to EBV, Kaposi sarcoma, TMA, HUS.	Dose reduction; improves the glomerular filtration rate and graft survival when compared to mTOR inhibitor.
Mycophenolate mofetil	2 g/day orally divided in two doses	Gastrointestinal: abdominal pain, nausea, vomits, diarrhea. Hematological: anemia, leukopenia, thrombocytopenia, increased risk of infections (especially viral), neoplasms.	Dose reduction not less than 1.5 g/day can decrease the gastrointestinal, hematological and infectious adverse reactions without an acute rejection rate increase.
Prednisone	0.5–1 mg/kg, orally divided in two doses with a taper of 5–10 mg/day indefinitely	Susceptibility to infections, obesity, osteonecrosis (avascular necrosis), hyperglycemia, hypertension, dyslipidemia, peptic ulcer, cushinoid features, long-term myopathy, osteoporosis, atherosclerosis, skin atrophy, cataracts.	Steroid withdrawal; better graft survival, lower risk of mortality, decrease in graft dysfunction with an improved metabolic and hemodynamic profile, although with contradictory results that may involve higher acute rejection rates proved by biopsy without affecting the long-term graft survival.
Alemtuzumab	30 mg/kg intravenously unique pre-transplant induction dose	Predisposition to severe infections (bacterial, viral, fungal) and increased risk of neoplasms.	Used as pre-transplant induction therapy allowing early steroid taper, CNI decrease or change to mTOR with a reduction in AR episodes in the first posttransplant year, without differences in graft survival in patients with low immunological risk and conventional therapy.
Bortezomib	1.3 mg/m^2 intravenously on day 1, 4, 8 and 11 for two cycles	Peripheral sensory neuropathies. Hematologic: anemia, leukopenia, thrombocytopenia Nausea, diarrhea, weakness.	Used as desensitization therapy, severe humoral rejection treatment, allowing an immunosuppression restart at an adjusted dose, being considered an immunosuppression minimization strategy.
Belatacept	10 mg/kg intravenously on posttransplant days 1, 5, 14 and then every 4 weeks indefinitely	Greater predisposition to lymphoproliferative disorders not associated to EBV, herpes virus and tuberculosis infections.	Adjuvant treatment with MMF and prednisone maintains a CNI-free immunosuppression with an increase in acute rejection in the first 6 months (posttransplant) but better long-term renal graft function compared with CsA.

Immunosuppressor	Used dose in renal transplant	Adverse reactions	Minimization strategy
Belimumab	Initial dose:120 mg intravenously, then 400 mg IV every 2 weeks indefinitely	Infusion related (bradycardia, myalgias, rash, urticarial, hypotension), depression, insomnia, nausea, diarrhea, bronchitis, pharyngitis, increased risk of viral infections.	Is in experimental phase, it can induce immunologic tolerance or mixed chimerism as an immunosuppression reduction strategy.

TMA, thrombotic microangiopathy; EBV, Epstein-Barr Virus; HUS, hemolytic uremic syndrome; mTOR, mechanistic target of rapamycin; CNI, calcineurin inhibitors; CsA, cyclosporine [50, 51, 53, 60, 61, 95, 97, 100, 101, 103].

Table 1. Immunosuppression in renal transplant, adverse reactions and minimization strategies.

Sawinski et al. [54], evaluated 88 clinical trials regarding CNI reduction strategies associated with MMF or imTOR in a meta-analysis (minimization of the CNI without suspending it, conversion to another immunosuppressant "imTOR," and withdrawal of the CNI in the post-transplant period or never used in the RT); finding the best results with the strategies in which the CNI was minimized, especially in the first 6 months without stopping it, with a lower incidence of AR (RR 0.80, 95% CI 0.68–0.95), graft loss (RR 0.71 95% CI 0.56–0.9), and better graft function and no differences in mortality (RR 0.87, 95% CI 0.66–1.15), compared to standard regimens with CNI. Nevertheless, it is important to mention that the majority of studies were based on CsA induction with basiliximab, thus more research is needed to determine the role with other immunosuppressants (TAC and thymoglobulin) and their doses, with the aforementioned strategies. Finally, a systematic review of 83 studies that included a total of 16,156 patients with a removing sample (RR 2.54; CI 95%: 1.56–4.12) or an avoiding sample (RR 2.16; CI 95%: 0.85–5.49) of CNI from the immunosuppression maintenance regimen, was associated to AR without a difference in graft loss (RR 0.96; CI 95%: 0.79–1.16), and with a lower incidence of hypertension in the CNI-abstained groups (RR 0.82, CI 95%: 0.71–0.95) [55].

5. Strategies for removing steroids from immunosuppression in kidney transplantation

Another tempting strategy for reduction of posttransplant immunosuppression is to withdraw or avoid the use of corticosteroids because of the numerous side effects, with the purpose of improving quality of life and reducing cardiovascular mortality.

This intervention has increased from 5 to 35% since the year 2000 until today, in RT recipients in the USA. Historically, the removal of steroids has been associated with the risk of precipitating AR [56, 57]; however, long-term safety in terms of patient and graft survival has been satisfactory with early steroid withdrawal (ESW); as Rizzaari shows [58] in a 10 year follow-up of 1241 RT recipients with graft survival, showing similar death in living donor RT recipients with maintained with steroids (79 *vs.* 73%) and with even an better survival in deceased donor RT (80 *vs.* 67%) with a report in their survival analyses free of AR, similar between the

groups with and without corticosteroids. Lopez Soler et al. [59], similarly reported in a cohort undergoing ESW with a 10-year follow-up that showed better graft survival ($p = 0.023$), lower risk of mortality (0.23, $p \leq 0.011$), and less graft failure (0.57, $p = 0.026$).

Similar to minimization of the CNI, numerous meta-analyses have been published regarding a population undergoing ESW, both in the adult and pediatric populations, concluding, in some, a higher rate of AR, especially of mild characteristics, without greater impact in the function or graft survival, and with satisfactory results in the metabolic and hemodynamic profile, reducing cardiovascular morbidity and mortality (**Table 1**) [19, 20, 60, 61].

Knight et al. [19], evaluated a meta-analysis of 34 clinical trials with 5637 patients in regimens that included withdrawal or nonuse of steroids at any time of the transplantation, and found that the withdrawal of steroids was associated with a higher incidence of AR (RR 1.56; 95% CI 1.31–1.87), but with a lower incidence of hypertension (RR 0.64; 95% CI 0.50–0.83), diabetes (RR 0.90; 95% CI 0.85–0.94), and hypercholesterolemia (RR0.96; 95% CI 0.67–0.87); concluding that AR had no impact on function or survival of the grafts because it was considered mild.

Zhang et al. [60], in a meta-analysis of 13 clinical trials in 3520 patients with ESW after transplantation found a higher incidence of AR; but when the trials that used TAC were exclusively analyzed, the statistical significance was lost and only remained in those that used CsA. Studies that involve corticosteroid withdrawal associated with TAC in the immunosuppression regimen, document the development of borderline changes in AR, especially in the early stage of transplantation, without impact on function or survival of the graft [23, 62].

The current use of immunosuppression induction with anti-DC25 antibodies (basiliximab) or lymphocyte depletion (thymoglobulin) combined with MMF and TAC has favored that the minimization or elimination of the use of posttransplant steroids be safe with cell type AR rates comparable to those that maintain the use of the posttransplant steroid [16, 17, 20–27, 29–31, 58, 60, 63–67].

The good results from minimization or suspension of some immunosuppressants are encouraging because one of the main associated causes with poor long-term kidney graft survival is directly or indirectly related to the side effects of immunosuppressants that cause long-term complications and even a higher cardiovascular mortality [4, 11, 43, 68–71].

Experience with this intervention in our transplant center has shown satisfactory short-term (12 months) results with similar AR rates in immunosuppression with and without steroids, with lower glucose levels, lipids, and better blood pressure parameters, which leads to less use of antihypertensive and lipid-lowering drugs in the group without steroids [17, 23]. Nonetheless, despite the acceptable results found with these strategies the community dedicated to transplantation is concerned about what happens with these long-term immunosuppression strategies, especially since presently one of the main causes of graft loss is the chronic antibody-mediated rejection mainly associated to sub-immunosuppression.

Nonetheless, the sub-immunosuppression generated by minimization strategies or suspension of an immunosuppressant in the posttransplant context causes uncertainty regarding the formation of antihuman leukocyte antigen (HLA) antibodies [donor-specific antibodies

(DSA) or nondonor-specific antibodies (NDSA)] over time, with an increased risk of antibody-mediated AR and graft loss. Some studies show results in the incidence of DSA with immunosuppression regimens considered less potent, which could cause the appearance of humoral AR and long-term graft loss [33–37]. Kreijveld et al. [72] showed that the reduction or removal of TAC from immunosuppression in the posttransplant period does not generate antibodies and does not even predict the development of AR. As for steroids, the mechanism of suppression of antibodies by the B lymphocyte with the use of these drugs has created the idea that avoiding or removing steroids in the posttransplant period favors the appearance of antibodies against the major histocompatibility complex (MHC) and against other renal donor antigens. Even so, information related to the formation of DSA with the minimization or suspension of steroids posttransplantation is scarce [73–75].

6. Antihuman leukocyte antigen antibodies in kidney recipients with steroid withdrawal

One of the leading causes of long-term graft loss is interstitial fibrosis and tubular atrophy (IFTA) and the appearance of DSA posttransplant, which seems to play an important role in graft dysfunction.

The B lymphocyte antibody suppression combined with the use of steroids has created the idea that avoiding or removing these drugs in the posttransplant period may induce the appearance of antibodies. Unfortunately, there is not sufficient evidence to uphold that the withdrawal of steroids contributes to the increase in production of antibodies or if it is associated to a higher rejection rate and chronic graft dysfunction, that being with steroids in the immunosuppression regimen. Delgado et al. [73], in a retrospective study of 43 kidney recipients during posttransplant antibody monitoring, showed that patients with steroid withdrawal did not develop DSA compared to the steroid maintained group posttransplantation. Drugs such as MMF and thymoglobulin, in addition to interacting with T lymphocytes, inhibit the formation of B lymphocyte antibodies, so it is possible that the immunosuppression regimens that use these drugs provide greater safety even when steroids are avoided or suspended after the transplantation. Furthermore, the avoidance or withdrawal of steroids may enhance the myelosuppressive effect of MMF, since steroids induce greater activity of the hepatic enzyme uridine diphosphate-glucoronosyltransferase that degrades MMF [76]. In addition, steroids induce the cytochrome P450 isoenzyme 34ª responsible for the metabolism of TAC, and so avoiding these drugs would favor the increase of TAC serum levels, thereby increasing their immunosuppressive effect [77]. These possible mechanisms suggest that the appearance of DSA induced by the suspension of steroids after transplantation could be no different than in the immunosuppression schemes maintained by these drugs.

In a clinical trial recently performed at our center with living donor kidney transplant recipients who underwent protocolized biopsies every 3 months, it was found that the presence of AR was no different between patients who had an early steroid removal compared to those in which the drugs were sustained. However, the suspension of steroids has generated uncertainty about the risk of developing DSA posttransplant, over the course of time. Due to this

concern, our team recently conducted a prospective cohort of 77 patients with low immunological risk (data not yet published) where findings revealed that the presence of cellular AR was a predictor for the formation of DSA against class II antigens, coinciding with the results of other authors [78]. There is currently little scientific evidence in which the absence of steroids in the posttransplant period may generate a greater presence of posttransplant DSA. Delgado et al. [73], observed that in a retrospective study of 43 kidney recipients during posttransplant antibody monitoring, patients with steroid suspension did not develop DSA compared to the group with maintained steroids. On the other hand, de Kort et al. [79] recently showed that in a population with steroids suspended using lymphocyte-depleting immunosuppressive induction (alentuzumab) and monotherapy with TAC, there was an increased risk for the development of DSA from an early posttransplant stage. Our study (data not yet published) also showed a higher incidence of DSA in patients with immunosuppression therapy without steroids appearing from a very early stage of the transplantation (<12 months). Unlike the study by de Kort et al. [79], 97% of our population undergoing steroid withdrawal used nonlymphocyte-depleting antibodies (basiliximab) with a double immunosuppression maintenance regimen based on MMF and TAC.

The immunoglobulin subclasses (IgG1/IgG3) capable of binding and activating the classical complement pathway (C1q) can predict the presence of antibody-mediated AR even with phenotypes of more severe damage (extensive microvascular inflammation and increased C4d deposition) and risk of kidney graft loss [80–83]. Undoubtedly, the measurement of antibody subclasses in patients subjected to a sub-immunosuppression state with minimization schemes or suspension of immunosuppression should be considered in order to discern whether the presence of these antibodies, according to the ability to fix complement, can generate chronic damage and lower the survival of the grafts. Finally, the benefits obtained from the nonsteroid schemes in the posttransplant stages in the lipid, metabolic, and blood pressure profiles, in our previously reported experience, should be considered for its possible risk of activating the immune system [17, 23].

7. Minimization strategies of mycophenolate mofetil in renal transplantation

Mycophenolate mofetil (MMF) has been established as the leading immunosuppressive regimen in most clinical trials and in almost 100% of the renal transplant centers in the world. With the initial use of CsA a daily dose of MMF was established at 2000 mg, while now, since the immunosuppressant regimen has changed to TAC significantly improving graft survival, the dose of MMF has not been established [84].

The MMF is an antiproliferative drug that requires de-esterification in gastrointestinal tissue for its absorption, thus releasing mycophenolic acid (MPA) that is freely absorbed and needs a pH > 5.5 to facilitate absorption in the small intestine. The most common use of MMF is still the prevention of AR in renal, pulmonary, cardiac, and hepatic organs, in adjunct with other immunosuppressive agents, which has shown to reduce AR by 20–40% in RT compared with azathioprine (AZA).

CsA and TAC have a different influence on enterohepatic circulation and the metabolism of MPA. The TAC increases serum levels of MMF and therefore exposure of the metabolite in the blood circulation in patients undergoing this immunosuppression regimen when compared to CsA, while the decrease in MMF dosage combined with TAC has not yet been well studied and no conclusive results have been established [85].

Clinical trials have tried to establish the MMF dosage. Doria et al. [86], included 901 patients with *de novo* RT, assigning three study groups with a MMF dose of <2000, =2000, and >2000 mg with thymoglobulin and an alemtuzumab-based induction, and no significant differences were found at 1 year follow-up regarding AR and graft loss; but they did find an increase, though not significant, in hematological complications related to leukopenia, anemia, and greater gastrointestinal disorders in patients with MMF doses of 2000 and >2000 mg.

These side effects have also motivated the establishment of adjusted dosing for certain populations. There are several controversies about whether reducing MMF dose modifies graft survival. Ji et al. [87], evaluated 128 patients with a low immunological risk at 12 months of follow-up, using immunological induction with basiliximab, methylprednisolone bolus (MPD) and TAC with a dosage of 0.1 mg/kg/day divided into two doses, PDN at 1 mg/kg/day at dose reduction, and MMF in different doses: = 500, <1500, and >1500 mg; finding, in the low dosage groups (=500 and <1500 mg), an increased number of cases of AR, renal graft dysfunction, and C4d deposition in follow-up biopsies, while the conventional dose group of MMF ≥ 1500 mg did not present any representative difference. Therefore, it is suggested that the dose should be individualized to the demographic characteristics of each population, under an integral evaluation of weight and height, and likewise that immunosuppression should not be reduced to doses less than 1 g of MMF per day nor suspension of the antimetabolite, since it jeopardizes the survival of the graft.

The side effects of MMF are divided into those due to gastrointestinal disease where diarrhea is the main manifestation with a frequency of up to 40–50% and in severe cases has been attributed as a cause of histologically inflammatory colitis type lesions similar to Crohn's disease.

Within the hematological side effects attributed to the drug there is leukopenia with or without neutropenia that can be potentiated by the use of other, concomitant drugs (Valganciclovir, trimethoprim with sulfamethoxazole, etc.) during the early period of RT. Other attributable side effects are hypogammaglobulinemia and severe anemia, especially in the first posttransplant months. The MMF has been associated with pneumonia due to pneumocystitis jirovecci, cytomegalovirus (CMV) disease, reactivation of Chagas disease, infection with Epstein-Barr virus (EBV), and risk of malignancy. On the other hand, patients with solid organ transplantation with hepatitis C seem to have better long-term outcomes with MMF therapy [88]. There is a strong association between the concentration of MPA, the pharmacological effects, and inter-individual variability between the MPA within the area under the curve (MPA AUC) estimated as the concentration of MMF after systemic elimination, enterohepatic recirculation, and the concentration before the dose (C 0) [89].

Two analysis tools have been used for the measurement of MPA plasma levels: high performance liquid chromatography (HPLC) and enzyme multiplied immunoassay technique (EMIT). The EMIT is less specific in the measurement of MPA than HPLC: the concentrations of MPA that are

obtained by the EMIT method are typically higher than those of HPLC. The overestimation of the MPA concentration by the use of EMIT is approximately 24–35%. The degree of overestimation varies depending on the patients' characteristics, the time elapsed since the transplantation, and time of the blood sampling. However, in pediatric RT recipients the EMIT assay showed a diagnostic efficacy comparable to HPLC to assess the risk of AR, leaving EMIT as an acceptable monitoring tool for MPA. Therefore, either HPLC or EMIT can be used, although HPLC is a more specific analytical tool for the accurate assessment of MPA and metabolites [90, 91].

This clinical data supports the need for therapeutic monitoring of MPA. However, this could result in higher costs and time since the precise measurement of MPA AUC 0–12 h requires multiple blood samples during the dosing interval, which can be expensive and clinically impractical [91].

It is well established that in RT recipients, MMF reduces the risk of AR and improves graft survival; nonetheless, the side effects that include diarrhea in up to 37.3%, hematological alterations (leukopenia, anemia, thrombocytopenia), and an increase in the incidence of infections in 23–25% during the first year of transplantation, make it necessary to reduce the dose of MMF. Such side effects can be avoided by individualizing immunosuppression in patients, and other studies have demonstrated that the minimization strategies of immunosuppression must be adjusted according to the race, gender, and anthropometric characteristics at each transplant center [92].

8. New strategies for minimization of immunosuppressive therapy in kidney transplantation

8.1. Alemtuzumab

New strategies in the minimization of immunosuppression involve the use of alemtuzumab (humanized monoclonal antibody that targets CD52 on lymphocytes) used as a reduction strategy in doses of CNI and immunosuppression without steroids [93, 94].

Chan et al. [95], reported in 82 patients treated with alemtuzumab (TAC as monotherapy) *versus* 42 patients with daclizumab, TAC, and MMF; all with ESR, with results of a low AR incidence at 6 months posttransplant, and without differences in the survival of the graft or in its function, confirming the minimization of immunosuppression as a therapeutic strategy with this drug (**Table 1**).

In 3-year posttransplant follow-up studies, alemtuzumab combined with ESR has shown reduction in AR episodes in patients with low immunological risk compared to basiliximab-based induction, while the presentation of AR was similar in those patients with high immunological risk in whom immunosuppressive induction was compared with thymoglobulin. The main advantage of the use of alemtuzumab as a strategy to reduce immunosuppression is found in the availability to reduce the used dosage of CNI and the subsequent conversion to maintenance immunosuppression based on imTOR, whose main objective is to avoid chronic nephrotoxicity and improve graft survival and long-term function (**Table 1**) [96, 97].

The therapeutic effect of alemtuzumab is not different from the immunological induction with thymoglobulin in the areas of AR incidence, delayed graft function, CMV infection, development of NODAT, and use of granulocyte colony stimulant [98].

8.2. Proteasome inhibitors

Proteasome nonselective inhibitors prevent the antibody-mediated AR of the graft. However, adverse effects outweigh the benefits by limiting their application in clinical practice. Up till now, the inhibition of immunoproteasomes is effective in experimental models in the context of autoimmune diseases being used for several weeks of treatment, without significant side effects. The ONX 0914, a selective proteasome inhibitor (B5i) of the LMP7 subunit, prevents chronic rejection in allogenic kidneys transplanted in rodents. The selective inhibition of immunoproteasomes by ONX 0914 and bortezomib reduces the number of plasma cells and B lymphocytes, and suppresses the formation of donor-specific antibodies in transplanted organs.

In renal grafts, T lymphocyte, B lymphocyte, and macrophage infiltration is reduced, as well as the complement deposit, interferon-γ, interleukin-17, and IgG [99].

Several series of cases have shown the efficacy of bortezomib in reversing the severe antibody-mediated rejection, establishing the maintenance therapy in posttransplant patients, and has even been used as a desensitization treatment in recipients with a positive cross test considered highly sensitized with satisfactory results; formulating guidelines to establish strategies for adjusting immunosuppression in long-term RT recipients. However, there are contradictory results. The BORTEJECT study [100] used bortezomib as a treatment for late antibody-mediated AR in 44 patients, with a follow- up of 3 years, with immunosuppression based on imTOR or CNI, with MMF 1–2 g/day and PDN, without finding a significant difference in the incidence of AR and renal function compared with placebo. Therefore, studies that evaluate the use of the drug in the induction and maintenance of immunosuppression are necessary to allow the minimization or optimization of the therapy used in selected cases (**Table 1**).

8.3. Belatacept

Belatacept (CTLA-4 Ig fusion protein) is a new drug with a mechanism of action that allows CNI-free maintained immunosuppression. Clinical studies show a higher incidence of T cell-mediated AR in the first 6 months after transplantation, but show better long-term renal graft function. Likewise, the use of belatacept shows a lower incidence of DSA formation and less graft damage compared to the use of CsA. The most relevant adverse reactions include: herpes virus infections, tuberculosis, and a higher frequency of posttransplant lymphoproliferative disorders.

Belatacept has not yet been compared to a TAC/MMF-based regimen, considered the immunosuppression maintenance standard in RT.

The current immunosuppressive treatment, far from being perfect, has contributed to the overall improvement of the renal graft and patient survival, which contributes to overcoming the barrier for the development of new therapeutic agents. Consequently, most of the

new drugs have failed in the course of transplantation, including janus kinase inhibitors (tofacitinib), sphingosine-1-phosphate receptor modulator (FTY720, fingolimod), protein kinase C inhibitor (sotrastaurin, AEB), inhibitors of adhesion anti-LFA-1 molecules (efalizumab), anti-ICAM-1, and the first generation of anti-CD40-ligand. Most of the current treatments, still in research and focused on the immunology of the transplant, are biological or cell-based treatments. The blocking of co-stimulation with the purpose to prevent T cell activation remains a point of interest. The ASKP1240, an anti-CD40 monoclonal antibody, has recently been tested in immunosuppression minimization regimens based on CNI dose reduction or suspension, compared to a control group based on standard dose TAC, finding higher AR and infection rates in the group treated with anti-CD40, so the future of this drug remains uncertain. More recently, CFZ533, a fully humanized monoclonal antibody has shown efficacy in primates, and clinical research is being initiated in humans (**Table 1**) [101].

8.4. Belimumab

Belimumab (human monoclonal antibody that inhibits B cell activating factor), approved for treatment in systemic lupus erythematosus (SLE), is in use in early-stage clinical studies for the prevention of antibody-mediated RA, as well as in patients sensitized with low titers of anti-donor-specific antibodies.

Cellular therapies represent an innovative therapeutic objective for the maintenance of long-term renal graft, and thus avoidance of adverse reactions related to the maintenance of immunosuppression. The premise of cell therapy is the induction of donor-specific nonresponse in the context of operational tolerance or mixed chimerism (**Table 1**) [100].

A single center study evaluated the autologous use of mesenchymal progenitor cells instead of the antibody-based induction with schemes based on low and high doses of CNI, comparing induction with basiliximab and standard maintenance with MMF-CNI. The induction of autologous mesenchymal progenitor cells resulted in a lower AR rate, a decrease in opportunistic infections, and better renal graft function 1 year after transplantation, concluding with conventional immunosuppressive maintenance [102].

Another study was carried out in hematopoietic progenitor cells transplant with HLA concordant kidney donors in adjunct with total lymphoid radiation and thymoglobulin, which resulted in persistent mixed chimerism with stable renal graft function and removal of all immunosuppressants in 50% of the patients (**Table 1**) [103].

The most recently used strategies include the use of a product based on hematopoietic stem cells "facilitating cells" co-administered with nonmyeloablative reconditioning in living donor kidney graft recipients, reaching, in five out of eight patients, a satisfactory donor-chimerism with successful immunosuppression maintenance withdrawal without evidence of AR or graft-versus-host disease [104].

The results of the previous studies, although very encouraging, should be validated in larger multi-centric controlled and randomized studies, from the safety-efficacy and cost–benefit points of view, compared with conventional immunosuppression therapy.

Conflict of interest

There are no conflicts of interest to report.

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Chapter 18

Diagnosis, Treatment, and Outcomes of Antibody-Mediated Rejection in Kidney Transplantation

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Additional information is available at the end of the chapter

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Abstract

Antibody mediated rejection remains an important barrier to optimal long-term outcomes after kidney transplantation. Donor specific antibody, while not the formidable barrier to transplantation it once was, remains a major risk factor for antibody mediated rejection and its consequences of premature graft failure. Recent advances in understanding of the cellular and molecular mechanisms of antibody production and antibody-mediated injury have led to refinements in diagnostic techniques, and have paved the way for the development of novel therapies to treat rejection and prolong allograft function. The purpose of this chapter is to review the current level at which we understand the pathophysiology of antibody mediated rejection, describe the current diagnostic criteria for antibody mediated rejection, and discuss available and emerging treatments as well as their outcomes.

Keywords: kidney transplantation, donor specific antibody, antibody mediated rejection

1. Introduction

The first successful kidney transplant was performed between identical twins by Joseph E. Murray and his colleagues at the Peter Bent Brigham Hospital in 1954 [1]. Since then, the field of kidney transplantation has progressed immensely owing to greater understanding of the immune mechanisms underlying allograft rejection at a cellular and molecular level and development of increasingly potent immunosuppressive drug therapies [2]. Today, kidney transplantation is considered the treatment of choice for patients with end stage renal disease (ESRD) since it is associated with lower mortality and cardiovascular morbidity while offering

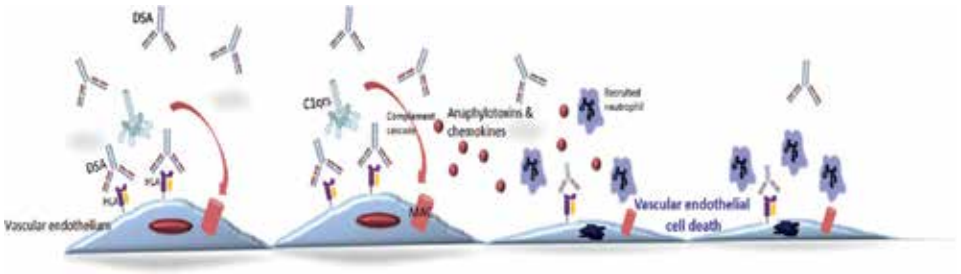

Figure 1. In AMR, DSAs bind to human leukocyte antigens on graft vascular endothelium. This is followed by activation of complement, membrane attack complex-mediated cellular injury and infiltration of mononuclear cells. Reproduced with permission from Montgomery et al. [4].

improved quality of life [3]. However, allograft rejection remains a major impediment to the longevity of renal allografts. Recognition of donor antigens as "non-self" by the host immune system elicits humoral and cell mediated immune responses that if left unchecked result in the destruction of the allograft (**Figure 1**).

2. Human leukocyte antigen (HLA) system and allograft rejection

Human leukocyte antigens (HLA) play a vital role in host defense against foreign pathogens and in immune surveillance of tumors. These antigens are encoded by the major histocompatibility complex (MHC) which is a family of genes that encompasses a 3.6 million base pair genomic region, 6p21, on the short arm of chromosome 6 [5]. The MHC complex is divided into three regions representing three classes of genes—classes I, II, and III. The HLA genes that are involved in the immune response belong to classes, I and II [6]. Class I antigens are expressed on all nucleated cells whereas class II antigens are expressed only on professional antigen presenting cells (APCs)—dendritic cells, B-cells, and macrophages [7]. In the setting of infection, pathogen derived foreign antigens are presented to T-cells as peptides on the surface of major histocompatibility complex (MHC) molecules expressed on the surface of APCs. The ensuing signaling cascade results in the activation, proliferation, and differentiation of naïve T lymphocytes into subtypes with distinct cytokine profiles. Type 1 helper T (Th1) cells drive the cellular immune response mediated by CD8+ cytotoxic T lymphocytes, and type 2 helper T (Th2) cells drive B-cell mediated humoral immune response [2].

The heterogeneity of human MHC molecules enables the immune system to protect us from a variety of foreign pathogens. However, in the context of transplantation between genetically distinct individuals, these MHC polymorphisms elicit immune responses that can result in rejection of the allograft [7]. Two pathophysiologically distinct flavors of renal allograft rejection are recognized—cell mediated, and antibody mediated rejection. Both these types of rejection can manifest as acute or chronic clinicopathologic variants. Acute cell mediated rejection is characterized by infiltration of the allograft by effector T cells resulting in the typical features such as tubulitis, interstitial inflammation and in more advanced cases, endothelial arteritis [2, 8].

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3. Clinical relevance of antibody mediated rejection

AMR is estimated to occur in 3–10% of transplant recipients and it represents 20–30% of episodes of acute rejection [7]. Although less common than cell mediated rejection, AMR is generally recognized to have a worse prognosis and requires different forms of therapy [9]. In the 1960s anti-HLA antibodies were recognized as a cause for allograft rejection following reports of hyperacute antibody mediated rejection in patients with antibodies reactive to donor lymphocytes [10, 11]. Patel and Terasaki's landmark study documented immediate graft failure in 24 of 30 (80%) of the patients with circulating donor reactive antibodies identified by a positive cytotoxicity crossmatch [12]. This led to the universal practice of antibody screening by complement dependent cytotoxicity (CDC) crossmatch prior to renal transplantation and the avoidance of transplantation in patients with a positive crossmatch. Therefore, until the mid-1980s, acute cellular rejection, as opposed to antibody mediated rejection (AMR), was considered the major barrier to successful [13]. The advent of calcineurin inhibitors (CNIs) in the 1980s led to a significant decline in incidence of acute rejection and a consequent improvement in short term graft survival rates [14]. Today, cellular rejection seldom causes graft loss [15]. However, contemporary data suggests that these gains have not led to sustained improvement in long-term graft survival [16]. Reasons for the lack of improvement in long-term graft survival have been a topic of much debate and most late graft losses were attributed to either chronic allograft nephropathy (CAN) or death with a functioning graft [17]. Although, the multifactorial nature of late renal allograft loss makes therapeutic intervention challenging [18] prevention and treatment of AMR holds the key to optimizing long term graft survival.

Exposure to non-self HLA by way of pregnancy, blood transfusion or transplantation may lead to the development of circulating anti-HLA antibodies. ESRD patients who are sensitized to HLA by prior exposure have a prolonged wait-time for transplantation and reduced transplant rates. Removal of pre-formed circulating donor specific antibodies (DSA) by various desensitization techniques allows transplantation of many of these biologically disadvantaged patients [19–21] However, such HLA incompatible kidney transplants recipients are at increased risk for developing AMR. A high percentage of episodes of AMR are difficult to treat and may cause immediate graft loss or delayed transplant glomerulopathy [22]. Therefore, AMR remains a significant impediment to the success of transplantation in this subset of patients.

4. Diagnosis of antibody mediated rejection

The Banff classification schema has been used internationally for scoring and classifying kidney transplant pathology findings since its first iteration was published in 1993. However, earlier versions dealt with AMR in an imprecise manner. The development of more sophisticated methods of detection of DSAs by means of solid-phase assays together with the sensitivity and specificity of C4d staining in peritubular capillaries in identifying AMR paved the way for rigorous morphological classification of AMR [23]. The cornerstones for the diagnosis for AMR are (1) Histologic evidence of acute tissue injury; (2) Evidence of current/recent antibody interaction with vascular endothelium; (3) Serologic evidence of DSAs. The updated 2015 Banff classification system recognizes acute active AMR and chronic active AMR and outlines detailed criteria for the diagnosis of each (**Table 1**) [24].

Acute/active AbMR	All three features must be present for diagnosis. Biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious for acute/active ABMR. Lesions may be clinically acute or smoldering or may be subclinical; it should be noted if the lesion is C4d-positive or C4d-negative, based on the following criteria: 1. Histologic evidence of acute tissue injury, including one or more of the following: • Microvascular inflammation ($g^a > 0$ in the absence of recurrent or *de novo* glomerulonephritis, and/or $ptc^b > 0$) • Intimal or transmural arteritis ($v^c > 0$) • Acute thrombotic microangiopathy in the absence of any other cause • Acute tubular injury in the absence of any other apparent cause 2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections or C4d >0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute T-cell mediated rejection (TCMR), borderline infiltrate, or infection; ptc ≥2 alone is not sufficient, and g must be ≥1 • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated 3. Serologic evidence of DSAs (HLA or other antigens) Biopsies suspicious for AMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing
Chronic active ABMR	All three features must be present for diagnosis. As with acute/active ABMR, biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious, and it should be noted if the lesion is C4d-positive or C4d-negative, based on the criteria listed: 1. Histologic evidence of chronic tissue injury, including one or more of the following: • TG ($cg^d > 0$), if no evidence of chronic thrombotic microangiopathy; includes changes evident by EM only • Severe peritubular capillary basement membrane multilayering (requires EM)c • Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of biopsy-proven TCMR with arterial involvement but are not required 2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥2 alone is not sufficient and g must be ≥1 3. Serologic evidence of DSAs (HLA or other antigens): • Biopsies suspicious for AMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing

C4d staining without evidence of rejection	All three features must be present for diagnosis: 1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections 2. g = 0, ptc = 0, cg = 0 (by light microscopy and by EM if available), v = 0; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this) 3. No acute cell-mediated rejection (Banff 1997 type 1A or greater) or borderline changes

g-glomerulitis, ptc-peritubular capillaritis, v-vasculitis, cg-chronic glomerulopathy (transplant glomerulopathy)

Table 1. Criteria for antibody mediated rejection as outlined by the Banff 2015 Meeting Work Group [24].

Glomerular and/or peritubular capillary infiltration with polymorphonuclear leukocytes and/or macrophages represents microvascular inflammation and is the classic histologic feature of acute tissue injury in acute AMR (**Figure 2**). However, intimal or transmural arteritis, acute TMA and ATN can also denote acute AMR (**Figure 3**). Splitting or double contouring of the GBM (transplant glomerulopathy) as well as severe multi-lamination of peritubular capillary basement membrane are histologic features of chronic AMR. Detection of inert complement split product, C4d in peritubular capillaries by IF or IHC indicates antibody interaction with vascular endothelium (**Figure 4**). However, recognizing that complement independent pathways may be involved in the etiopathogenesis of AMR, the Banff classification also sets forth certain criteria (listed in **Table 1**) that allow for the diagnosis of acute or chronic AMR in patients without detectable C4d staining. Demonstration of circulating DSAs is a pre-requisite for diagnosis of AMR. Noting that non-HLA DSAs may result in clinical and histopathologic findings indistinguishable from AMR, Banff criteria for serologic evidence of DSAs require detection of either DSAs directed against donor HLA or "other" antigens [24].

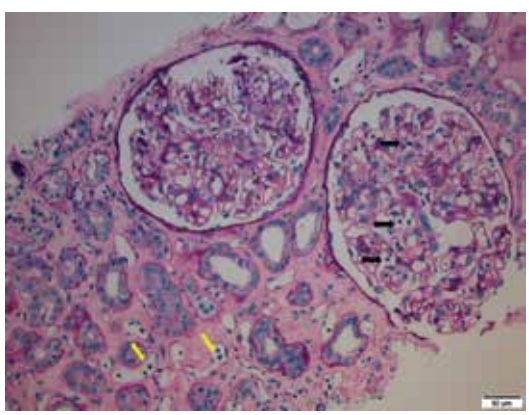

Figure 2. Black arrows show infiltrating polymorphonuclear leukocytes in glomerular capillary loops (glomerulitis) in a patient undergoing acute oliguric antibody mediated rejection. Yellow arrows point to demarginated polymorphonuclear leukocytes in peritubular capillaries (peritubular capillaritis). Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.

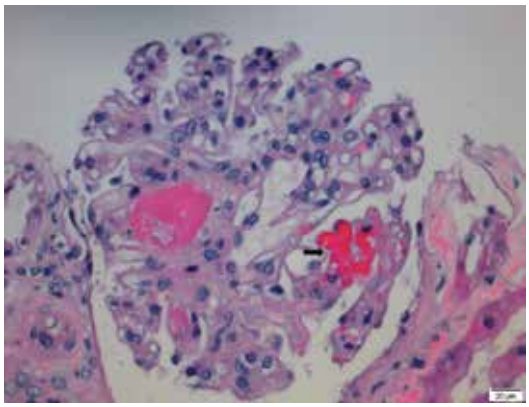

Figure 3. Platelet fibrin thrombi in glomerular capillary loops (black arrow) due to acute thrombotic microangiopathy in a patient with acute antibody mediated rejection. Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.

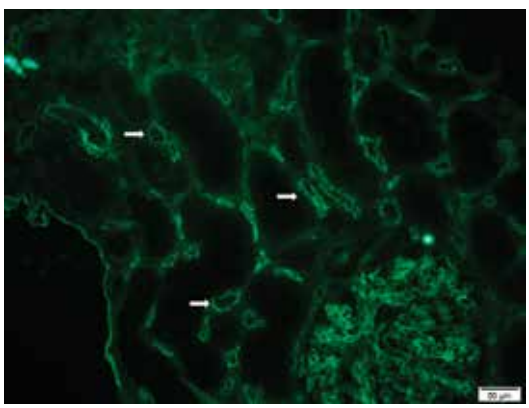

Figure 4. Immunofluorescence staining showing diffuse linear C4d positivity in peritubular capillaries (white arrows). Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.

5. HLA antibody detection assays

5.1. Calculated panel reactive antibody (cPRA)

In the complement-dependent cytotoxicity (CDC) assay, recipient serum is mixed with donor lymphocytes and complement is supplemented. Presence of DSAs is indicated by the appearance of cytotoxicity. The CDC crossmatch was principal technique for detecting DSAs in kidney transplant candidates till the mid-1980s [12, 15]. The panel reactive antibody (PRA) assay, a simple test that predicts the likelihood of a transplant candidate finding a HLA compatible donor (with a negative CDC crossmatch). This test involves treating a panel of cells derived from a pool of individuals representative of the local donor population with recipient serum and noting the percentage of cells that develop cytotoxicity. Therefore, a patient with a high PRA percentage can be predicted to be HLA incompatible with a majority of the potential donors. Consequently, such patients have prolonged wait times for transplantation

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and may die waiting for a compatible organ [25]. The emergence of more sensitive solid phase assays that employ HLA antigen-coated beads have revealed a greater prevalence of pre-sensitization to HLA in potential transplant recipients than was previously appreciated. Using these sensitive techniques of HLA antibody detection (in recipient serum) in conjunction with modern HLA typing methods (to determine donor HLA typing), we can now estimate a transplant candidate's calculated panel reactive antibody (cPRA). This is an alternative to standard PRA testing. Transplant centers can designate HLA antigens to which the patient has been sensitized as "unacceptable." The cPRA is computed from HLA antigen frequencies among kidney donors in the United States and represents the percentage of actual organ donors that express any "unacceptable" HLA antigens [25]. One of the key elements of the Organ Procurement and Transplantation Network's (OPTN) new Kidney Allocation System (KAS) introduced in December 2014 is to allocate additional points to waitlisted kidney transplant candidates based on a CPRA sliding scale [26]. The aim of this policy is to increase access to transplantation for sensitized candidates [27]. Soon after the new KAS came into effect, the proportion of transplants being performed in patients with CPRA >98% rose from 2.4 to 13.4% [28]. Therefore, the new KAS appears to be accomplishing the goal of equitable allocation of scarce deceased donor organs to highly sensitized patients who are biologically disadvantaged.

5.2. HLA antibody detection assays

Techniques for detection of HLA antibodies in transplant candidates have evolved considerably since the 1960s when the traditional cell-based complement dependent cytotoxicity (CDC) assay was first developed. Modern flow-cytometry and solid phase assays are far more sensitive than the CDC crossmatch. In the CDC crossmatch, recipient serum is incubated with donor lymphocytes, along with complement. A positive crossmatch is said to occur when DSAs present in the recipient serum bind the corresponding antigens expressed on the surface of donor T or B lymphocytes and activate complement leading to cell lysis [29]. The flow cytometry crossmatch (FCXM) developed in the 1980s was shown to be more sensitive than the CDC crossmatch and can detect lower strength, but clinically significant antibodies that are imperceptible to the CDC crossmatch [30]. In the FCXM, like the CDC crossmatch, donor-specific anti-HLA antibodies, if present in the recipient serum, bind HLA molecules on the surface of donor T or B lymphocytes. The flow cytometry technique detects these HLA bound anti-HLA antibodies by means of secondary fluorescein-labelled antihuman IgG [29]. The advent of solid phase assays for HLA antibody screening in the 1990s has redefined what it means to be sensitized to HLA. There are two varieties of solid phase assays – enzyme linked immunosorbent assay (ELISA) based methods and single antigen bead based methods. Single antigen bead based assays may employ either a flow cytometry platform or a Luminex platform to detect HLA [31]. The Luminex platform employs fluorochrome impregnated microbeads that are coated with specific HLA molecules. Donor-specific anti-HLA antibodies, if present in the recipient serum, bind HLA molecules coated on the surface of the beads. The microbeads are then incubated with phycoerythrin (PE)-labeled anti-human IgG antibodies. The Luminex dual laser system identifies the specificity of the bound anti-HLA antibodies[Ref]. The Luminex based assay has now become the most popular method of HLA antibody detection, both due to its superior sensitivity, as well as its ability to identify the antigenic specificity of the detected HLA antibody [29].

6. Treatments for antibody mediated rejection

The success of desensitization techniques, which enabled transplantation in the setting of pre-existing DSA, represented a breakthrough in the ability to offer transplantation to highly-sensitized patients, who previously had little hope of receiving a transplant [32–34]. But early success was tempered by observations of antibody rebound, early AMR, and suboptimal long-term graft survival [35, 36]. Thus the ability to successfully perform incompatible transplants and optimize long-term outcomes is contingent upon the ability to successfully treat AMR. The first reported efforts at allograft rescue in the setting AMR employed similar techniques as were used for desensitization, namely, techniques that remove or reduce circulating antibody [37]. While removal of antibody remains the cornerstone of AMR therapy, improved understanding of the pathophysiological mechanisms of antibody production and antibody mediated injury have yielded several adjunctive treatment options which are now in various stages of application or new development. For treatment of AMR, no standard protocols exist. Published reports are generally small patient series, and reported techniques vary based on center-specific experience and expertise, as well as center-specific access to emerging therapies [38–40]. Thus, randomized-controlled trial data do not exist for most of these treatments. Meta-analyses are limited by patient heterogeneity, treatment regimen heterogeneity and sample size [39, 41]. Below are brief descriptions of currently existing treatment modalities, though it is important to understand that these are rarely, if ever, used as monotherapies. Most AMR treatment strategies employ a technique for antibody removal in combination with adjunctive agents to minimize antibody production and/or act at the level of the graft to minimize antibody-mediated injury.

6.1. Therapeutic plasma exchange

Though often referred to simply as "plasmapheresis," the procedure utilized in the treatment of AMR is more accurately described as therapeutic plasma exchange (TPE). While plasmapheresis [42] technically describes plasma removal without replacement, TPE entails plasma removal with replacement of a substitute colloid component. A 1–1.5 L plasma volume exchange generally removes approximately 70% of plasma components, including anti-HLA antibodies [43]. For immunoglobulins, the durability this treatment differs dependent upon the tissue compartments in which each immunoglobulin subclass resides. IgM, which resides solely in the intravascular space, and does not significantly repopulate by re-equilibration following TPE, much unlike IgG and IgA. Re-equilibration into the intravascular space generally means that for IgG present in high concentration initially in the serum, multiple TPE treatments are required to make measurable impact on the circulating concentration [44–46]. Rates of antibody removal with TPE, as well as characteristics of rebound following treatment vary with antibody subclass and specificity, and mechanistically this remains poorly understood [47]. TPE was one of the first reported successful strategies for treatment AMR and remains a cornerstone of most current treatment protocols [37, 38, 48, 49].

6.2. Immunoadsorption

Immunoadsorption (IA) is a therapy not available worldwide, but where applied has been used successfully in both desensitization and treatment of AMR. IA has the benefit of specifically removing circulating IgG, while sparing desired plasma protein components such as clotting factors [50]. IA can rapidly and efficiently deplete IgG after a small number of treatments [51–53]. A single randomized controlled trial reporting IA plus pulse steroid compared to pulse steroid alone as treatment for AMR was stopped early after an excessive number of graft failures in the control group [54].

6.3. Intravenous immune globulin (IVIG)

The precise mechanisms of IVIG action both in desensitization and in treatment of AMR remain unclear, but there is evidence to support that it is multimodal [55, 56]. IVIG has been shown to have inhibitory effects on B-cells [57–59], antigen presenting cells [60], and on complement [61, 62]. IVIG in the treatment of AMR has been reported as high-dose therapy (1–2 gm/kg) used without plasma exchange treatments [63–65], and more commonly, as low-dose therapy (100 mg/kg/dose) used in combination with TPE [39, 40, 66].

6.4. Splenectomy

Removal of the spleen, the largest lymphoid organ in the body, is postulated to deplete the plasma cell reservoir, and thereby yield a rapid decrease in circulating HLA antibody in patients with severe acute rejection [67, 68]. Due to its associated morbidity, splenectomy is generally reserved as a rescue therapy [67, 69–71] when all other less invasive interventions are failing and a graft is at risk for imminent loss. Potentially less morbid alternatives to operative splenectomy, including angioembolization or splenic irradiation, may prove to be beneficial in select patient situations [72].

6.5. B-cell and plasma cell targeted medical therapies

Rituximab (Rituxan®, Genentech) is a monoclonal antibody directed against the B-cell CD20 antigen [73]. This recombinant antibody is constructed as a chimeric protein with human IgG_1 constant regions linked to murine anti-human CD20 variable regions [74]. Binding of rituximab to CD20 leads to antibody-dependent complement-mediated cytotoxicity and apoptosis of the bound cell. Rituximab thereby depletes the memory B-cell population and this is hypothesized to, in turn, reduce the plasma cell population and decrease HLA-antibody production [75–77]. Rituximab has been used as an adjunctive therapy in combination with various treatment modalities including: IVIG [78], TPE plus steroid pulse [79, 80], and TPE plus IVIG [66, 81]. While rituximab was perhaps the earliest used adjunctive agent in the treatment of AMR, to date only a single randomized controlled trial of its use has been performed, in which it was compared to placebo in addition to standard therapy (TPE with low dose IVIG). No difference was observed in this underpowered study though there was a trend toward improved outcomes with the addition of rituximab [82].

Whereas the postulated effect of rituximab on antibody-production is indirect, bortezomib (Velcade®, Takeda Oncology) acts directly at the level of the antibody-producing plasma cell. Bortezomib is a proteasomal inhibitor that depletes circulating plasma cells by inducing apoptosis [83–85]. The first reported use bortezomib in transplant recipients was in a small series where graft salvage was attempted in the cases of AMR refractory to therapies including TPE, IVIG, and rituximab [86]. Following bortezomib therapy, circulating DSA strength has been reported to decrease substantially [87], though interestingly, class I and class II DSAs may be not be reduced with equal efficacy [88]. Bortezomib, like rituximab, has been used in combination with TPE, with and without steroids and rituximab [89–92] and is thoroughly reviewed elsewhere [85].

6.6. Complement inhibition

The realization that the tissue injury associated with AMR was, at least in part, mediated by the complement cascade led to the hypothesis that complement inhibition may afford tissue-level protection while TPE or other antibody removing techniques were implemented. The first reported use of the terminal complement inhibitor eculizumab (Soliris®, Alexion Pharmaceuticals) was in a patient with severe accelerated oliguric AMR who was deemed an inappropriate candidate for a rescue splenectomy [93]. With TPE, IVIG, rituximab, and eculizumab, recovery of renal function was achieved. Several reports describe the use of eculizumab as a salvage therapy, either in lieu of or in combination with splenectomy [94–96], but reports of successful salvage are not universal [97]. Eculizumab's mechanism of action of action led to studies of its pre-emptive use in incompatible kidney recipients at high risk for AMR, and while eculizumab may decrease the incidence of AMR [98], it does not prevent it [99].

Additional complement inhibitors have since become available and are being evaluated for their relative efficacy in AMR. C1-esterase inhibitor (C1-INH) is an endogenous protein that is a more proximal inhibitor of the complement cascade and is commercially available as a purified plasma preparation (Berinert®, CSL Behring, and Cinryze®, Shire). A recently reported double-blinded randomized controlled trial of C1-INH as an add-on to standard TPE therapy for AMR suggested a benefit in terms of improved long term renal allograft function in those who received C1-INH [100]. Like eculizumab, C1-INH may have promise in the prevention of AMR in high-risk patients [101], or as a graft protective agent in the setting of severe or treatment refractory AMR [102].

6.7. IL-6 inhibition

IL-6 is a pro-inflammatory cytokine with properties that activate numerous cell lines including B-T- and plasma cells. Tocilizumab (Actemra®, Genentech)is a humanized monoclonal antibody which blocks IL-6 signal transduction by binding and inhibiting the IL-6 receptor [103]. In animal models, IL-6/IL-6R signaling has been found to promote renal injury [104] and may be associated with the injury of acute rejection [105]. In human studies, it may affect a decrease in HLA antibody production [106]. A recent trial of tocilizumab in patients with refractory chronic AMR reported improved long-term graft survival rates in those who received tocilizumab [107].

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7. Outcomes and unanswered questions

Generally reported estimates of the incidence of AMR are around 7% for all recipients [108], and may be as high as 50% among recipients of HLA-incompatible grafts [109, 110]. Despite improved abilities to diagnose and treat AMR, it remains an important cause of premature graft loss [111, 112]. Clinically silent AMR identified on biopsy in the setting of normal renal function, if left untreated, is associated with a two-fold increased risk of graft loss [109, 113]. If the AMR is clinically apparent and associated with graft dysfunction, the risk of graft loss can increase to six-fold [109]. Even when recognized and treated promptly, AMR portends recurrent AMR, and ultimately, chronic AMR and transplant glomerulopathy [114, 115].

The pathophysiology of AMR and the molecular mechanisms of antibody-mediated injury have never been better understood, however the fact that such heterogeneity is observed clinically from case to case suggests that much remains yet to be clarified. The spectrum of AMR severity, acuity, and treatability is broad and not easily predictable even when clinical parameters appear relatively constant. While some lines of evidence suggest that any DSA [116, 117] even historical DSA not present at transplant [111], has the potential to be harmful, others have reported clinically silent DSA that, although detectable, has no apparent impact the incidence of rejection or on long-term outcomes [118]. Whether sensitization alone, not just DSA, is an independent risk factor for AMR, is unclear [115]. Whether this variability lies in the DSA specificity, in differential expression of the target HLA molecules on the allograft, or on other factors, remains to be determined. Multiple lines of evidence suggest that complement activating, C1q-binding DSA are associated with greater risks for rejection and for worse outcomes [119–122], compared to non-C1q binding DSAs. The ability to identify and test for the more virulent DSAs may prove to be of benefit in terms of surveillance and directing treatment. There is evidence that class II DSA is associated with worse long-term outcomes [123–125], and poorer responses to treatments [126] compared to class I DSAs. What underlies this difference, remains uncertain. Whether, and how, antigens vary in terms of their immunogenicity and risks for inciting AMR, remains to be determined. Whether any of the available therapies is optimally suited for different DSA patterns or specificities, or AMR phenotypes, also remains to be determined.

Perhaps the most effective means of minimizing the risks of AMR may be in maximizing efforts to prevent it. Experience with HLA-incompatible transplant recipients have demonstrated, both in single-center and multi-center series, that long-term outcomes are inversely correlated with the starting crossmatch strength [19, 20]. Thus careful attention paid to donor selection, and making any effort possible to minimize incompatibility, can pay great dividends in the long-term post-transplant [115]. And while prevention will not always be feasible, the ability to more readily and accurately detect AMR will enable more rapid treatments and improve the chances of their success. Just as new agents are being developed to remove antibody [127], and interrupt the pathways that impart antibody-mediated injury, so too are innovative, increasingly specific, and less invasive procedures for the diagnosis of AMR. The ability to identify AMR, and perhaps even characterize AMR phenotypes based on gene expression profiles in biopsy tissue [128, 129] should allow a clearer determination of AMR severity and ultimately help guide therapy. The identification of serum and/or urinary biomarkers [130–133] should enable better surveillance, earlier diagnosis of AMR, and

prompt treatment to prevent irreversible tissue injury. Ultimately, optimizing the diagnosis and treatment of AMR will lead to greater graft longevity and thus, better utilization of this vastly limited resource.

Conflict of interest

The authors have no conflicts of interest relevant to this publication.

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Chapter 19

Clinical and Pathological Review of Post Transplant Lymphoproliferative Disorders

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Additional information is available at the end of the chapter

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Abstract

Posttransplant lymphoproliferative disorder (PTLD) is a rare but potentially serious complication following transplantation with an overall incidence of PTLD of 1–5% in solid organ transplant (SOT) recipients and 1% in hematopoietic stem cell transplant (HSCT) recipients. The clinical and pathological spectrum of PTLD is broad; however, most cases of PTLD occur within the first year after transplantation and are associated with EBV. Clinical features that independently predict rates of response and survival have not been systematically studied for PTLD. Patients whose PTLD expressed CD20 or EBV have shorter intervals to PTLD onset, whereas late-onset cases of PTLD are typically EBV negative. Phenotypic characterization of PTLD reveals potential reliance on EBV or NF-kappaB signaling instead of B-cell receptor signaling, which links PTLD to other subgroups of EBV-related lymphomas, highlighting new potential treatment approaches. PTLD can be a life-threatening post-HSCT complication due to the impact of the patient's underlying disease (malignant or nonmalignant) as well as the type and intensity of the conditioning regimen. EBV-negative PTLD is more often a delayed phenomenon post-HSCT compared to EBV-positive PTLD. Further investigations are needed to better understand the role of EBV in the pathogenesis of different forms of PTLD in the immunosuppressed patients.

Keywords: posttransplant lymphoproliferative disorder (PTLD), Epstein-Barr virus (EBV), immunosuppression (IST)

1. Introduction

PTLD is an uncommon complication of hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) with a broad clinical and pathological spectrum ranging from an acute, self-limited illness resembling infectious mononucleosis with histologically innocuous polyclonal lymphoid proliferation to aggressive, life-threatening tumors resembling aggressive lymphomas in immunocompetent individuals. The overall incidence of PTLD is about 1–5% in solid organ transplant (SOT) recipients and 1% in hematopoietic stem cell transplant (HSCT) recipients. Most cases of PTLD occur within the first year after transplantation. Late-onset PTLD, occurring years after transplantation, is often associated with more monoclonal lesions and a worse prognosis [1].

The clinical outcome of untreated aggressive PTLD after HSCT is very poor, suggestive of the biologic heterogeneity between the PTLD and de novo DLBCL. The mortality associated with PTLD induced by EBV infection can be reduced by monitoring EBV by polymerase chain reaction and by preemptively giving rituximab. The prompt initiation of preemptive therapy and early diagnosis of EBV disease are associated with decreased mortality [2, 3].

There is no consensus opinion as to whether a particular subtype of HSCT is associated with increased incidence of PTLD and whether salvage therapy provides survival benefits in patients developing early compared to late PTLD though, empirically, regimens that would reduce EBV-specific T cells, e.g. in vitro or in vivo T-cell purging, are believed to increase the risk of EBV-associated PTLD. Clinical features that independently predict rates of response, progression-free survival, and overall survival have not been systematically studied for PTLD presenting as aggressive non-Hodgkin lymphoma.

From a pathological standpoint, PTLD that represents a spectrum of lymphoid proliferations can be classified as nondestructive, polymorphic, monomorphic, and classical Hodgkin lymphoma types [29] based on morphology, immunophenotype, and clonality. Monomorphic and classical Hodgkin PTLDs are then classified based on their resemblance to de novo in lymphomas in immunocompetent individuals, e.g., diffuse large B-cell lymphoma, Burkitt lymphoma, plasmacytoma, plasma myeloma, peripheral T-cell lymphoma, NOS, hepatosplenic T-cell lymphoma, etc. The majority of monomorphic PTLDs resemble aggressive non-Hodgkin lymphoma, and most indolent lymphomas such as low-grade follicular lymphoma and EBV-negative extranodal marginal zone (MALT) lymphoma are not considered PTLD. However, a small number of EBV+ MALT lymphomas have been reported in the post-transplant setting and are considered a bona fide PTLD [29, 30].

2. PTLD in SOT vs. HSCT

Compared to the general population, the SOT patients have a 30–60-fold increased risk of developing PTLD. Most cases of PTLD occur within the first year after transplantation. EBV DNAemia occurs after transplantation in significantly more SOT recipients than HSCT

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patients. Correspondingly, more SOT patients also develop PTLD than HSCT patients and also significantly later posttransplant compared to HSCT recipients. The median length of time between transplant and diagnosis of PTLD for SOT patients is 2.8 years versus 121 days for HSCT patients [1, 4].

2.1. PTLD in SOT

In adults, incidence rates range 1–3% in kidney and liver transplants, 1–6% in cardiac transplants, 2–6% in combined heart-lung transplants, 4–10% in lung transplants, and up to 20% in small intestine transplants. The variation in rates among the types of organs transplanted is likely related to the degree and duration of immunosuppression as well as the number of EBV-positive donor lymphocytes in the graft. PTLD is more common in lung and small bowel transplants. Duration of the posttransplant period is important because PTLD is most likely to develop in the first year following transplantation, with an incidence of 224 per 100,000 but falls to 54 per 100,000 in the second year and 31 per 100,000 in the sixth year. PTLD represents a heterogeneous group of non-Hodgkin lymphomas that vary clinically and are ill-defined morphologically [5–8].

2.2. PTLD in HSCT

PTLD develops in approximately 1% of patients post HSCT, among which the majority of cases occur within the first year after allogeneic stem cell transplantation (alloSCT). It is highly related to EBV reactivation. Risk factors that associate with high incidence of EBV-related PTLD include elderly patients (aged ≥50 years at transplantation), T-cell depletion-containing regimens, antithymocyte globulin (ATG) use, and grafts derived from unrelated or HLA-mismatched donors. PTLD can also develop in patients who received autologous stem cell transplants, but the frequency is much lower than alloSCT [9, 10]. PTLD in ASCT cases occurs in younger age group, with shorter duration of onset than solid organ transplantation.

3. Diagnostic markers

3.1. CD20 positivity

The prognostic role of CD20 expression and Epstein–Barr virus (EBV) positivity in PTLD after SOT is poorly understood. In a retrospective study, a total of 45 pediatric SOT patients (28 heart, 11 liver, and 6 kidney) were diagnosed with PTLD 45 months after SOT. Of the 40 evaluable PTLD cases (11 monomorphic, 19 polymorphic, 5 early lesions, and 5 rare subtypes), 32 (80%) had detectable EBV, and 28 (70%) were classified as CD20 (+). Patients whose PTLD expressed CD20 or EBV had shorter intervals between SOT and PTLD onset (28 vs. 64 or 77 months for CD20 and EBV, respectively) ($P < 0.02$). Patients with CD20 (+) tumors had higher 5-year PTLD-related EFS (83.7% vs. 28.6%, $P < 0.001$) and OS (95.8% vs. 56.3%, $P = 0.01$). EBV expression was unrelated to PTLD-related EFS or OS. CD20 expression is thus

found to be associated with timing of development of PTLD and predicts survival in pediatric PTLD in SOT [11].

3.2. Other diagnostic markers

Comprehensive phenotypic characterization of PTLD reveals potential reliance on EBV or NF-kappaB signaling instead of B-cell receptor signaling. Several signaling pathways, cells of origin of PTLD, and their relation to viruses were analyzed by immunohistochemistry and in situ hybridization. Most PTLDs are of activated B-cell origin. Two-thirds of cases show an Epstein-Barr virus (EBV) infection of the neoplastic cells. NF-kappaB signaling components are present in the majority of cases, except for EBV-infected cases with latency type III lacking CD19 and upstream B-cell signaling constituents. Proteins involved in B-cell receptor signaling like Bruton tyrosine kinase are seen only present in a minority of cases. Phosphoinositide 3-kinase (PI3K) is found to be expressed in 94% of cases and the druggable PI3K class 1 catalytic subunit p110 in 76%, while other signal transduction proteins are expressed only in occasional cases. Unsupervised cluster analysis has revealed three distinct subgroups: (I) related to EBV infection, mainly latency type III and lacking CD19, upstream B-cell signaling, and NF-kappa constituents; (ii) related to EBV infection with expression of the alternative NF-kappaB pathway compound including RelB, CD10, and FOXP1 or MUM1; and (iii) unrelated to virus infection with expression of the classic NF-kappaB pathway compound p65 [12]. EBV and NF-kappaB are important drivers in PTLD in contrast to B-cell receptor signaling. The main signal transduction pathway is related to PI3K. This links PTLD to other subgroups of EBV-related lymphomas, highlighting also new potential treatment approaches [4].

The diagnosis of PTLD relies on comprehensive morphologic examination, immunophenotyping, genetics, and EBV status. Most of PTLDs are of B-cell origin. EBV plays an important role in the pathogenesis of PTLD. The duration of disease onset is shorter in EBV-positive cases.

3.3. EBV viremia and EBV detection by EBER

The majority of EBV infections that occur after transplantation, especially in adults, are clinically silent reactivations. This leads to a subsequent delay in the diagnosis of PTLD. A positive correlation between the degree of EBV DNAemia and the development of PTLD has significant implications for the importance of monitoring viral load after transplantation. In a study done by Holman et al., the risk of PTLD in viremic patients significantly increased with the peak quantity of EBV DNAemia [2, 13]. Since the occurrence of PTLD is significantly related to the viral load, constant monitoring and quantification of EBV-DNA load are utilized as prognostic markers for the development of PTLD.. In solid organ transplant (SOT) recipients, approximately 50% of patients develop detectable EBV DNAemia, but only a much smaller subset develops PTLD.

3.4. EBV antigens

The broad EBV latency profile (LMP1+/EBNA 2+) is found to be expressed in 59% of EBV (+) PT-DLBCL and is associated with a more elaborated inflammatory response than intermediate

latency (LMP1+/EBNA 2-) with a role for innate and tolerogenic immune response in EBV + PT-DLBCL. EBV signature is the most important factor in the pathogenesis of EBV (+) PT-DLBCL [14].

3.5. Genomic profiling of PTLD

Clinical, pathological, and molecular genetic characteristics of PTLD show that EBV-positive and EBV-negative PTLDs have distinct gene expression profiling with clustering related to EBV status than immune status. Except for decreased T-cell signaling, EBV-negative PTLDs are inseparable from EBV-negative IC-DLBCL. In contrast, an EBV viral response signature is clearly shown to segregate EBV (+) PT-DLBCL from EBV (−) PT-DLBCL [14, 16].

3.6. PTLD diagnostic algorithm

Systematic morphological, immunophenotypic, and genetic analysis of each PTLD case should be performed. In DLBCL type, one may apply BCL-6, CD10, and MUM-1 immunostains in order to establish the cell of origin according to the Hans algorithm [17]; but the value of this assignment is not well established in this setting. Based on EBV protein expression, the latency type of EBV infection is defined as LMP1-/EBNA2- (type I, restricted), LMP1+/EBNA2- (type II, intermediate), and LMP1+/EBNA2+ (type III, broad). The stromal infiltrate can be estimated semiquantitatively based on the ratio of tumor cells and stromal cells in the entire tissue section. In situ hybridization: EBER (EBV-encoded RNA) in situ hybridization is considered the standard for diagnosis of EBV infection and should be performed in all PTLD cases. PTLD cases are defined as EBV (+) if EBER was expressed in all tumor cells in which RNA was preserved [14].

4. EBV-positive PTLD vs. EBV-negative PTLD

Epstein-Barr virus-positive (EBV (+)) and EBV-negative (EBV (−)) PT-DLBCL have distinct gene expression profiles, and the transcriptomic profile of EBV (−) PT-DLBCL is similar to that of DLBCL in immunocompetent individuals (IC-DLBCL) and supports the hypothesis that EBV (−) PT-DLBCL are de novo lymphomas. EBV (+) and EBV (−) PT-DLBCL have distinct aCGH profiles and shared only one recurrent imbalance. EBV (−) PT-DLBCL, however, display at least ten aberrations recurrent in IC-DLBCL, among which characteristic gain of 3/3q and 18q and loss of 6q23/TNFAIP3 as well as 9p21/CDKN2A. The most prevalent aberration in EBV (+) PT-DLBCL is due to gain/amplification of 9p24.1 targeting PDCD1LG2/PDL2. FOXP1 oncogene and the tumor suppressor CDKNA2 implicated in EBV (−) DLBCL do not play a critical role in the pathogenesis of EBV (+) PT-DLBCL [14].

5. Nondestructive vs. monomorphic PTLD

Destructive PTLD, which has typically not correlated with other specific risk factors, has recently been shown to be associated with older recipient age and prolonged receipt of

calcineurin inhibitors. Furthermore, recent data has contributed to and, in some instances, shed light on previous debate concerning the role of viruses other than EBV and the level of HLA mismatch as risk factors for PTLD [15]. Gene association studies focusing on key cytokines and their receptors have identified several polymorphisms that may prove useful to identify patients at risk, with distinction for early nondestructive and late occurring monomorphic PTLD.

6. Monomorphic PTLD vs. polymorphic PTLD

The term PTLD represents a spectrum of B-cell hyperproliferative states that can be classified as nondestructive PTLD, polymorphic PTLD, monomorphic PTLD, and classical Hodgkin lymphoma, all of which may be associated with Epstein–Barr virus (EBV).

Nondestructive lesions are classified as reactive plasmacytic hyperplasia, florid follicular hyperplasia, and infectious mononucleosis-like PTLD. These lesions are frequently associated with an acute illness similar to mononucleosis, with polyclonal B-cell proliferation, but without evidence of malignant transformation by definition, the lymphoid architecture is preserved.

Polymorphic PTLDs are polyclonal or monoclonal lymphoid infiltrates with evidence of destructive growth patterns including necrosis, destruction of underlying lymphoid architecture, and nuclear atypia. However, polymorphic PTLD does not otherwise meet all criteria for B-cell or T-/NK-cell lymphomas as characterized in immunocompetent patients [1].

Monomorphic PTLDs are monoclonal lymphoid proliferations. Monomorphic PTLD most commonly resembles aggressive B-cell lymphomas such as Burkitt lymphoma/high-grade B-cell lymphoma or diffuse large B-cell lymphoma (DLBCL), as seen in immunocompetent patients. Likewise, classical Hodgkin-like lymphoma variably resembles those observed in immunocompetent patients. Small B-cell lymphomas such as follicular lymphoma, small lymphocytic lymphoma, and EBV-negative marginal zone lymphomas that occur in the post-transplant setting are not characterized as PTLD. A small group of EBV+ MALT lymphoma is now recognized as *bona fide* PTLD [30].

7. Pediatric PTLD

PTLD occurs more commonly in pediatric patients than in adults. The higher incidence in children is thought to result from being EBV-naïve recipients. PTLDs can arise during post-transplant period after both myeloablative and nonmyeloablative allogeneic hematopoietic cell transplantation. EBV is frequently expressed in PTLD in patients with both HSCT and SOT. Posttransplant lymphoproliferative disorder (PTLD) has been associated with high mortality, but recent anecdotal survival appears better. NAPRTCS registry had 235 registered PTLD cases from 1988 to 2010 which showed that survival has improved with more recent PTLDs in children following kidney transplants [6, 18].

8. Role of immunosuppression (IST)

The degree and duration of immunosuppression play a major role in the development of PTLD. Cytotoxic T cells provide a defense mechanism against EBV-infected B cells in immunocompetent individuals. However, when T-cell function is impaired, this defense mechanism is lost, therefore promoting the development of PTLD.

8.1. ATG and PTLD

In vivo T-cell depletion (TCD) with antithymocyte globulin (ATG) or alemtuzumab (AL) is commonly used in HSCT. TCD facilitates engraftment and reduces the incidence and severity of graft-versus-host disease (GvHD). As reduced intensity conditioning (RIC) and matched unrelated donor transplants (MUD) are now being performed more frequently, ATG and AL have become integral components of preparative regimens. Although ATG and AL provide safer T-cell depletion, delayed T-cell reconstitution following TCD accounts for infectious complications and increased mortality.

EBV PTLD is predominantly derived from donor B cells before reconstitution of the EBV-specific cytotoxic T lymphocyte (CTL) response. It can, however, occur later in the most severely immunocompromised patients with additional risk factors such as donor and recipient mismatch, graft manipulation with T-cell depletion, as well as the degree and duration of immunosuppression.

In a study by Buyck et al., an overall incidence of 6.3% for EBV PTLD was reported in 89 patients with severe aplastic anemia. A marked increase in the incidence (13.3%) was noted in patients exposed to ATG, with 5 of 43 patients developing EBV PTLD [19].

9. Other rare subtypes of PTLD

Although the majority of monomorphic PTLDs fall into the category of diffuse large B-cell lymphoma, other types of lymphoma may also occur. These include plasma cell myeloma, classical Hodgkin lymphoma, or HL-like PTLD, EBV+ MALT lymphoma as well as various T-cell lymphomas with or without EBV infection.

9.1. Burkitt PTLD

A variety of lymphomas can develop as PTLD, although some types appear infrequently and remain poorly understood. PTLD-Burkitt lymphomas behave aggressively and require intensive chemotherapeutic intervention. These display the typical histological features of Burkitt lymphoma but are markedly positive for EBV. While BL-PTLD is a rare entity, it is a discrete form of PTLD with a high EBV expression and should be treated as a high-grade lymphoma. BL-PTLD historically represents a small, but significant, proportion of PTLD cases. BL-PTLD represents 15% of PTLD patients for pediatric heart, lung, and heart-lung transplants from

1982 to 2009, with a 1.1% overall incidence among pediatric transplant heart-lung recipients, 14% of our pediatric renal PTLD patients, 1.6% among kidney recipients, and 0.71% pediatric liver-transplant recipients, as reported in a single institution study. BL-PTLD is a more aggressive type of PTLD and does not respond to a trial of decreased immunosuppression like P-PTLD and some M-PTLDs. BL-PTLD does require cessation of conventional immunosuppression during treatment with multiagent lymphoma-specific chemotherapy. Bone marrow involvement remains a poor prognostic factor, despite the use of lymphoma-specific chemotherapy in these cases [20–24].

BL after organ transplantation is often found in extra-nodal sites; it involves the central nervous system more frequently than it does in immunocompetent patients. In 70% of BL occurring after organ transplantation, genes or gene products related to EBV can be demonstrated within the tumor cells. The EBV status of the tumor is of important prognostic significance: EBV-positive BL occurring in organ transplant patients usually responds well to reduction or cessation of immunosuppressive therapy; in some cases permanent complete remissions can be achieved even without chemotherapy. In contrast, patients with EBV-negative BL have a very poor prognosis and rarely respond even to aggressive chemotherapy protocols [23].

9.2. T cell PTLD

The etiology of posttransplant T-cell lymphomas remains unclear. Similarities with posttransplant B-cell proliferations are the predominant extranodal presentation and the finding that the time of occurrence is influenced by the type of immunosuppression. In contrast with posttransplant B-cell proliferations, only a minority of the cases are associated with EBV. Most tumors appear to be monoclonal. Prognosis is generally poor, but tumor presentation with localized disease might have a somewhat better prognosis. Ambiguity about the pathogenesis of T-PTLD and the lack of accepted diagnostic criteria may contribute to the rarity and inconsistent characterization of T-PTLD in the literature. While there is a general impression that T-PTLD is very difficult to cure, several recently reported cases demonstrate that these tumors can be very treatment responsive with the use of different chemotherapy regimens than those typically used to treat B-PTLD, such as the intensive ALL-type treatments we employed, and/or the use of different strategies for immunosuppression. Most T-PTLDs are not EBV-driven; thus, reduction of immunosuppression may not be effective as a sole treatment strategy and may be less critical for management of T-PTLD than it is in EBV-driven B-PTLDs.

T-PTLD cases may sometimes exhibit a bimodal response to therapy, with initial eradication of the bulk nodal disease with regimens typically used to treat ALL but persistence of low-level clonal T cells in the marrow, CSF, and lung. Due to small patient numbers, different strategies of treatment may be needed compared to B-lineage PTLD [25, 26].

9.3. HL-PTLD

PTLD that resembles Hodgkin lymphoma (HL-PTLD) has been reported infrequently. These cases have variable numbers of Reed-Sternberg-like (RS-like) cells and highlight differences in the phenotype that may distinguish these from true Hodgkin lymphoma (HD). These

occur 8 months to 13 years following transplant. The large cells of HL-PTLD are pleomorphic B cells that react strongly for CD20 and/or CD79a and express CD30 but are usually negative for CD15 and have few mitoses. They are positive for EBV early RNA (EBER) using an EBER-1 probe, as are some of the background small lymphocytes. HD-PTLD is managed by withdrawal of immunosuppression and variably treated with rituximab to chemotherapy [27]. HL-PTLD and HD appear to be two related but immunophenotypically and biologically distinct forms of lymphoproliferation in posttransplant patients and may require different protocols for their management [27, 28]. Overall survival at 2 and 5 years was 86% with 81% of patients surviving event-free. Rituximab monotherapy has not been shown to lead to long-term remission, but treatment with classical HL chemotherapy is effective and tolerable. New treatment modalities such as CD30-targeted or EBV-specific agents may diminish toxicity.

10. DLBCL vs. PTLD

Within the PT-DLBCL series, EBV (+) cases were different from EBV (−) cases. The fact that all EBV (+) PT-DLBCL cases are of activated B-cell (ABC) origin whereas 45% EBV (−) PT-DLBCL cases were of GCB origin might contribute to the observed difference in survival. Overall, EBV (−) PT-DLBCL was similar to DLBCL arising in immunocompetent individuals regarding median age at diagnosis (63 versus 65 years). The amount of stromal infiltration was significantly higher in IC-DLBCL than PT-DLBCL (12/13 and 12/33 cases contained ≥15% stroma, respectively, $P = 0.0012$). Geographical necrosis was almost exclusively observed in EBV (+) PT-DLBCL (46%), compared to EBV (−) PT-DLBCL (11%). In contrast, there was no obvious difference in the absolute amount of stromal infiltration between both groups. IDO1 was variably expressed in the tumor and/or stromal cells of 7/22 EBV(+) PT-DLBCL. The Epstein–Barr virus broad latency profile (LMP1+/EBNA2+) was most frequently expressed in PT-DLBCL (n = 13/22; 59%) and associated with a more elaborate inflammatory response than intermediate latency (LMP1+/EBNA2−).

11. Therapy and prevention of PTLD

Prevention of PTLD involves limiting the duration and degree of immunosuppression while still maintaining the adequacy of the donor graft. Achieving a balance of reduction in immunosuppression and preventing graft rejection or graft-versus-host disease can be challenging.

Antiviral prophylaxis may also play a role in preventing PTLD, though data are scarce, particularly in SOT recipients. The use of antivirals such as acyclovir, valganciclovir, and ganciclovir is common for HSV, CMV, and EBV prophylaxis, though utilization varies according to institutional guidelines and protocols.

Many transplant centers will preemptively reduce immunosuppression and/or administer the anti-CD20 agent rituximab when EBV reactivation (i.e., ≥1000 EBV genome equivalents per mL) has occurred in the posttransplant setting.

11.1. Treatment of nondestructive lesions

Reduction of immunosuppression is typically recommended for patients with early lesions. If there is residual disease or reduction in immunosuppression is not tolerated or allowable due to concerns regarding the donor graft, rituximab may be utilized.

11.2. Treatment of polymorphic PTLD

A combination of reduction in immunosuppression and rituximab is recommended for patients with polymorphic PTLD.

11.3. Treatment of monomorphic PTLD

For patients with monomorphic PTLD, treatment involves rituximab alone or in combination with systemic chemotherapy, in addition to reduction in immunosuppression. Single-agent rituximab may be utilized in patients with minimal PTLD-related symptoms or low disease burden or if poor performance status or other medical comorbidities preclude the use of systemic chemotherapy. The most commonly employed chemotherapy regimen is R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone); rituximab is omitted in cases of CD20-negative PTLD.

11.4. Conclusion(s)

PTLD can be a life-threatening post-HSCT complication due to the impact of the patient's underlying disease (malignant or nonmalignant) as well as the type and intensity of the transplant conditioning regimen. EBV-negative PTLD is a delayed phenomenon post-HSCT as compared to EBV-positive PTLD. Biomarkers that measure the extent of immunosuppression may have a role in avoiding PTLD and other posttransplant complications. Further investigations are needed to better understand the role of EBV infection in the pathogenesis of the different forms of PTLD in the immunosuppressed patients.

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Chapter 20

Metabolic Drug Interactions with Immunosuppressants

Katalin Monostory

Additional information is available at the end of the chapter

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Abstract

Organ transplantation has become a routine clinical practice for patients with end-stage disease of liver, kidney, heart, or lung. Although improved immunosuppressant therapy substantially contributes to the success of transplantation, clinicians continue to face challenges because of wide interindividual variations in blood concentrations resulting in subtherapeutic or supratherapeutic levels. Many undesired side-effects or therapeutic failure of immunosuppressants as a consequence are the results of differences or changes in drug metabolism. Considering genetic and nongenetic factors, such as co-medication, can refine the immunosuppressant therapy, facilitating personalized treatments to individual recipients. This review provides an up-to-date summary of functional polymorphisms of enzymes involved in the metabolism of immunosuppressants with low molecular weight and of the clinical significance of metabolic drug interactions between immunosuppressive agents and other drugs in therapeutic regimens of transplant recipients.

Keywords: drug metabolism, genetic polymorphism, phenoconversion, calcineurin inhibitors, mTOR inhibitors, corticosteroids, inosine monophosphate dehydrogenase inhibitors

1. Introduction

Many undesired side-effects or therapeutic failures of drugs are the results of differences or changes in drug metabolism. A patient's drug metabolizing capacity, highly influenced by genetic variations or alterations in the expression and activities of drug-metabolizing enzymes, can substantially modify the pharmacokinetics of a drug and eventually its efficacy or toxicity [1]. Even if the routine clinical practice applies blood concentration guided dosing,

the interindividual variability in drug metabolism calls for personalized medication primarily for drugs with narrow therapeutic index [2, 3]. The identification of genetic and nongenetic factors that can potentially affect the pharmacokinetics of a particular drug is a prerequisite of tailored pharmacotherapy [4, 5].

2. Genetic and nongenetic variations of drug-metabolizing cytochrome P450 (CYP) enzymes

CYP enzymes are the key players in the metabolism of most drugs; therefore, interindividual and intraindividual variations in CYP activities are of significant importance in clinical practice. The pharmacokinetic variability can divide the population into poor, intermediate, extensive, and ultra-rapid metabolizer phenotypes. The loss-of-function mutations in CYP genes result in permanent poor metabolism, whereas nongenetic (internal or environmental) factors can substantially modify the expression and activities of CYP enzymes, evoking transient poor or extensive/ultra-rapid metabolism [6, 7]. The clinical relevance for many CYP genetic variants, regarding drug efficacy, adverse drug reactions, or dose requirement, has been clearly evidenced [6–9]; however, the heritable genetic polymorphisms are not the only determinant factors in interindividual differences in drug metabolism. CYP genotype determines the potential for the expression of functional or nonfunctional enzymes; and nongenetic host factors (age, sex, and disease states) and environmental factors (nutrition, medication, smoking, and alcohol consumption) can alter the expression and activities of CYP enzymes [10]. Homozygous wild genotype, predicted to be translated to functional CYP enzyme, can be transiently switched into poor or extensive metabolizer phenotype, due to phenoconversion [1, 11]. Consequently, both the CYP genotype and the current CYP expression or activity should be considered for the estimation of a patient's drug-metabolizing capacity.

The prevalence of loss-of-function or gain-of-function alleles is generally 1–10%; however, the distribution of the common CYP variants varies among different ethnic populations. CYP3A enzymes, responsible for the metabolism of approximately 40% of the drugs on the market, including many immunosuppressant agents, display great genetic and nongenetic variations. For CYP3A5, substantial interethnic differences in allelic variants have been demonstrated. The prevalence of *CYP3A5*3* allele (6986A > G), resulting in splicing defect and nonfunctional CYP3A5 protein, is 88–97% in white (Caucasian), 66% in Asian, and 12–35% in African populations; consequently, a higher average proportion of functional CYP3A5 in the total hepatic CYP3A pool is expected in subjects of black origin [7, 12]. On the other hand, the enormous, even more than 100-fold interindividual variability in the expression and activity of CYP3A4 is attributed to nongenetic factors rather than genetic polymorphisms [13]. *CYP3A4*1B* allele, which has a frequency of 3–5% in white populations, but a much higher frequency in African population (50–82%) has been reported to result in increased transcription; however, the clinical significance of *CYP3A4*1B* to CYP3A4 function seems to be doubtful [14, 15]. *CYP3A4*22* allele with the prevalence of 2.5–8% in white and of 4% in Asian populations displays low hepatic CYP3A4 expression and results in decreased CYP3A4 activity [16]. Although the association between *CYP3A4* genotype and pharmacokinetic behavior of CYP3A-substrates has been extensively studied, no clear phenotype-genotype relationship has been described for CYP3A4.

Beside the genetic polymorphisms, one of the major sources of interindividual or intraindividual variability in drug metabolism is concomitant medication and co-morbidities, evoking phenoconversion, notably CYP induction and enzyme inhibition [17]. CYP induction leads to an increase in the expression and activity of CYP enzymes and contributes to the increased elimination of drugs metabolized by the particular enzyme. Several pathways involving the activation of various nuclear receptors (PXR pregnane X receptor, CAR constitutive androstane receptor, and glucocorticoid receptor) have been reported to enhance the transcription of CYP3A genes and to contribute to the complex regulation of CYP3A enzymes by drugs such as rifampicin, phenobarbital, carbamazepine, and synthetic or natural steroids [18–21]. Reduced drug concentration as a consequence of CYP3A induction leads to the lack of the pharmacological effect and drug failure. Phenoconversion converting genotypic extensive metabolism into phenotypic poor metabolism of drugs may occur during inflammation (sterile or infection-induced inflammation). Elevated release of proinflammatory cytokines (IL-6, IL-1β, TNF-α) has been associated with downregulation of several drug-metabolizing CYPs, including CYP3A enzymes. The mechanism of downregulation is the repression of PXR and CAR that are involved in transcriptional regulation of CYP3A expression [22–26]. As a consequence, transient poor metabolizer phenotype is developed, significantly increasing the risk of adverse drug reactions and impacting the clinical outcome [1, 27]. Likewise, co-medication can also give rise to poor metabolism. Several drugs or food components (e.g., bergamottin) are known to inhibit the function of drug-metabolizing CYPs; therefore, the concomitant treatment with a CYP inhibitor is expected to increase the exposure of those pharmacons that are metabolized by the particular enzyme. As a consequence of CYP inhibition, the risk of increased exposure and drug-induced adverse reactions can be anticipated, primarily for drugs with narrow therapeutic index, such as tacrolimus and ciclosporin.

By recognizing individual differences in drug metabolism, personalized drug therapy adjusted to the patient's drug-metabolizing capacity can help to avoid the potential side effects of drugs. The graft and recipient survival are highly influenced by drug-metabolizing capacity of the liver, and it is essential to predict potential drug-drug interactions and to tailor medication at both early and late postoperative periods.

3. Metabolism of immunosuppressants

In recent decades, transplantation (liver, kidney, heart, and lung) has become a routine procedure for patients with end stage disease. Advances in surgical techniques and postoperative therapy have led to increasing numbers of transplantation and extended survival among these patients. The final outcome of transplantation and the long-term graft function have been improved mainly due to the development of potent and specific immunosuppressive drugs. Immunosuppressants efficiently decrease the risk of rejection, blocking the recipient's immune system and protecting the transplanted organ. Because of the narrow therapeutic indexes and increased risk of adverse drug reactions, it is essential to apply personalized immunosuppressive therapy adjusted to patient's drug-metabolizing capacity.

Immunosuppressants are generally classified according to their molecular mode of action; however, in terms of metabolic drug interactions, two main categories must be distinguished

Immunosuppressant	Pharmacology	Adverse effects	Enzymes responsible for the metabolism
Calcineurin inhibitors:			
Ciclosporin	Selective inhibition of T-cell dependent immune response: Calcineurin inhibition, Inhibition of cytokine production	Nephrotoxicity, hepatotoxicity, Hyperlipidaemia, hypertension, Tremor, hyperkalaemia, hypomagnaesemia, Hypertrichosis, gingiva hyperplasia	CYP3A4/5
Tacrolimus	Selective inhibition of T-cell dependent immune response: Calcineurin inhibition, Inhibition of cytokine production	Nephrotoxicity, hypertension, diabetes, cholestasis, diarrhea, Tremor, hyperkalaemia, hypomagnaesemia	CYP3A4/5
mTOR inhibitors:			
Sirolimus	Inhibition of B- and T-cell proliferation	Thrombocytopenia, anaemia, leukopenia, lymphocele, pneumonitis Hyperlipidaemia, Stomatitis aphtosa, wound-healing complications	CYP3A4/5
Everolimus	Inhibition of B- and T-cell proliferation	Thrombocytopenia, anaemia, leukopenia, lymphocele, pneumonia Hyperlipidaemia, hypertonia, wound-healing complications	CYP3A4/5
Purine analogues:			
Azatioprin	Inhibition of purine metabolism	Bone marrow suppression, leukopenia, anaemia, thrombocytopenia, myeloid dysplasia, Cholestasis, hepatotoxicity	Thiopurine S-methyl-transferase, Xantine oxidase
Inosine monophosphate dehydrogenase inhibitors:			
Mycofenolate mofetil **Mycofenolate**	Selective inhibition of inosine monophosphate dehydrogenase, Inhibition of B- and T-cell proliferation	Vomiting, diarrhea, abdominal pain muscle weakness, Anaemia, leucopenia	UDP-glucuronyl transferase, CYP3A4/5
Corticosteroids:			
Prednisone **Methyl-prednisolone**	Inhibition of T-cell migration and production of T-cell lymphokines	Adrenal cortex suppression Hypercholesterolemia, diabetes, hypertension, osteoporosis, osteonecrosis, cataracta, skin atrophy	CYP3A4/5

Table 1. Immunosuppressants with low molecular weight.

according to their molecular weights (agents with low or high molecular weights). High-molecular-weight agents, such as polyclonal and monoclonal antibodies (e.g., thymoglobulin, basiliximab, belatacept), that are not substrates for drug-metabolizing enzymes, are metabolized in common protein degradation pathways (intracellular catabolism by endosomal-lysosomal system) [28]; therefore, they are not subjects of metabolic drug interactions and not subjects of this review. In the metabolism of immunosuppressants with low molecular weight, drug-metabolizing CYP enzymes are involved which may entail metabolic drug interactions (**Table 1**).

3.1. Calcineurin inhibitors

For solid organ transplant recipients, the mainstay of the immunosuppressive regimens is calcineurin inhibitor (CNI) therapy with ciclosporin or tacrolimus which selectively blocks several signaling processes, resulting in the inhibition of T-cell activation and proliferation (**Figure 1**) [29, 30]. These drugs effectively treat allograft rejection; however, they display large interindividual variability in their pharmacokinetics, requiring monitoring of blood concentrations for optimal safety and therapeutic efficacy.

Ciclosporin A is an 11-amino acid cyclopeptide that blocks the production of IL-2 by inhibition of calcineurin and, as a consequence, the activation of T-cells (**Figure 1**) [31]. Ciclosporin undergoes extensive metabolism by CYP3A enzymes, producing more than 30 metabolites. The major metabolic pathways are *N*-demethylation to 4-*N*-demethyl ciclosporin, hydroxylation at several positions (1-, 6-, 9-monohydroxy and 1,9- or 6,9-dihydroxy-metabolites), and oxidation to carboxylic acid [32]. Some of the metabolites (e.g., 1,9-dihydroxy-ciclosporin,

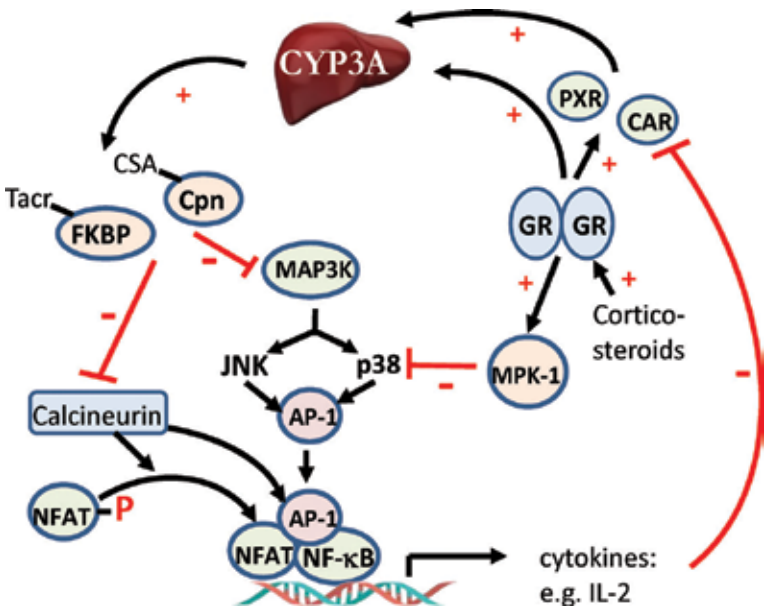

Figure 1. Molecular action of calcineurin inhibitors and corticosteriods. AP-1 activator protein 1, CAR constitutive androstane receptor, CSA ciclosporin, FKBP tarcolimus binding protein, GR glucocoticoid receptor, IL-2 interleukin 2, JNK c-Jun N-terminal kinase, MAP3K mitogen-activated protein 3 kinase, MPK-1 mitogen-activated protein kinase 1, NFAT nuclear factor of activated T-cells, PXR pregnane X receptor, Tacr tacrolimus.

1c9-dihydroxy-ciclosporin, 1-carboxy-ciclosporin) are toxic contributing to the nephrotoxic and hepatotoxic properties of the parent compound [33, 34]. Consequently, high CYP3A activity increases the rate of ciclosporin metabolism and decreases the immunosuppressive effect, which requires dose modification [35]. However, high CYP3A activity also increases the toxic metabolite formation and the risk of nephrotoxicity and hepatotoxicity. Therefore, immunosuppressive strategy must consider the blood concentrations of both ciclosporin and the toxic metabolites, especially if they are accompanied with symptoms indicating nephrotoxicity or hepatotoxicity.

Immunosuppressive properties of tacrolimus are similar to ciclosporin; however, for the same pharmacological effect, significantly lower blood concentration of tacrolimus is required than that of ciclosporin. Tacrolimus, the 23-membered macrocyclic lactone, is converted by demethylation, hydroxylation, and ring rearrangement to at least 15 metabolites, and only a minor proportion of tacrolimus dose is eliminated as unchanged parent drug [36]. Metabolism of tacrolimus leads to the inactivation of the molecule, except for the major 13-*O*-demethyl and the minor 31-*O*-demethyl metabolites. The 13-*O*-demethyl-tacrolimus possesses some immunosuppressive effect; however, it is about one tenth as active as tacrolimus, whereas the 31-*O*-demethyl metabolite displays an immunosuppressive activity comparable to tacrolimus [37, 38]. On the other hand, high blood concentration of 15-*O*-demethyl-tacrolimus metabolite has been reported to be associated with nephrotoxicity and myelotoxicity and with higher incidence of infections [39]. Similarly to ciclosporin, tacrolimus is metabolized by CYP3A enzymes, anticipating great interindividual and intraindividual differences in pharmacokinetics of tacrolimus: (1) CYP3A activity of enterocytes contributes to the first-pass metabolism of tacrolimus; (2) substantial interindividual differences in hepatic CYP3A activity result in great variability in the rate of tacrolimus metabolism, which requires continuous drug monitoring and dose modification primarily in the early postoperative period; (3) concomitant treatment with CYP3A inhibitors is the potential source of metabolic drug interactions; (4) genetic polymorphisms of CYP3A5 also contribute to the high interindividual variability. Since the relative contribution of CYP3A5 to tacrolimus biotransformation is significantly higher than that of CYP3A4 [40], the recipients carrying wild type *CYP3A5*1* allele or transplanted with liver grafts carrying *CYP3A5*1* are able to metabolize tacrolimus more rapidly than CYP3A5 nonexpressers [35, 41].

3.2. mTOR (mammalian target of rapamycin) inhibitors

The mTOR inhibitors prevent cell proliferation by blocking cell cycle progression from the G1-phase to the S-phase. The immunosuppressive activity is mediated *via* blocking mTOR protein kinases, resulting in inhibition of growth factor–mediated T-cell proliferation in response to IL-2 trigger [42]. Sirolimus is a 31-membered macrolide, whereas everolimus is a sirolimus derivative having a 2-hydroxyethyl chain substitution at position 40. Although the chemical structures of sirolimus and everolimus are similar to that of tacrolimus, the mechanism of action of mTOR inhibitors is distinct from calcineurin inhibitors, which allows the application of combination regimens. Additionally, the main advantages of mTOR inhibitors are their nonnephrotoxic properties; therefore, mTOR inhibitors in combination with reduced dose calcineurin inhibitors can augment the calcineurin inhibitor–induced nephrotoxicity [43–45].

The structural similarities can explain some common metabolic pathways of mTOR inhibitors and tacrolimus, such as *O*-dealkylation and hydroxylation at several positions [42]. Sirolimus is primarily metabolized by CYP3A enzymes and by CYP2C8 at lower extent, producing hydroxylated and *O*-demethylated metabolites (e.g., 12-hydroxy-, 16-*O*-demethyl-, 39-*O*-demethyl-, 27–39-*O*-didemethyl- and dihydroxy-sirolimus as major metabolites) [46, 47]. The metabolism of sirolimus leads to inactivation, despite the fact that some metabolites display some pharmacological activity less than one tenth of the parent drug. Everolimus is also metabolized by CYP3A and CYP2C8 enzymes; however, the elimination rate of everolimus is more rapid than sirolimus (with 30 h *vs.* 62 h elimination half-lives, respectively). Everolimus is *O*-demethylated and hydroxylated at several positions (forming both mono- and dihydroxy-metabolites); furthermore, a ring-opened metabolite is also formed from everolimus [46]. Everolimus-induced adverse effects are associated with the exposure rather to the parent compound than to its metabolites.

3.3. Antimetabolite purine analogues

One of the oldest agents with immunosuppressive activity introduced for kidney transplant recipients was the purine analogue 6-mercaptopurine, which acts by inhibiting purine nucleotide synthesis and, as a consequence, cell proliferation. The prodrug of 6-mercaptopurine, azathioprine with more favorable side-effect profile was later introduced to prevent rejection. Azathioprine is converted to 6-mercaptopurine by nonenzymatic cleavage of the thioether in enterocytes and hepatocytes or in erythrocytes. The major active metabolites, 6-thioguanine nucleotides, are formed *via* 6-thioinosine monophosphate in natural purine synthetic pathways. Inhibition of cell proliferation is mediated by incorporation of the thiopurine nucleotide analogues into DNA (and RNA), causing DNA damage [48]. 6-Mercaptopurine, independently from either direct administration or production from azathioprine, undergoes metabolic inactivation by xanthine oxidase and thiopurine S-methyl transferase and is excreted in the urine, leaving less parent compound available to form thiopurine nucleotides [49]. Due to genetic polymorphism, the thiopurine S-methyl transferase activity is highly variable in patients; namely, those subjects who carry one or two nonfunctional thiopurine S-methyl transferase alleles are unable to tolerate normal doses of azathioprine and can experience serious myelosuppression [50]. Therefore, genotyping assay is recommended before starting azathioprine therapy to identify high-risk patients, and dosage reduction or alternative therapy is recommended for these patients.

3.4. Inosine monophosphate dehydrogenase inhibitors

Mycophenolic acid is a selective inhibitor of inosine monophosphate dehydrogenase, which is responsible for *de novo* biosynthesis of guanosine monophosphate, one of the building blocks of DNA. Depletion of the guanosine pool in the cell arrests the lymphotic cell proliferation and suppresses the subsequent immune response triggered by allogenic transplanted organ [51]. In several rapidly dividing cells (e.g. enterocytes), an alternative salvage pathway exists for purine synthesis in addition to *de novo* synthetic pathway; however, lymphocytes seem to be dependent on the *de novo* pathway. Consequently, mycophenolic acid is able to selectively block proliferation of T- and B-cells. Mycophenolic acid is available as enteric-coated mycophenolate sodium and as mycophenolate mofetil ester prodrug that is extensively hydrolyzed to the active metabolite mycophenolic acid by carboxylesterases.

Mycophenolic acid is primarily metabolized by UDP-glucuronyl transferases (UGT1A7/8/9, UGT2B7), forming the major 7-*O*-mycophenolic glucuronide that is pharmacologically inactive and to the minor acil-glucuronide that has pharmacological activity comparable to the mycophenolic acid [52, 53]. The major proportion of the glucuronide conjugates is excreted in urine, whereas a smaller proportion that is eliminated *via* bile is metabolized by bacteria in the gut, and the deconjugated mycophenolic acid can be reabsorbed (enterohepatic circulation) [54]. Furthermore, in patients' blood and urine, a minor demethylated metabolite (6-*O*-demethylmycophenolic acid) was also detected that was proved to be produced by CYP3A enzymes.

3.5. Corticosteroids

At the beginning of transplantation history, glucocorticosteroids were the primary immunosuppressive agents in the rejection prophylaxis strategy, and nowadays, they are still the first-line agents for treatment of graft rejection. The high-dose glucocorticoids given in peritransplantation are tapered to low doses in the maintenance phase, aiming the steroid-free immunosuppression regimens because of serious adverse effects of glucocorticoids developing in long-term therapy. Acute rejection is generally treated with methylprednisolone, whereas the maintenance therapy applies either methylprednisolone or prednisone. Corticosteroids activate the cytosolic glucucorticoid receptor and modulate several cellular functions, including transcription of genes involved in proliferative and inflammatory processes. The activated receptor inhibits the transcription of NF-kB and activator protein 1 dependent genes, including proinflammatory cytokines (**Figure 1**). This process leads to the depletion of T-cells and macrophage dysfunction [55].

Regioselective and stereospecific hydroxylation of corticosteroids at several positions (at carbon 2, 6, 7, 15, 16, and 21) are catalyzed by CYP3A enzymes. Additionally, dual effect of corticosteroids on CYP3A enzymes has been demonstrated: (1) corticosteroids can competitively inhibit the function of CYP3A [56], and (2) they can induce CYP3A transcription. Activated glucocorticoid receptor upregulates the expression of nuclear receptors (PXR and CAR) that are involved in transcriptional regulation of CYP3A genes. Moreover, the proximal promoter region of CYP3A4 gene contains glucocorticoid responsive element, which directly binds activated glucocorticoid receptor [18, 57]. As a consequence of increased expression and activity of CYP3A enzymes, metabolic drug interactions can be expected upon concomitant treatment with drugs that require CYP3A activity for their metabolism.

3.6. Novel investigational immunosuppressant agents

Although calcineurin inhibitor–based immunosuppression efficiently prevents rejection, adverse reactions of ciclosporin and tacrolimus, primarily nephrotoxicity, prompt the discovery of novel agents with immunosuppressive activity [58]. Two investigational agents with low molecular weight should be mentioned: voclosporin and sotrastaurin. Voclosporin, a next-generation calcineurin inhibitor, is an analogue of ciclosporin with a single carbon extension added to the amino acid-1 of ciclosporin. Voclosporin displays higher binding affinity to cyclophillin A than ciclosporin leading to more potent inhibition of calcineurin [59]. Furthermore, it has a favorable safety property that it appears to be less toxic than currently available calcineurin

inhibitors. Similarly to ciclosporin, voclospiron is a substrate for CYP3A enzymes, anticipating pharmacokinetic/metabolic drug interactions with those agents that interact with ciclosporin as well [60]. However, voclosporin is no longer pursed in transplantation. Sotrastaurin is protein kinase C inhibitor that effectively inhibits IL-2 production with the mechanism different from calcineurin or mTOR inhibition. Although sotrastaurin displayed some potential in preventing allograft rejection in animal studies, high efficacy and safety failure rate were observed in clinical trials involving kidney and liver transplant patients [61, 62]. Therefore, further development of sotrastaurin in transplantation has been halted.

4. Significant metabolic drug interactions with immunosuppressants

4.1. Combined immunosuppressive therapy

Transplant recipients' immunosuppressive therapy is often a multidrug therapy, primarily in the early postoperative period, which constitutes a challenge for clinicians to consider the complexity of drug interactions. Due to the fact that the metabolism of immunosuppressants with low molecular weight is catalyzed by the same enzymes (CYP3A4 and CYP3A5), the blood concentrations, elimination half-lives, and consequently, the efficacy or toxicity of certain immunosuppressant agents are expected to be modified during concomitant treatment. Therefore, during multidrug therapy or during withdrawal of any of the immunosuppressive drugs, special attention is required for optimal dosing for therapeutic concentrations. Each modification in immunosuppressive regimens can lead to changes in blood concentration of a drug (**Table 2**).

Calcineurin inhibitors are often applied in combination with mTOR inhibitors. Since both mTOR inhibitors and calcineurin inhibitors are substrates of CYP3A enzymes and can inhibit CYP3A activities, reduction of calcineurin inhibitor doses is recommended. Standard doses of ciclosporin were observed to decrease the clearance of sirolimus or everolimus more substantially than the doses of tacrolimus [45]. The major drawback of calcineurin inhibitor therapy is the risk of nephrotoxicity which appears to be dose dependent. The combination of low calcineurin inhibitor doses with mTOR inhibitors was found to be beneficial regarding retaining low rejection rates and lowering the risk of nephrotoxicity [44, 63]. To avoided renal dysfunction, the complete substitution of calcineurin inhibitors for mTOR inhibitors was attempted; however, the substitution showed an increase in graft failure in patients treated with merely mTOR inhibitors [64].

Corticosteroids have been demonstrated to induce the expression of the efflux pump transporter ABCB1 (P-glycoprotein) playing a main role in intestinal drug absorption and of CYP3A enzymes responsible for the metabolism of the majority of drugs [18, 65]. Therefore, the concomitant treatment of calcineurin inhibitors or mTOR inhibitors with corticosteroids can be expected to decrease the blood concentrations of tacrolimus/ciclosporin or of sirolimus/everolimus. Although the evidence for clinically significant interactions between corticosteroids and ciclosporin or mTOR inhibitors is limited, clear clinical effect of corticosteroids on tacrolimus exposure has been demonstrated [66, 67]. This also implies that dose reduction or cessation of corticosteroids leads to an increase in blood concentrations of tacrolimus, requiring dose

Immunosuppressant	Drug interactions	Consequences
Ciclosporin Tacrolimus	sirolimus, everolimus	Increased blood levels of ciclosporin and mTOR inhibitors; increased risk of nephrotoxicity
	prednisolone	Decreased blood levels due to enhanced metabolism of ciclosporin/tacrolimus, increased risk of rejection
	Antifungals:	
	ketoconazole	Increased blood levels of ciclosporin/tacrolimus; replacement of ketoconazole to other azole derivatives
	fluconazole, voriconazole, itraconazole	Inhibition of CYP3A4; dose reduction of ciclosporin, tacrolimus is necessary
	Antibiotics:	
	clarithromycin, erythromycin, azithromycin	Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus
	rifampicin	CYP3A4 induction; enhanced metabolism of ciclosporin, tacrolimus; increased risk of rejection
	Antiviral agents:	
	ritonavir	Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus
	Lipid-lowering agents:	
	fluvastatin, simvastatin, atorvastatin	Increased statin exposure by ciclosporin; incrased risk of myopathy and rhabdomyolysis
	Antihypertensive agents:	
	diltiazem, verapamil, amlodipine	Irreversible inhibition of CYP3A4, formation of metabolic intermediate complex; Increased blood levels of ciclosporin / tacrolimus
	nifedipine	Reversible, competitive inhibition CYP3A4
	carvedilol	Inhibition of ABCB1 transporter; increase absorption of oral ciclosporin
	Antidiabetic agents:	
	troglitazone, rosiglitazone	CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection
	Psychopharmacons:	
	carbamazepine, valproic acid	CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection
	fluvoxamine	Inhibition of CYP3A4; contraindicated
	Herbs:	
	St John's wort	CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection
	grapefruit, pomelo	Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus

Immunosuppressant	Drug interactions	Consequences
Sirolimus Everolimus	ciclosporin	Increased blood levels of ciclosporin and mTOR inhibitors; increased risk of nephrotoxicity
	prednisolone	Decreased blood levels due to enhanced metabolism of sirolimus/everolimus, increased risk of rejection
	Antifungals:	
	ketoconazole	Increased blood levels of mTOR inhibitors; replacement of ketoconazole to other azole derivatives
	fluconazole, voriconazole, itraconazole	Inhibition of CYP3A4; dose reduction of sirolimus, everolimus is necessary; voriconazole – sirolimus combination is contraindicated
	Antibiotics:	
	clarithromycin, erythromycin, azithromycin	Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus
	rifampicin	CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection
	Antiviral agents:	
	ritonavir	Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus
	Antihypertensive agents:	
	diltiazem, verapamil, amlodipine	Irreversible inhibition of CYP3A4, formation of metabolic intermediate complex; Increased blood levels of sirolimus/everolimus; verapamil-sirolimus combination is associated with increased blood levels of verapamil
	Antidiabetic agents:	
	troglitazone, rosiglitazone	CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection
	Psychopharmacons:	
	carbamazepine, valproic acid	CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection
	Herbs:	
	St John's wort	CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection
	grapefruit, pomelo	Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus
6-mercaptopurine Azathioprine	allopurinol	Inhibition of xantine oxidase; myelotoxicity
Mycophenolate	Ciclosporin	Inhibition of enterohepatic circulation, decrease in blood levels of mycophenolic acid

Immunosuppressant	Drug interactions	Consequences
	Antiviral agents:	
	ganciclovir, valganciclovir	Mycophenolate-glucuronide inhibits renal tubular secretion of ganciclovir; increased blood levels of ganciclovir and increased risk of toxicity (nephrotoxicity, neutropenia, leukopenia)
Prednisolone	*Antifungals*:	
Methylprednisolone		
	ketoconazole, fluconazole, voriconazole, itraconazole	Increased blood levels of corticosteroids Inhibition of CYP3A4
	Antibiotics:	
	rifampicin	CYP3A4 induction; enhanced metabolism of corticosteroids
	Antiviral agents:	
	ritonavir	Irreversible inhibition of CYP3A4; increased blood levels of corticosteroids

Table 2. Clinically relevant pharmacokinetic drug interactions with immunosuppressants.

adjustment [68]. Interestingly, CYP3A5 nonexpressers with *CYP3A5*3/*3* genotype are more susceptible to glucocorticoid induction than *CYP3A5*1* carriers [69]; thus, more pronounced increase in tacrolimus exposure can be expected in CYP3A5 nonexpressers after glucocorticoid withdrawal.

Clinically significant interaction between mycophenolic acid, the active metabolite of mycophenolate mofetil, and ciclosporin has been reported [70]. The mycophenolate-glucuronide metabolite eliminated into bile undergoes enterohepatic cycling because of intestinal bacterial metabolism and reabsorption of mycophenolic acid. The enterohepatic circulation, contributing to overall pharmacokinetics of mycophenolic acid by 37% in human, is inhibited by concomitant administration of ciclosporin but does not interfere with tacrolimus or sirolimus [71, 72]. In ciclosporin-mycophenolate combination therapy, the reduced blood concentration of mycophenolic acid is necessary to ameliorate by increasing dose of mycophenolate mofetil. Furthermore, special attention on optimal dosing is required during switching ciclosporin-mycophenolate to tacrolimus-mycophenolate therapy and *vice versa*.

4.2. Metabolic drug interactions between immunosuppressants and post-transplant medication

4.2.1. Treatment and prevention of infections

Environmental circumstances and immune deficiencies due to immunosuppression therapy make recipients susceptible for infections that are one of the leading complications after organ transplantation; therefore, prevention and management of infections is a major task

primarily in the early postoperative period. Since fungal infections are a threatening cause of morbidity and mortality, the antifungal prophylaxis is an important element of posttransplant medication. The antifungal azole-derivatives are potent (some of them are very strong) CYP3A inhibitors, predicting potential metabolic drug interactions with calcineurin inhibitors, mTOR inhibitors, or corticosteroids. The most potent CYP3A inhibitor is ketoconazole, able to increase blood concentrations (AUC) of ciclosporin (> 4-fold), tacrolimus (> 2-fold), sirolimus (11-fold), everolimus (15-fold), and methylprednisolone (> 2-fold) [73, 74]. Because of the substantial increase in blood concentrations of several immunosuppressants that can be avoided by drastic reduction of immunosuppressant doses and because of other adverse effects of ketoconazole, the concomitant medication is discouraged. Fluconazole, itraconazole, and voriconazole are alternative regimens for antifungal therapy or prophylaxis; however, all three drugs are azole derivatives and have the capability to inhibit CYP3A function, albeit at a lower extent than ketoconazole [75–77]. Although the continuous immunosuppressant monitoring is highly recommended and dose adjustment (reduction) is generally required, the antifungal treatment with fluconazole, itraconazole, or voriconazole can be safely applied except for voriconazole-sirolimus combination [78]. Because of an extreme (7-fold) increase of sirolimus blood concentrations as a consequence of concomitant use of voriconazole, this combination is contraindicated. Amphotericin B, the nonazole type antifungal agent, does not influence CYP activities; therefore, no metabolic drug interactions can be expected in concomitant treatment with immunosuppressants. However, the widespread use of amphotericin B is limited because of its toxicity profile, primarily because its nephrotoxic side-effect can contribute to the renal injury by ciclosporin or tacrolimus.

Organ transplant patients are at high risk for developing bacterial infections that occur in 20–40% of transplants. Potential sources of infection are from hospital and community exposures, as well as from endogenous flora of patients. Among the antibiotics used for treatment of infections, the macrolide erythromycin and clarithromycin have been reported to interact with immunosuppressive agents. These macrolides are CYP3A substrates and bind to CYP3A4 enzymes, leading to a complex formation that completely inactivates CYP3A4 enzyme [79–82]. The *in vitro* findings were confirmed by clinical observations that blood concentrations of ciclosporin/tacrolimus or sirolimus/everolimus increased as a consequences of concomitant treatment with erythromycin or clarithromycin [73, 83–86]. Page et al. [87] and Mori et al. [88] have reported some potential of azithromycin for drug interaction with ciclosporin and tacrolimus; however, *in vitro* experiments demonstrated that azithromycin poorly interfere with CYP3A4 [89]. When concomitant therapy with these macrolides is necessary, blood concentrations of calcineurin inhibitors or mTOR inhibitors should be carefully monitored, and the immunosuppressant doses should be adjusted. In contrast, the macrolide rifampicin is a potent CYP3A4 inducer and can activate PXR, resulting in a substantial increase in CYP3A4 expression [90]. The increased CYP3A4 activity consequently enhances the metabolism and elimination of calcineurin inhibitors, mTOR inhibitors, and corticosteroids [91–93]. However, blood concentration–guided dose-adjustment of immunosuppressants should be applied carefully because increased metabolism can evoke elevation of toxic metabolite formation (e.g., ciclosporin).

A significant cause of graft failure still remains viral infections, which are acquired as new infection or reactivation of latent viruses. After transplantation, cytomegalovirus (CMV) is the

most common viral infection in recipients, primarily in those CMV-seronegative patients who were transplanted with graft from CMV-seropositive donors, resulting in viral reactivation. For prophylaxis and treatment of CMV infection, aciclovir, ganciclovir, and valganciclovir (the prodrug of ganciclovir) are generally applied. None of these antiviral drugs influences the function of drug-metabolizing CYPs or UDP-glucuronyl transferases, and consequently, they do not modify the pharmacokinetic properties of immunosuppressants. Aciclovir and ganciclovir are eliminated primarily in the urine as unchanged compounds. Increased risk of nephrotoxicity and leukopenia has been reported in patients who were co-medicated with a drug that can reduce renal clearance of aciclovir or ganciclovir. During co-administration with mycophenolate or mycophenolate-mofetil, mycophenolate-glucuronide and aciclovir or ganciclovir can significantly compete for renal tubular secretion, resulting in an increase in aciclovir/ganciclovir and mycophenolate-glucuronide exposure, as well as the risk of nephrotoxicity or leukopenia [94–96]. Management of potent metabolic drug interactions between antiviral protease inhibitors and immunosuppressants is a major challenge because most of the protease inhibitors are clinically significant CYP3A4 inhibitors. Ritonavir-boosted therapies require substantial reduction of immunosuppressant doses (to 5–20% for ciclosporin; to 1–3.5% for tacrolimus) with continuous monitoring of blood concentrations [97–101].

4.2.2. Treatment of dyslipidemia

Dyslipidemia is often developed as an adverse impact of immunosuppressive therapy [102]. Ciclosporin, mTOR inhibitors, and prednisone are mainly implicated in lipid alterations. For treatment of hypercholesterolemia, the basic guidelines for dyslipidemia recommend diet and HMG-CoA reductase (hydroxymethyl-glutaryl-CoA reductase) inhibitor statins with special considerations for transplant patients. Although both ciclosporin and most statins (atorvastatin, fluvastatin, simvastatin, lovastatin) are primarily metabolized by CYP3A4 and metabolic drug interactions are likely occur, statins do not evoke increased ciclosporin exposure [103–106]. In contrast, ciclosporin induces significant elevation of statin blood concentrations which can be explained by the ten-fold higher molar concentrations of ciclosporin than statins. In combination with ciclosporin, the blood levels are increased in a statin-dependent manner, e.g., lovastatin is increased to a much greater extent than atorvastatin [104, 107]. Dose reduction of lipid-lowering agents is recommended to avoid myopathy or rhabdomyolysis. The blood concentrations of macrolide immunosuppressants (tacrolimus, sirolimus, and everolimus) are similar to that of statins [108, 109]; therefore, the lack of clinically relevant interactions between macrolides and statins is not unexpected.

4.2.3. Antihypertensive agents

Organ transplantation and immunosuppressive therapy (e.g., ciclosporin, prednisone) frequently trigger hypertension or worsen the preexisting disease in patients. While most of the antihypertensive agents (β-adrenoceptor blockers, α1-adrenergic receptor antagonists, central α2-adrenergic receptor agonists, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers) are not expected to influence the pharmacokinetics of immunosuppressants, medication with diltiazem, verapamil, or amlodipine requires special consideration and

frequent monitoring of immunosuppressant blood concentrations. The metabolism of all three Ca-channel blockers is primarily catalyzed by CYP3A4, anticipating potential drug interactions with immunosuppressants. Furthermore, significant inhibition of CYP3A4 by diltiazem, verapamil, and amlodipine has been demonstrated with an additional inhibitory property of metabolite intermediate complex formation that catalytically inactivates CYP3A4 and CYP3A5 enzymes [79, 80, 82, 110–114]. The inactivation of CYP3A enzymes by comedication with these antihypertensive drugs consequently leads to a permanent increase in blood concentrations of calcineurin inhibitors or mTOR inhibitors [115–122]. In transplant recipients comedicated with sirolimus and verapamil, an increase of blood concentrations of both sirolimus and verapamil was observed [123]. Furthermore, in patients carrying wild-type *CYP3A5*1* allele, concomitant treatment with amlodipine significantly decreased tacrolimus clearance, and along with the changes in tacrolimus pharmacokinetics, an increase in amlodipine blood concentrations was also observed [124]. The metabolism of the Ca-channel blocker nifedipine is catalyzed almost exclusively by CYP3A enzymes, and competition for the active site of CYP3As may be expected if nifedipine and CYP3A substrate calcineurine inhibitors or mTOR inhibitors are concomitantly applied. In contrast, no evidence for pharmacokinetic drug interactions has been provided in transplant recipients treated with nifedipine and ciclosporin/tacrolimus or sirolimus/everolimus. Carvedilol is often used for treatment of hypertension in transplant patients, and pharmacokinetic drug interaction between carvedilol and ciclosporin has been observed that required 10–20% reduction of ciclosporin doses to maintain the blood concentrations within the therapeutic range [125, 126]. The major metabolic pathways of carvedilol are catalyzed by CYP2D6 and CYP1A2 rather than by CYP3A4 [127]; however, inhibition of CYP3A enzymes by carvedilol does not account for pharmacokinetic drug interaction with ciclosporin. Carvedilol has been demonstrated to block the function of the ABCB1 transporter protein (ATP-binding cassette B1; previously called as Pgp) [128]. In the intestinal wall, ABCB1 transporter pumps pharmacons or other xenobiotics passed into the enterocytes back into the gut lumen. The inhibition of ABCB1-mediated transcellular transport in the intestine by carvedilol is responsible for the increased absorption of ciclosporin. Under careful monitoring of ciclosporin blood concentration, the ABCB1 inhibition by carvedilol can be beneficial in ciclosporin-sparing therapy for transplant patients. Since the absorption of tacrolimus and mTOR inhibitors is also mediated by ABCB1, similar pharmacokinetic drug interactions between these immunosuppressants and carvedilol are presumably developed as with ciclosporin.

4.2.4. Antihyperglycemic therapy

Hyperglycemia developing posttransplant diabetes mellitus is generally medication related. Corticosteroids can evoke reduction of glucose tolerance, whereas ciclosporin and tacrolimus directly block insulin-release by islet cells. The metabolism of the sulfonylurea type antidiabetic agents (e.g., tolbutamide, glipizide, glibenclamide, and glimepiride) is mediated by CYP2C9; therefore, metabolic drug interactions with immunosuppressants are not expected in patients treated with any of these oral hypoglycemic drugs. Although the thiazolidinedione type troglitazone and rosiglitazone are not CYP3A substrates, they can induce the expression of CYP3A enzymes by activation of the nuclear receptors, PXR and CAR [129–132]. Enhanced transcription results in an increase in CYP3A activities and the metabolism of calcineurin inhibitors,

mTOR inhibitors and corticosteroids, increasing the risk of rejection [133]. Immunosuppressant dose adjustment is required to avoid subtherapeutic blood concentrations, and careful monitoring of immunosuppressant blood concentrations is recommended during withdrawal of troglitazone or rosiglitazone and during switching to other antihyperglycemic agent.

4.2.5. Psychiatric medication

The most common psychiatric disorders encountered in transplant patients are anxiety, depression, mood disorders, behavior problems, and insomnia that are reversible in most cases; however, they often require psychotherapy with antidepressants, mood stabilizers, anxiolytic agents, or even with antipsychotics. Many of these pharmacons are metabolized by enzymes other than CYP3A4 and do not influence the drug-metabolizing activities of CYP3A4; consequently, metabolic drug interactions with immunosuppressants cannot be expected. Nevertheless, the CYP3A4 inducing or inhibitory properties of some of these psychopharmacons should be considered. The mood stabilizer carbamazepine and valproic acid have been clearly evidenced to be able to activate CAR and PXR. The nuclear receptor activation leads to an increase in transcription of *CYP3A4* gene and CYP3A4 metabolic activity [134, 135], anticipating decrease of immunosuppressant blood concentrations [136]. To reduce the risk of organ rejection, adjustment (increase) of immunosuppressant doses is required with continuous monitoring of immunosuppressant blood levels. Furthermore, the CYP3A4 deinduction process can last for about 2 weeks after cessation of carbamazepine or valproic acid [137]; thus, careful monitoring of immunosuppressant blood concentrations during withdrawal is essential. The comedication with the antidepressant fluvoxamine is contraindicated because of its strong inhibitory properties for CYP3A4 substrates and potential drug interactions with ciclosporin/tacrolimus or with sirolimus/everolimus [80, 138, 139]. For psychotherapeutic agents that are CYP3A substrates (haloperidol, quetiapine, clonazepam, midazolam, alprazolam), continuous monitoring of immunosuppressant blood levels is highly recommended to avoid metabolic drug interactions.

4.2.6. Treatment of hyperuricemia

The metabolic drug interactions with ciclosporin/tacrolimus, sirolimus/everolimus, and corticosteroids are generally associated with reversible or irreversible inhibition of CYP3A activities, as well as with transcriptional induction of CYP3A4 and CYP3A5 expression. Clinically significant drug interaction occurs during simultaneous therapy with azathioprine (or 6-mercaptopurine) and allopurinol, the antihyperuricemic agent [140, 141]; however, it involves enzyme other than CYP3As. The metabolism of both 6-mercaptopurine and allopurinol is catalyzed by xantine oxidase, anticipating metabolic drug interactions and developing serious adverse reactions. As a consequence of inhibition of xantine oxidase by allopurinol, myelotoxicity is evoked by the accumulation of 6-thioguanine-nucleotide metabolites of azathioprine. The risk of bone marrow depletion is increased in patients with low thiopurine methyl-transferase activity. To avoid the serious myelosuppression during treatment of hyperuricemia and gout, substantial reduction of azathioprine dose (by at least 50%) is required when allopurinol is given concomitantly, or alternative agents other than allopurinol should be considered [142–144].

4.3. Metabolic drug interactions between immunosuppressants and herb components

Pharmacokinetic herb-drug interactions can also significantly influence the outcome of immunosuppressive therapy and long-term graft survival [145]. St John's wort (*Hypericum perforatum*) extract and grapefruit juice are well described as modifiers of pharmacokinetic properties of ciclosporin and tacrolimus [146–148]. St John's wort extract is a herbal product for treatment of symptoms of mild or moderate depression, including anxiety, fatigue, and sleeping problems. The extract contains a number of biologically active components, e.g., hyperforin of high interest. Hyperforin has a strong affinity for PXR and significantly increases the expression and activities of CYP3A4 enzyme, which is involved in metabolism of many drugs [149, 150]. Consequently, chronic consumption of St John's wort extract can decrease the blood concentrations of CYP3A substrates, such as calcineurin inhibitors, mTOR inhibitors, and corticosteroids [151–154]. In addition, St John's wort extract has been reported to induce the expression of ABCB1 transporter that reduces the absorption of ABCB1-ligand drugs from the gut. The hyperforin contents of commercially available St John's wort preparations are variables that appear to significantly affect the extent of pharmacokinetic interactions [150, 155]. Coadministration of ciclosporin with St John's wort extract has been reported to lead a 40–60% decrease of ciclosporin blood concentrations, increasing the risk of rejection; therefore, substantial dose adjustment is required [151, 152, 155–159]. Since clinicians are often unaware of concomitant consumption of herbal supplements, transplant patients should be informed about the drug interaction potential of St John's wort that can endanger the success of organ transplantation.

Concomitant intake of grapefruit (*Citrus paradisii*) or pomelo (*Citrus grandis*) has been demonstrated to increase the bioavailability of immunosuppressants [147, 160, 161]. Some components of these citrus fruits, bergamottin and naringenin responsible for the bitter taste, can inhibit the activities of CYP3A4 and CYP3A5 enzymes both in the intestinal wall and in the liver, resulting in significant reduction of first-pass metabolism of CYP3A substrates, including ciclosporin and tacrolimus [162–164]. Significant reduction of ciclosporin/tacrolimus doses is necessary to avoid the risk of nephrotoxicity or other adverse events associated with immunosuppressive therapy. The furanocoumarin bergamottin is a "suicide substrate," namely it is metabolized by CYP3A4 to an epoxid metabolite that covalently binds to and inactivates the enzyme [165]. The flavonoid naringenin was found to be a less-potent CYP3A4 inhibitor than bergamottin [166]; however, during consumption of grapefruit, the inhibitory effects of naringenin and bergamottin are added together. Since clear evidence of bergamottin content and CYP3A4 inhibitory potential of citruses other than grapefruit and pomelo was provided [167], the transplantation centers do not recommend citrus consumption for transplant patients during immunosuppressive therapy.

5. Concluding remarks

Although success of organ transplantation is continuously improving, several short- and long-term complications can adversely affect the outcome. One of the most significant factors influencing the long-term graft and patient survival is the appropriate immunosuppressive

therapy. Subtherapeutic blood concentrations of immunosuppressive drugs can evoke acute or chronic graft injury mediated by immunological mechanisms, whereas overdosing leads to over-suppression of the immune system that consequently develops serious infections, as well as adverse and even life-threatening side effects. Because of the narrow therapeutic indexes, dosing of most of the immunosuppressive agents is applied under careful monitoring of their blood concentrations. The knowledge of the potential factors that can modify immunosuppressive therapy, as well as pharmacokinetic and metabolic drug interactions, can decrease the fluctuation of immunosuppressant blood concentrations, can facilitate to avoid the serious adverse events, can improve the therapeutic outcome for transplant patients, and can reduce the medical costs.

The appropriate and tailored immunosuppressive medication is a great challenge and requires careful and continuous attention, because unrecognized simple interactions can induce serious complications. As such during administratrion of clarithromycin or antifungal agents without dose reduction of calcineurin inhibitors or mTOR inhibitors, blood concentrations of immunosuppressants can substantially exceed the therapeutic range within some days. Without dose modification, a reverse outcome is expected during comedication with anticonvulsants (valproic acid and carbamazepine) or with rifampicin resulting in subtherapeutic blood concentrations of immunosuppressants and increasing the risk of organ rejection. The lack of mycophenolate dose reduction during cessation of ciclosporin or replacement of ciclosporin to another immunosuppressant can also evolve development of serious adverse reactions. It is anticipated that the special attention and the knowledge of potential drug interactions can prevent the majority of misdosing-induced adverse events.

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Conflict of interest

The author declares that there is no conflict of interest.

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One of the most interesting and at the same time most challenging fields of medicine and surgery has been that of organ donation and transplantation. It is a field that has made tremendous strides during the last few decades through the combined input and efforts of scientists from various specialties. What started as a dream of pioneers has become a reality for the thousands of our patients whose lives can now be saved and improved. However, at the same time, the challenges remain significant and so do the expectations.

This book will be a collection of chapters describing these same challenges involved including the ethical, legal, and medical issues in organ donation and the technical and immunological problems the experts are facing involved in the care of these patients.The authors of this book represent a team of true global experts on the topic. In addition to the knowledge shared, the authors provide their personal clinical experience on a variety of different aspects of organ donation and transplantation.

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