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New approaches to increase intestinal length: Methods used for intestinal regeneration and bioengineering

Ali Shirafkan, Mauro Montalbano, Joshua McGuire, Cristiana Rastellini, Luca Cicalese

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Abstract

Inadequate absorptive surface area poses a great challenge to the patients suffering a variety of in-

testinal diseases causing short bowel syndrome. To date, these patients are managed with total parenteral nutrition or intestinal transplantation. However, these carry significant morbidity and mortality. Currently, by emergence of tissue engineering, anticipations to utilize an alternative method to increase the intestinal absorptive surface area are increasing. In this paper, we will review the improvements made over time in attempting elongating the intestine with surgical techniques as well as using intestinal bioengineering. Performing sequential intestinal lengthening was the preliminary method applied in humans. However, these methods did not reach widespread use and has limited outcome. Subsequent experimental methods were developed utilizing scaffolds to regenerate intestinal tissue and organoids unit from the intestinal epithelium. Stem cells also have been studied and applied in all types of tissue engineering. Biomaterials were utilized as a structural support for naive cells to produce bio-engineered tissue that can achieve a near-normal anatomical structure. A promising novel approach is the elongation of the intestine with an acellular biologic scaffold to generate a neo-formed intestinal tissue that showed, for the first time, evidence of absorption *in vivo*. In the large intestine, studies are more focused on regeneration and engineering of sphincters and will be briefly reviewed. From the review of the existing literature, it can be concluded that significant progress has been achieved in these experimental methods but that these now need to be fully translated into a pre-clinical and clinical experimentation to become a future viable therapeutic option.

Key words: Bioengineered intestine; Tissue engineered; Scaffolds; Organoids; Stem cells; Intestinal elongation techniques

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Core tip: Several methods were used to elongate the short and insufficient segment of intestine in patients suffering short bowel syndrome. These methods include transplantation of an intestinal graft, intestinal elongation, and techniques to create a bioengineered segment of intestine. Innovations in using stem cells, organoid units of intestine and bio-scaffolds allow the modern medicine to engineer segments of functional intestinal tissue in animal models. However, to reach the goal of implanting a fully functional bioengineered intestine in human improvements are still required. This article will review various methods to approach this condition from surgical techniques to elongate the intestine to the application of stem cells and bio scaffolds for creating three dimensional intestinal structure.

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INTRODUCTION

Intestinal absorptive function is the result of fine regulation between different cell types and signaling, cooperating within this organ. Intestinal failure is the consequence of various diseases that limit intestinal length or function. These include, but are not limited to: Intestinal atresia, gastroschisis, pseudo-obstruction, motility disorders, Crohn's disease, mesenteric thrombosis, intestinal necrosis, trauma and lead to short bowel syndrome. When the remaining portion of the intestine is functionally insufficient, intestinal failure results and this is characterized by fluid imbalance, electrolyte loss and altered nutrients absorption^[1]. Total parenteral nutrition (TPN) has been used as a treatment option, however, hepatic insufficiency, catheter related thrombosis and sepsis are the most significant limiting factors^[2-5].

Intestinal transplantation offers a physiologic cure in the treatment of these patients as an alternative treatment^[6]. Limitations of intestinal transplantation include sepsis and infections, chronic immunosuppression to avoid rejection and shortage in optimal organ donors^[7]. Various techniques have been proposed to develop a safe and functional method to take advantages of bioengineering in the field of intestinal elongation. In this article, we will review the current knowledge on this subject, explain the limitation and benefits of each method and finally elaborate on the future direction and goals.

In general, the methods in intestinal tissue engineering can be classified into the following groups:

Surgical techniques that can physically elongate the patient's intestinal length; development of intestinal tissue using stem cells (SCs) in culture; development of organoid units from intestinal cells implanted on biologic materials *in vivo* and then incorporated in continuity with the intestine; utilization of biologic scaffold *in vivo* to obtain a neo-formed intestinal segment.

SURGICAL TECHNIQUES

Early surgical procedures to address short bowel syndrome attempted to increase nutrient absorption prolonging food transit time. Those procedures included vagotomy and pyloroplasty procedures, reversing small intestine segment, pouch formation, and prejejunal or preileal colon transposition^[8-14]. In the early 1980s, Bianchi^[15] described a reproducible technique to increase the length of the small intestine. Briefly, the procedure consisted in dividing an intestinal loop longitudinally in the midline where the vessels alternately go to one or other side of the loop from the mesentery. Then each side would be sutured to form a hemiloop. The final step was to anastomose the newly formed loops iso-peristaltically. As a result, the length of that bowel loop would be doubled, however, the diameter was halved. The advantage of this procedure was preservation of all available mucosa while tailoring the intestine length^[15,16].

An alternative approach, called serial transverse enteroplasty (STEP), was introduced in early 2000. Following intentional dilatation of the small bowel, surgical stapling would be performed in an alternating direction from side to side in a "zig-zag fashion" perpendicular to the long axis of the bowel to elongate the existing small intestine. This procedure would be basically equivalent of the Bianchi procedure, however STEP had several theoretic advantages. The procedure was easier to perform and there was no need for anastomoses. Additionally, the intestine would never be opened, and the mesentery would never be jeopardized. In contrast, the over-all theoretical increase in length would depend on the amount of bowel dilatation and the size of the created intestinal lumen^[17].

However, the patients who had undergone the Bianchi procedure would wean off TPN more than those with STEP, and they eventually would require intestinal transplants more than those with STEP. In addition, STEP was shown to be associated with higher rates of complication^[18]. A study describes results from 38 patients who underwent STEP procedure for different diagnosis including intestinal atresia, gastroschisis with or without volvulus and necrotizing enterocolitis. Overall, the mean intestinal length increased considerably. The percentage of total calories tolerated enterally also increased. The most common complication was: Staple line leak, obstruction and

abscess. It should be acknowledged that both these procedures have an acceptable short-term outcome while bridging the patients to intestinal transplants and do not seem to constitute a permanent treatment for intestinal failure^[19].

SCS

SCs application in regenerative medicine is relatively new. The peculiarity of SCs differentiation is based on their plasticity and mainly on the microenvironment in which they are placed. Recently, it was shown that bone marrow derived hematopoietic stem cells (HSCs) after transplantation in mice, lethally irradiated with ⁶⁰Cobalt, induce regeneration of gastrointestinal tissues^[20]. Bone marrow mesenchymal stromal cells (BMMSCs) are able to mitigate lethal intestinal injury and their intravenous injection will increase the level of intestinal growth factors in the blood and induce regeneration of the intestinal SCs niche of the irradiated host^[21].

Utilizing soluble growth factors, like epidermal growth factor (EGF) and hepatic growth factor (HGF), in the culture medium of intestinal SCs improves results obtained by increasing the homing of transplanted cells^[22]. Supporting stem cell application, Qu *et al.*^[23] reported that transplantation of BMMSCs and soluble stem cell factors cooperate in regeneration of GI mucosa in a rat model in which indomethacin-induced GI injury was performed.

Hori *et al.*^[24] in 2002 seeded autologous mesenchymal stem cells (MSCs) on a collagen sponge graft to evaluate intestinal regeneration. Despite a complete mucosa was developed, they did not induce regeneration of the muscle layers. To develop smooth muscle cells with peristaltic features, Yoshida *et al.*^[25] employed induced pluripotent stem cells (iPSCs) from mice to induce differentiation of the muscularis into active and functional intestinal smooth muscle cells. However, they were not able to control the produced differentiated cells, since they include cardiac-like cells, mucosal cells and smooth muscle cells.

The intestine is a complex organ composed by many cell types. Today, no SC sources permit the generation of all cell types. During the last years, many studies analyzed stem-cell differentiation mechanisms. Studies on population of muscle-derived stem cells confirmed that they are capable of self-renewal and multi-lineage differentiation including the ability to differentiate into intestinal smooth muscle cells^[15,16].

Neuronal progenitor cells are present both in the central nervous system as well as enteric nervous system (ENS). Advances in cell culture techniques allowed isolation of enteric stem/progenitor cells and glial precursor cells. Several groups were able to isolate the neuronal crest-derived cells by sorting according to the markers for Sox10, p75 and Nestin. Following transplantation of these cells in the aganglionic bowel

of mice Ret (-/-), the ENS was rebuilt^[26].

Interestingly, it has been shown that inducing the CNS-neuronal progenitor cells with gut-derived soluble growth factors, will cause these cells to acquire enteric neuronal phenotype^[27]. Likewise, transfected BMMSCs with glial cell-derived neurotrophic factor (GDNF) and Neurotrophin-3 (NT-3) genes, resulted in differentiation of BMMSCs into neuron-like cells with expression of neuronal markers as MAP-2 and GFAP^[28,29].

In 2011, Spence *et al.*^[20] mimicked embryonic intestinal development in an *in vitro* model by using a series of specific growth factors at different time points and they successfully induced human pluripotent stem cells (PSCs) to differentiate into the new intestinal epithelium tissue and crypt-villus units. In order to mimic the natural intestinal peristalsis and physiology *in vitro*, Kim *et al.*^[30] developed a microfluidic "Gut-on-a-Chip" technology that exposed established epithelial cell lines to physiological peristalsis motions and liquid flow. This particular condition spontaneously induced morphogenesis of three-dimensional intestinal villi. However, these studies supported SCs applications, these *in vitro* models can only partially reiterate the whole *in vivo* intestinal complexity including absorptive or enteric barrier functions, and are far from offering a complete intestinal tissue that could be utilized in an *in vivo* model.

SCS AND BIO-SCAFFOLDS

SCs use has been improved by the attempt to create a three-dimensional (3-D) gel supporting structure system *in vitro* but this remains a major challenge for translational studies. McCracken *et al.*^[29] enhanced the 3-D tissue culture model. They transformed the PSCs implanted on a matrigel layer for a period of one to three months into intestinal mesenchyme and epithelium.

Generation of 3-D milieu provides a microenvironment with superior cell-cell interaction and communication that mimic an *in vivo* condition. For this aim, tissue engineering has used biocompatible scaffolds. Polymeric materials have two main characteristics; they are bio inert and easily biodegradable while they support all cell functions including adhesion, proliferation and differentiation.

Many studies supported that, these scaffolds provide a matrix for the seeding of cells in high density, which promotes reorganization of a functional tissue in a shorter time-frame. Biodegradable materials must give a perfect mechanical support until cells become able to produce extracellular matrix and other cellular factors. Then they are obligated to be wiped out gradually while being replaced by cellular and extracellular components. Persistence of these materials in the body and prolonged exposition to them can trigger an inflammatory response in the implantation site. Kim *et al.*^[31] used biodegradable

matrices of polyglycolic acid (PGA) fibers, and seeded smooth muscle cells in tissue culture dishes (static seeding) and a cell suspension in spinner flasks (stirred seeding). They observed that seeding with dynamic model produced more uniform distribution and resulted in a neo-formed tissue with higher cellularity and greater elastin deposition. In the course of optimization of the tissue engineering methods, Qin *et al.*^[32] isolated intestinal smooth muscle cells from rats and seeded them in small intestinal submucosa (SIS) that is an acellular porcine-derived collagen-based matrix. SIS were implanted in an adult rat jejunal interposition model. Cell-seeded SIS displayed significantly improvement in contracting ability in respect to the SIS when no cells are seeded. However, there were no organized smooth muscle cell layers. Totonelli *et al.*^[33] and Maghsoudlou *et al.*^[34] used a detergent enzymatic treatment (DET) procedure to wash the cellular components of the rat's intestine and to construct a natural acellular intestinal scaffold for regeneration of new intestinal tissue. The yielded scaffolds preserved the native architecture and connective tissue components.

Nakase *et al.*^[35] used a mixture of autologous smooth muscle cells from the stomach wall of a canine model with collagen solution, which was poured into a sponge to develop a collagen scaffold. Then, these structures have been implanted into the isolated defects of ileum as a patch graft. After 12 wk, the patch turned into relatively well-developed regenerated epithelium, villi and a smooth muscle layer in the lamina propria, however, the lack of contraction of these grafts presented as a significant problem.

Autologous MSCs from bone marrow were used by Hori *et al.*^[24] and seeded onto collagen scaffolds to induce the regeneration of a muscular layer. One month after implantation, they observed regeneration of the intestine with a muscular layer at the reconstructed site by - smooth muscle actin positive cells; however, this layer was thin and disappeared by 16 wk.

To stimulate proliferation of smooth muscle layer and angiogenesis, Lee *et al.*^[36] used basic fibroblast growth factors (bFGF). They compared two different concentrations of local administration of bFGF with the control. They found that incorporation of bFGF into the collagen coating layer of scaffolds would result in a significantly higher density of cells and blood vessels. They also found that when the bFGF is incorporated in encapsulated poly D, L-lactic-co-glycolic acid microsphere, it is more effective than its simple employment in collagen scaffolds suggesting that the addition of specific growth factors improves scaffold performance.

Previously, Zakhem *et al.*^[37] utilized a composite chitosan/collagen scaffold three-dimensional matrix to support the smooth muscle cells to restore lost innervation. They grew the rabbit colonic circular smooth

muscle cells (RCSMCs) on chitosan-coated plates with a ratio of 1:1 and observed that cells maintained their morphology and physiologic functionality over time. The muscle constructs contracted in response to acetylcholine and potassium chloride and they relaxed in response to vasoactive intestinal peptide. Furthermore, they showed that this scaffold supports neo-innervation of non-innervated smooth muscle cells^[38].

In 2015, Zakhem *et al.*^[38] showed that neural progenitor cells derived from the appendix and small intestine, will differentiate into mature functional enteric neurons, should they be incorporated in bio-engineered internal anal sphincters. Raghavan *et al.*^[39,40] found that according to the extracellular matrix microenvironment of culture medium, enteric neuronal progenitor cells, will generate excitatory or inhibitory neuronal subtypes. Microenvironment enriched with collagen I and laminin resulted in contraction pattern, collagen IV induced a nitrergic neuronal population (neurons where transmission is mediated by nitric oxide) and laminin and/or heparin sulfate resulted in a balanced expression of relaxant and contractile motor neurons.

ORGANOID UNITS ON BIO-SCAFFOLDS

Another approach to regenerate intestinal tissue employs the use of organoids. Haffen *et al.*^[41] in the 1980s, demonstrated that intestinal crypt cells require interacting with mesenchymal cells for survival, proliferation and differentiation. Then Organ *et al.*^[42] isolated progenitor cells from the intestinal crypt and seeded them onto sheets of polyglycolic acid. They observed generation of stratified epithelium suggestive of fetal intestinal development. Of the limitations of this technique was the absence of epithelial-mesenchymal cell-cell interaction, which is thought to be of importance in organogenesis. Subsequently, Tait *et al.*^[43] demonstrated that dissociated post-natal small intestinal epithelium of rats, will generate small intestine-like structures when transplanted in the subcutaneous plane of adult rats. They confirmed that those small aggregates of intestinal epithelium and stroma are able to generate the required signals for 3-D regeneration of intestinal tissue. Then Choi and Vacanti^[44], developed a villus structure with a core of mesenchymal stromal cells overlaid with epithelium called "Organoid Unit". They believed that these units possess the epithelial-mesenchymal interaction required for mucosal regeneration. They seeded the organoid units isolated from neonatal rat intestine, and seeded them on poly glycolic acid scaffolds. They implanted them into the rats' omentum and observed that cysts were generated after 8 wk, composed of columnar epithelium, Paneth's cells, goblet cells, and crypt-villus-like structures.

To improve their previous work, Choi *et al.*^[45] later demonstrated that by collagen coating the scaffolds,

the cells engraftment will enhance significantly and cyst sizes will be larger. Since it was known that the small intestine is a dynamic organ and responds differently to various factors, Vacanti's lab, also investigated the effect of massive small bowel resections, partial hepatectomy and portocaval shunt on the development of organoid units. These interventions would increase the serum level of the epithelial growth factor (EGF) and hepatocyte growth factor (HGF). Interestingly, they observed that the length and diameter are larger and the villus numbers, height, area and mucosal surface are significantly greater in the group with resected small bowel^[46]. As the next step, to evaluate the effect of incorporation of these organoid units in the intestine, they anastomosed the units side-to-side to the jejunum after three wk of implantation. They demonstrated that anastomosis had no complication. It also had trophic effects on the villus number, height, and surface length^[47]. However, they also described a patchy distribution of the obtained neo mucosa^[48].

Later, Grikscheit *et al.*^[49,50] adapted the organoid unit transplantation technique to develop tissue engineered colon. They produced organoid units from the rats' sigmoid colon and implanted them into the omentum. Then, these organoids were anastomosed to the ileum of the rats that previously underwent ileostomies. After 41 d, they found the rats had less stool transit time and moisture content. Histology also showed a normal large intestine architecture including epithelium, vasculature, ganglion cells, and muscularis propria.

To evaluate the function of the tissue engineered small intestine (TESI), Grikscheit *et al.*^[51] replaced small intestine with these TESIs. After development of TESIs, they anastomosed them side-to-side to the duodenum, when the rats had 95% of their small bowel resected. Forty days post operation, they found an appropriate architecture and a well formed muscularis mucosa with appropriately distributed Aurbach and Meissner's plexus and increased blood levels of B-12.

Following the successful results of TESI in rat model, Sala *et al.*^[52] transitioned this model in mice to take advantage of transgenic tools available in this species for studying the processes involved in formation of tissue engineered intestine. They found that TESI contains all four differentiated epithelial cell types present in the native small intestine including Goblet, Paneth, Enteroendocrine, and microvilli. They also confirmed that TESI contains innervated muscularis as well as presence of intact stem cell niche.

These investigators, also studied as a preclinical model an autologous-derived organoid unit transplantation in a large animal model. They generated organoid units from a short segment of jejunum of a swine model and implanted them onto omentum to the autologous host. They found that the TESIs replicated the native intestine with all epithelium, muscularis

mucosa and stem cell niche^[53].

Levin *et al.*^[54] investigated the possibility of development of organoid units from the postnatal human small intestine. They implanted organoid units, loaded on polyglycolic acid scaffolds in mice omentum. After 4 wk, they found all TESIs were of human origin with all differentiated cell types of mature human small intestine as well as muscularis and nerve tissue. This study was critical since the majority of the patients acquire the pathology after birth and the tissue engineering should be able to develop the tissues from post-natal stem cells. Then, recently they confirmed that both TESIs derived from human and mice developed intact epithelium with ultrastructural components of tight junctions, microvilli, ion transporter/channels, brush border enzymes similar to native tissue^[55].

SCAFFOLDS

Observing the development of a neomucosa after patching the intestinal defects with abdominal wall or serosa of the adjacent colon, brought hope in using these methods for expanding the small bowel absorptive area^[56-59]. Due to the limited availability of the tissues as well as anatomical restrictions, Thompson *et al.*^[60] investigated the outcome of the patching with prosthetic materials at 8 wk. They studied the outcome of patching the ileal defects of antimesenteric borders of rabbits' intestine by using a variety of prosthetic materials including knitted Dacron, PGA mesh and polytetrafluoroethylene (PTFE). They also performed an interposition in the distal ileum with a Dacron tube in another group of animals. They only observed development of thin neomucosa covering 15% of the defect with the patches and no neomucosa formation in interposition tubes. They concluded that the use of prosthetic material was not useful for clinical management of short bowel syndrome^[60].

Biological Scaffolds derived from extracellular matrixes of different types of tissues are being applied in tissue engineering to replicate the organs both structurally and functionally. In intestinal tissue engineering, these biocompatible materials are thought to increase the intestinal mucosal surface area and absorption.

Chen *et al.*^[61] used scaffolds derived from submucosal extracellular matrix of porcine small intestine "small intestine submucosa" (SIS) to evaluate the regeneration of small bowel in dogs. SIS has been previously used to create vascular grafts, abdominal wall, bladder, tendons, and dura mater in animals^[62-66]. They applied the SIS as a patch to repair a partial defect created in the small bowel wall. They observed development of mucosal epithelium, smooth muscles and serosa, however, the layers were not architecturally well organized. They also tried to interpose SIS as a tubular segment in the small intestine, which was unsuccessful and all animals died

postoperatively due to obstruction or leakage^[61].

Then, Wang *et al.*^[67] interposed rat derived SIS between an isolated ileal loop in a rat model. They found development of a well-organized three-layer small intestine including mucosa, smooth muscle and serosa after 24 wk, however, there were no signs of innervation.

Another type of scaffolds applied is a collagen-rich membrane derived from submucosal layer of the pig's small intestine called "Surgisis". Since it is bio-compatible, resistant to infection and contains growth factors, it seemed prudent to use it as a bioscaffold for small intestine regeneration^[68-74].

Cicalese *et al.*^[75] utilized an acellularized matrix of connective tissue obtained from the dermis of cadaveric donors to develop "acellular dermal matrix" (ADM) with preserved proteins of basement membrane, elastin and collagen fibers. We hypothesized that this matrix will be vascularized by host capillaries and stem cells either circulating or derived from the adjacent crypts would induce tissue regeneration. We implanted these ADMs into the rats' intestine either in continuity of the functioning bowel loops or as a blind-ended pouch in a defunctionalized jejunal limb. The blind-ended pouch group immediately showed full thickness ingrowth of capillaries, myofibroblasts and a fully regenerated mucosa at 6 mo. Despite the first group developing peritonitis in the first week without any signs of mucosa or muscular development, in subsequent studies, and using a ticker ADM placed immediately in continuity with the resected intestine, we were able to obtain successful generation of a neo-normal intestinal segment without obstructions or abscesses similar in morphology to the blind-end pouch group.

Similarly, Ansolani *et al.*^[74] utilized a three-centimeter long tubular Surgisis graft to interpose it in an isolated ileal loop in a rat model. After 24 wk, they found a neovascularized, well-developed layers of serosa, smooth muscle and mucosa. This biomaterial showed to offer a promising alternative in small intestine regeneration, however, the fact that it was not placed in continuity with the functional intestinal tract and there was no confirmation of absorption were the limiting factors.

Recently, we studied the function of such obtained bioengineered intestinal segment transplanting on the rats' proximal jejunum a Surgisis scaffold. Besides performing a detailed anatomic and functional evaluation, we measured the absorptive function of this neo intestine *in vivo*. The structural characteristics of the bio artificial intestinal segment was comparable to normal intestine while we also observed brush border development with preserved microvilli as well as the presence of water and ion transporter/channels. In order to unequivocally demonstrate absorption, the animals underwent to a laparotomy after 12 wk from the primary surgery. Upon isolated of the newly formed intestinal segment and its vascular pedicle, we

evaluated the absorption of D-Xylose from that specific surface area alone, which confirmed comparable absorption with normal intestine^[75]. These promising results providing absorptive functional evidence for the first time *in vivo*, offer the basis for investigation of this method in a large animal model and its possible rapid translation into the clinical settings.

FUTURE DIRECTIONS

Through the years, significant improvements have been made in the development of new methods to create neo-formed bioengineered intestinal tissue. In the last few years, we have assisted an increment of interest in the field. At this time, most of the proposed models described in the literature present several limitations to translate into human. The main limitations are due to the complexity of some models. For example, the need to perform multiple surgeries to re-implant in continuity with the intestine preformed omental organoids. Moreover, many of the methods described are still rudimental and do not offer a complete structure that can be used in a clinical application. Even more limiting, most methods do not offer evidence of *in vivo* absorptive function. We believe that constitute a minimum and fundamental requirement to embark in using any neo-formed bioengineered intestinal structure in a clinical setting to treat intestinal failure. On these bases, we believe that the simpler model that we have described and proven functional *in vivo* utilizing an acellular biologic scaffold placed immediately in continuity with the short intestinal segment appears to be more promising to translate into clinical application for patients with intestinal failure. With these new approaches, if proven successful in a preclinical model, a breakthrough could take place in development of bio-artificial organs.

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High-risk corneal allografts: A therapeutic challenge

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Abstract

Corneal transplantation is the most common surgical procedure amongst solid organ transplants with a high survival rate of 86% at 1-year post-grafting. This high success rate has been attributed to the immune privilege of the eye. However, mechanisms originally thought to promote immune privilege, such as the lack of antigen presenting cells and vessels in the cornea, are challenged by recent studies. Nevertheless, the immunological and physiological features of the cornea promoting a relatively weak alloimmune response is likely responsible for the high survival rate in "low-risk" settings. Furthermore, although corneal graft survival in "low-risk" recipients is favourable, the prognosis in "high-risk" recipients for corneal graft is poor. In "high-risk" grafts, the process of indirect allorecognition is accelerated by the enhanced innate and adaptive immune responses due to pre-existing inflammation and neovascularization of the host bed. This leads to the irreversible rejection of the allograft and ultimately graft failure. Many therapeutic measures are being tested in pre-clinical and clinical studies to counter the immunological challenge of "high-risk" recipients. Despite the prevailing dogma, recent data suggest that tissue matching together with use of systemic immunosuppression may increase the likelihood of graft acceptance in "high-risk" recipients. However, immunosuppressive drugs are accompanied with intolerance/side effects and toxicity, and therefore, novel cell-based therapies are in development which target host immune cells and restore immune homeostasis without significant side effect of treatment. In addition, developments in regenerative medicine

may be able to solve both important short comings of allotransplantation: (1) graft rejection and ultimate graft failure; and (2) the lack of suitable donor corneas. The advances in technology and research indicate that wider therapeutic choices for patients may be available to address the worldwide problem of corneal blindness in both "low-risk" and "high-risk" hosts.

Key words: "High-risk" grafts; Graft rejection; Systemic immunosuppression; Cell-based immunomodulation; Keratoprosthesis; Collagen-based hydrogels

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Core tip: Corneal grafts enjoy a high acceptance rate when performed in "low-risk" host graft beds. This is associated with a relatively weak alloimmune response. However, in "high-risk" hosts where the immunologically quiescent homeostatic environment of the cornea is compromised prior to graft procedure, heightened immune responses significantly increase the risk of graft rejection. Clinical approaches such as tissue matching and long-term immunosuppression could be beneficial in preventing graft rejection especially in "high-risk" settings. In addition, promotion of transplant tolerance by cell-based therapies and use of corneal "substitutes" such as collagen-based hydrogels are promising alternatives for "high-risk" recipients.

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INTRODUCTION

Corneal transplantation is the most common and successful form of solid organ transplantation^[1]. It is considered the primary treatment to restore vision to patients with corneal blindness - a leading cause of blindness worldwide^[1]. In the year 2014-2015, 3520 cases of corneal transplantation were performed in the United Kingdom compared to 2069 cases of kidney and 842 liver transplantations^[2]. The corneal graft survival rate is 86% at 1-year for penetrating keratoplasty (PK), despite the fact that corneal grafts are rarely tissue matched for histocompatibility leukocyte antigens (HLA) and systemic immunosuppressant medications are not routinely used^[3]. However, the 15-year graft acceptance declines to 55%, which is similar to survival rates in other forms of solid organ transplantation^[3,4]. More importantly, corneal grafts performed in "high-risk" recipients have a much reduced acceptance rate with a 5-year survival of 54.2% compared to 91.3% in recipient eyes that have not been overtly inflamed. The

"high-risk" recipients were defined by the Collaborative Corneal Transplantation Studies Research Group as two or more quadrants of the cornea vascularized or a previous graft had been rejected^[5,6]. Unfortunately, any previous inflammatory response in the ocular surface such as corneal infectious diseases (e.g., herpetic simplex keratitis or trachoma), severe trauma, alkali burn and previously failed graft place the host cornea at risk of corneal neovascularization^[7,8]. Furthermore, "high-risk" recipients not only experience higher graft failure rate but also present with more frequent acute rejection episodes compared to "low-risk" grafts^[7].

It is worth emphasizing here the difference between corneal graft failure and corneal graft rejection. In brief, clinical corneal graft failure is the irreversible loss of graft clarity, and rejection is one of the causes of corneal graft failure. However, the loss of graft clarity can be due to a number of reasons including infection, surgical trauma, glaucoma, aging as well as rejection, which is an exclusively immunological event. Graft rejection is moreover the most common cause of graft failure accounting for over 30% of cases^[3,4]. The characteristic features of corneal graft rejection in which there is an immunological response against donor antigens are graft oedema, keratic precipitates on the endothelium of the transplanted graft and the presence of rejection lines [formed due to accumulation of inflammatory cells on corneal epithelium or endothelium (Khodadoust line)] together with the presence of inflammatory cells in the anterior chamber (AC) of the eye^[9,10]. This review article focuses on the mechanism of corneal graft rejection revealed through experimental studies as well as current and potential treatments for corneal graft rejection.

EXPERIMENTAL CORNEAL ALLOGRAFT

The immunological responses mediating corneal graft rejection have been studied extensively using animal models, and especially in the well-established murine model of full-thickness orthotopic corneal transplantation. Similar to human corneal grafting, murine corneal allografts performed in an uninflamed graft bed, despite being mismatched for both major and minor histocompatibility complex antigens, half of the grafts failed, whereas in the inflamed "high-risk" graft bed, almost all of the grafts failed and with an increased tempo depending on the level of major histocompatibility complex (MHC)/non-MHC antigen mismatch^[11,12].

The rejection mechanism of corneal allograft

Corneal allograft rejection represents a form of delayed-type hypersensitivity (DTH) response, predominantly mediated by allospecific CD4+ T cells. The response can affect one or more of the three cellular layers in the cornea (epithelium, stroma and endothelium)^[13-15]. However, the endothelial layer is

the main target in PK with graft failure occurring when > 50% of the corneal endothelium is lost^[16,17]. As the corneal endothelium possesses limited regenerative property and is the essential layer responsible for maintaining corneal deturgescence, alloimmune responses directed at the corneal endothelium eventually result in stromal and epithelial oedema and with irreversible corneal opacification^[16].

During the surgical procedure, trauma to corneal tissues induces local production of cytokines and chemokines such as interferon (IFN)- γ , interleukin (IL)-1 β , IL-6, IL-10 and CXCL2 which initially peaks at day 3-5 post graft procedure^[18]. Meanwhile, infiltration of innate immune cells occurs into the cornea including dendritic cells (DC), macrophages, natural killer (NK) cells and neutrophils^[19]. A unique feature of corneal allograft compared to other forms of solid organ transplantation is that the rejection response is mediated almost exclusively through the indirect pathway as the healthy central donor cornea possesses low numbers of antigen presenting cell (APC). Therefore, the activation of naïve T cells occurs predominantly through host APC newly recruited from the bone marrow and presenting donor antigenic peptides, including HLA antigens to host naïve T cells. In contrast, the direct pathway involves the direct recognition of alloantigen on donor origin APC which have migrated from the graft tissue to the local draining lymph nodes (DLN), by host naïve T cells^[20,21]. Newly recruited bone marrow APC after processing antigens from the corneal allograft then migrate *via* lymphatic vessels to the DLN where they activate naïve T cells and mediate immune rejection against corneal graft.

Corneal allograft rejection is predominantly mediated through CD4+ Th1 cells that secrete cytokines IFN- γ , tumour necrosis factor (TNF)- α and IL-2^[14,22]. In the rejected graft, abundant neutrophils, macrophages and CD4+ T cells are present^[23]. Furthermore, studies have suggested that CD4+ T cells may function directly as effector cells mediating graft rejection as adoptive transfer of allogeneic CD4+ T cells to beige nude mice (impaired T cell production, but do produce macrophages) resulted in graft rejection even when macrophages were depleted^[24]. Although *in vitro* experiments showed the ability of allo-specific CD4+ T cells to induce apoptosis of corneal endothelial and epithelial cells, investigations of the involvement of perforin or Fas-induced apoptosis by CD4+ T cells have eliminated both mechanisms^[24]. In addition, allografts deficient in Fas-ligand (FasL or CD95L) demonstrated 100% rejection, further indicating that mechanisms other than Fas-FasL were used by CD4+ T cells in mediating graft rejection while FasL expressed in the cornea was more likely to promote immune privilege^[25]. Nevertheless, prolonged exposure to proinflammatory Th1 type cytokines IFN- γ , TNF- α and IL-1 was shown to induce apoptosis of corneal endothelium and upregulation of inducible

nitric oxide synthase, the latter generating nitric oxide which causes direct cytotoxicity to endothelial cells^[26]. In addition, inhibition of inducible nitric oxide synthase showed protection against cytokine-mediated corneal tissue damage as well as prolonged allograft survival when administered systemically^[26,27]. However, studies investigating the role of Th17 cells in mediating corneal allograft rejection have shown controversial results. While some studies showed that IL-17 demonstrated pathological effect during early corneal allograft rejection^[28], recent findings have suggested that Th17 cells are involved in promoting allograft acceptance in the early post graft stages followed by a Th1 dominant response mediating graft rejection^[29,30]. Interestingly, further investigation also indicated that enhanced expression of IL-17 at a late stage (> 45 d) post corneal allograft impaired graft survival. Late stage anti-IL-17 treatment not only reversed corneal opacity but also reduced the level of neovascularization^[30]. Strikingly, IL-17 knockout mice that received anti-IFN- γ treatment failed to reveal any significant difference in graft survival compared to wild type mice. This indicates that mechanisms other than Th1 and Th17 cells were involved, which may be due to the redundancy of the immune system promoting an alternative and exaggerated Th2 response capable of mediating graft damage^[29,31].

Is the success of unmatched corneal allografts due to immune privilege?

The relatively high acceptance of corneal allografts compared to other forms of solid organ transplantation has been largely ascribed to the immune privilege of the eye^[32,33]. Immune privilege was a term coined by Sir Peter Medawar in the 1940s where skin allografts placed in the AC of the eye evaded immunological rejection but only if the graft was not invaded by blood vessels^[34]. Extensive study of this phenomenon ascribed immune privilege especially in the context of corneal allograft to: (1) the reduced expression of MHC class I molecules in corneal tissue and the lack of constitutive MHC class II expression; (2) the absence of both blood and lymphatic vessels in the cornea; (3) the lack of "passenger leukocytes" in the cornea; (4) presence of immunoregulatory molecules in the AC and on corneal cells; and (5) anterior chamber-associated immune deviation (ACAID) induced post corneal allograft^[32,33]. However, recent studies have shown that the corneal tissue possesses a population of MHC II + leukocytes with increased numbers towards the peripheral cornea^[20,35-40]. Furthermore, corneal neovascularization rapidly develops post corneal grafting; within 1 wk, both blood and lymphatic vessels are already invading the donor cornea thus providing access of immune cells to the cornea as well as increasing homing of APC to the DLN. Furthermore, vessels persist regardless of the fate of the graft (Figure 1)^[11]. This means that unmatched corneal allografts

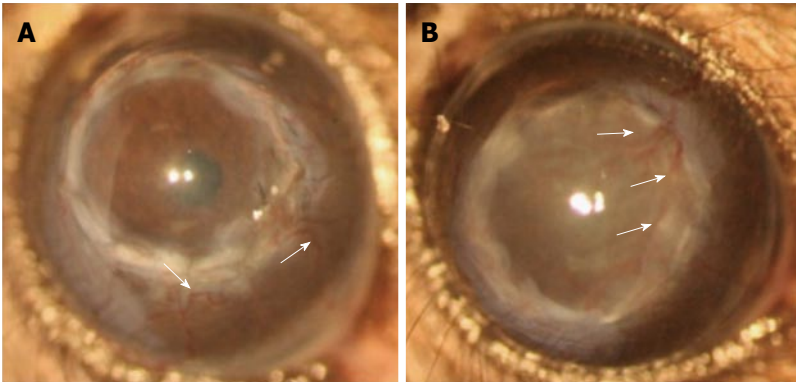


Figure 1 Corneal allografts in C57BL/6 mice. (A) Accepted and (B) rejected corneal allografts (Balb/c donor) in C57BL/6 mice demonstrating invasion of blood vessels (arrows); the rejected graft shows more blood vessels invading the donor graft.

are accepted in 50% cases indefinitely despite the presence of blood and lymphatic vessels and infiltration of host immune cells.

In contrast to immune privilege, which describes the local acceptance of grafts within the eye, ACAID is a systemic immune response. ACAID is an unusual suppression of the systemic immune system whereby alloantigen placed in the AC of the eye elicits a regulatory response in the spleen, which upon further exposure suppresses the immune response to the alloantigen (*e.g.*, skin graft), and prevents graft rejection^[41]. This phenomenon has been shown to be mediated through CD8+ T regulatory cells (Treg) generated in the spleen^[33]. It was believed that ACAID is induced not only when alloantigen is inoculated into the AC but also post corneal allograft due to shedding of alloantigenic materials from graft endothelial cells^[42]. However, growing evidence suggested that Treg induced after corneal allograft show a phenotype of CD4+CD25+Foxp3+ whereas effector Treg in ACAID is CD8+ Treg^[13,43,44]. Furthermore, blockade of CD8+ T cells only abrogated ACAID but with no effect on corneal allograft survival while blockade of IL-17A which reportedly impaired allograft induced Treg suppressive function also reduced corneal graft survival, but did not alter the induction of ACAID^[43,45].

It is clear therefore that most of the proposed mechanisms to explain the phenomenon of immune privilege have proven not to be true. Instead, the prolonged acceptance in "low-risk" corneal allograft compared to other solid organ transplants may simply be due to the effect of an overall weak indirect alloimmune response as a result of the low levels of alloantigen acting together with local and systemic regulatory mechanisms. First, the insufficient strength of the alloimmune response in the initial stages of allosensitization is likely due to the limited number of donor derived passenger leukocytes particularly in the central cornea, and low expression of histocompatibility antigens. In addition, while other forms of solid organ transplants are rich in vascular networks and donor passenger leukocytes undergo both acute (direct

pathway) and chronic (indirect pathway) rejection^[46], corneal allograft rejection is predominantly mediated through the indirect pathway^[47-50]. In the healthy cornea, the majority of MHC II + cells are CD11b+ and CD11c+ cells distributed at the peripheral cornea whereas the central cornea which is used as donor cornea during corneal allograft procedure was believed to be devoid of MHC II + cells but contains a population of MHC class II negative immature DC and Langerhans cells^[20,36-39]. Recently, studies using CD11c-eGFP mice have shown that a reduced number of MHC II +CD11c+ cells are present in the central cornea and exclusively located in the corneal epithelial basal layer beneath which a layer of MHC II +CD11b+ cells were also observed^[40]. However, the expression level of MHC class II molecules on these cells was found to be at a relatively low level indicating that these cells together with MHC class II negative DC and Langerhans cells are more likely to promote immune tolerance rather than immunity^[40]. We reported that in a "low-risk" setting, there was no evidence of donor leukocyte migration to the DLN^[20]. Therefore, corneal allograft rejection in "low-risk" setting is exclusively mediated by indirect allorecognition. The lack of both blood and lymphatic vessels in initial stages post graft may delay the infiltration of host innate immune cells including APC, thus becoming a limiting factor for initiating a sufficient rejection response before the development of an established vessel network. Second, while new vessels invade the graft, other regulatory mechanisms including the induction of Treg come into play. It was found that rather than changes in frequency, the expression level of Foxp3 was significantly higher in the DLN of accepted allografts compare to either rejected or syngeneic grafts^[44]. Moreover, adoptive transfer of Treg has been shown to promote corneal graft survival^[51], associated with production of IFN- γ and IL-17A^[45,52]. It was shown that IL-17A is required for the effective suppressive function of Treg in promoting allograft survival and unusually supports a protective role for Th17 cells during corneal allograft rejection^[45]. Interestingly, IFN- γ was required for generation of Treg

under fully MHC and minor histocompatibility antigen mismatched condition, whereas IFN- γ inhibited the generation of allospecific Treg when only MHC or minor histocompatibility antigen was mismatched^[52]. These somewhat puzzling findings suggest that possibly the balance between Th1, Th17 and Treg responses largely dictates the outcome of the graft. Consequently, when an effective peripheral tolerance response fails to be induced, the default balance favours a Th1 response and as such, promotes allograft rejection.

Lastly, the physiological milieu of the cornea and the anterior segment of the eye possess many immunoregulatory molecules that protect the cornea from immune mediated attack. For instance, FasL is expressed extensively in ocular compartments including all three cellular layers of the cornea^[53,54]. Several studies have reported that FasL expressed in the eye is responsible for inducing apoptosis of infiltrating Fas-bearing leukocytes, especially lymphocytes. Furthermore, its expression in particular on corneal endothelial cells plays an important role in corneal allograft survival, since donor corneas lacking FasL in the endothelium and stroma but not epithelium were rejected vigorously compared to normal FasL expressing donor corneas^[25,53,55,56]. Moreover, the interaction of Fas-FasL induced apoptotic cell death was shown to be an important mechanism in the induction of immunological tolerance to antigens injected into the AC, as in the absence of apoptotic cell death, immune tolerance failed to be elicited^[55]. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is also capable of inducing apoptosis of various tumour cells and its functional expression was demonstrated in corneal tissue^[57]. Overexpression of TRAIL in donor corneal tissue has been shown to significantly delay graft rejection, accompanied by an increased number of apoptotic cells in the graft^[58]. However, other groups in attempts to establish a correlation between TRAIL expression and allograft survival have not found an effect^[13].

Programmed death ligand-1 (PD-L1 or B7-H1) is another molecule with similar functions to FasL and TRAIL by promoting apoptosis of infiltrating PD-1 positive CD4 and CD8 T lymphocytes^[59]. PD-L1 belongs to the B7 superfamily providing costimulatory signals to T cells and is constitutively expressed in both murine and human corneal tissues^[59-61]. Its blockade or deficiency is associated with increased corneal graft rejection whereas strong ligation between PD-L1 and PD-1 revealed prolonged allograft survival^[59-62].

Complement regulatory proteins were found to be expressed by corneal tissues and in the AC, which protects the cornea from being the target of complement-fixing antibodies^[63,64]. One such molecule strongly expressed in the corneal epithelium is decay-accelerating factor (DAF) which function is to inhibit complement deposition on the cell surface, thus preventing autologous complement activation^[63,65]. Further studies

have suggested that DAF shows regulatory properties towards the T cell response^[66]. DAF deficiency on donor or recipient cornea accelerated graft rejection together with increased numbers of IFN- γ producing T cells, reduced levels of transforming growth factor (TGF)- β and IL-10^[66]. Furthermore, NK cells attack cells that lack the expression of MHC class I molecules and the poor expression of MHC class I by corneal endothelial cells makes them prone to NK cells mediated tissue damage^[13,67]. However, studies have shown that the AC contains NK cell inhibitory factors such as macrophage migration inhibitory factor and TGF- β , which prevent corneal endothelial cells becoming targets for NK cells^[13,68,69]. Galectin-9 was demonstrated as another immunosuppressive molecule constitutively expressed on corneal tissues, which significantly promoted corneal allograft survival by inducing apoptosis of alloreactive T cells^[70].

Many other immunoregulatory molecules present in the anterior segment of the eye have also been demonstrated to have potential in prolonging corneal allograft survival including alpha-melanocyte stimulating hormone, calcitonin gene-related peptide, vasointestinal peptide, somatostatin or indoleamine dioxygenase^[71-73].

Elevated innate and adaptive immune responses in "high-risk" corneal allograft promote graft rejection

Although clinically and experimentally, there are many causes of a "high-risk" graft bed, a common denominator is an already activated immune system both systemically and locally (cornea and eye-DLN) providing a proinflammatory milieu unlike the situation in "low-risk" dormant recipients. In general, murine corneal allografts performed in "high-risk" recipients not only experience over 95% graft rejection rates compared to 50% in "low-risk" recipients, but in addition grafts are usually rejected rapidly, 2 wk post-surgery compared to 3-4 wk in uninflamed corneas^[12]. As early as 24 h post corneal allograft, increased levels of chemokine mRNA expression including CCL2 and CXCL2 were observed in "high-risk" recipients compared to "low-risk" recipients^[74]. No difference in the number of infiltrating leukocytes was observed between "high-risk" and "low-risk" recipients at day 1 suggesting the source of the early increased chemokine levels was from resident corneal cells^[74]. Increased numbers of infiltrating macrophages and neutrophils in "high-risk" recipients were found at day 3 recruited by CCL2 and CXCL2 which leads to a dramatic increase in chemokine levels in the "high-risk" group at day 6 post graft with a broader spectrum of chemokines including CCL2-CCL5, CCL11, CXCL2 and to a lesser extent CXCL10^[74]. Furthermore, the local proinflammatory environment in "high-risk" recipients post-surgery contains high levels of vascular adhesion molecules further increasing the recruitment of both innate immune cells and memory T cells to the cornea^[75].

Accordingly, the increased levels of innate leukocytes especially macrophages and DC which serve as APC together with pre-existing vascularization significantly increases the number of APC reaching the DLN within a shorter period compared to "low-risk" recipients. In addition, although the presence of donor APC in the DLN as well as their ability to upregulate expression of MHC class II post "high-risk" allograft were reported in several studies, it remains controversial whether direct pathway-activated allospecific T cells play a role in mediating corneal allograft rejection^[76] or rather promotes tolerance to the allograft^[77]. Depletion of leukocytes from donor corneas prior to "high-risk" corneal allograft as well as using CCR7^{-/-} donor corneas failed to demonstrate a significant difference in allograft survival^[77,78]. Thus, these studies indicate that the frequency of donor APC is unlikely to be sufficient to mediate significant acute graft rejection through direct antigen presentation during corneal allograft rejection. Therefore, it remains likely that the heightened innate immune responses leading to increased infiltration of host APC presenting alloantigen to host T cells is (indirect pathway) responsible for the increased rejection of "high-risk" grafts, as well as "low-risk" grafts as described in previous sections.

Neovascularization is the common feature that distinguishes "high-risk" and "low-risk" host graft beds. In "high-risk" corneal allografts, despite vascularization of the cornea prior to the graft procedure, further vascularization is also induced after grafting^[79]. Lymphatic vessels in the cornea act as conduits for efferent migration of APC to DLN while blood vessels provide afferent access of inflammatory leukocytes to the cornea; infiltrating leukocytes then act as a further source of pro-angiogenic factors. Studies have shown that inhibition of either blood or lymphatic vessels was able to significantly prolong graft survival comparable to "low-risk" recipients suggesting that either disruption of efferent or afferent access of leukocytes can suppress alloimmune responses^[80-82]. Furthermore, although the definition of "high-risk" recipients included corneas with two or more quadrants with evidence of vascularization, clinically the incidence of graft rejection has been shown to increase with increased levels of vascularization present prior to the corneal graft procedure^[83], further suggesting that increased corneal vascularization shifted the balance towards immune rejection.

The adaptive immune response was also shown to be elevated in various ways among "high-risk" recipients. One of the consequences of an increased innate immune response is the increased number of APC with the ability to activate naïve T cells. Indeed, the DTH response in "high-risk" recipients was found significantly accelerated compared to "low-risk" recipients^[12,47]. Furthermore, the allograft was rejected promptly if the recipient had been previously sensitized with a previous corneal graft or skin graft^[84]. It was clearly shown that in "high-risk" recipients

which previously experienced graft rejection, the effector/memory T cell response promoted accelerated rejection of regrant of the same donor origin^[85]. It is also possible that memory T cells due to a previous infectious disease of the cornea such as herpes keratitis becomes activated by bystander mechanisms, when a subsequent corneal graft procedure is performed (Kuffova *et al*, in press). Thus, two types of increased adaptive immune responses are present in "high-risk" recipients to promote graft rejection, namely, enhanced activation of allospecific T cells as well as reactivation of memory T cells due to previous immune mediated conditions of the cornea such as infection or previous graft.

PREVENTION OF ALLOGRAFT REJECTION

Tissue matching - controversies and justifications

Tissue matching is not routinely performed clinically for patients undergoing corneal transplantation due to its remarkable success rate in "low-risk" recipients^[3,86,87]. However, the markedly poorer prognosis of "high-risk" grafts suggests this should be reconsidered, although, the controversy has not been resolved^[6,7,88]. Some of the studies addressing this issue are reviewed below: In clinical practice, matching for HLA class I antigens under "low-risk" and HLA class II antigens under "high-risk" conditions have both been shown to significantly reduce the risk of rejection^[89,90]. In a pre-clinical model, minor H antigen incompatibility has been shown to have higher rates of rejection even in "low-risk" grafts than MHC mismatches, and similarly, improvement in prognosis of "high-risk" grafts were demonstrated in a clinical study as well, when matched for minor H antigens^[91,92]. Differences in donor-recipient blood groups may also contribute to graft rejection in "high-risk" recipients as ABO antigens are expressed in the corneal epithelium and endothelium^[93]. ABO and Rh \pm incompatibility were shown to have a significant influence on corneal allograft rejection in earlier clinical studies^[6,94], but recently, no influence in allograft failure due to immune rejection was shown in a 5-year follow up clinical study in "low-risk" corneal transplants. However, conflicting results were reported in "high-risk" cases^[93,95]. The major reasons for differences in success rates of allografts in humans are thought to be due to surgical techniques, competency of surgeons and properly distinguished risk factors associated with graft bed^[96]. Furthermore, a recent review identified the lack of specificity and low sensitivity in tissue typing methods compromise the quality of HLA matching in different centres performing clinical studies^[97].

A possible reason behind the high success rates of acceptance of corneal allograft in "low-risk" recipients without tissue matching is, regardless of the technical factors discussed above, the relative

weakness of the alloimmune response (as discussed above), which is relatively easily controlled with daily application of topical steroidal drops. This concept is supported by the observation that more frequent graft rejection “episodes” and eventual graft failure develop after topical steroids are discontinued in “low-risk” graft recipients (e.g., after first year post corneal transplantation)^[98-100].

The shortage of donor corneas worldwide, the high demand and the long wait time for the “right” donor match restricts the wider application of corneal grafts, while on some occasions, it has to be performed as an emergency procedure with high risk of failure^[101,102]. As the immunological events behind the “high-risk” grafts lead inevitably to irreversible graft failure, a treatment protocol is currently being developed which will assess and compare the HLA matching along with longer wait time for the surgery, but may be associated with more favourable graft survival outcome especially in “high-risk” graft recipients^[101].

Support for tissue matching comes from experimental studies using a “high-risk” regrant model, with single antigen disparity, in which antigen-specific memory T cell activation was directly correlated with accelerated graft rejection. Thus matching is advised to prevent risk of rejection by ensuring that a donor regrant has no or minimal concordance with the original graft^[85].

Use of immunosuppressive agents

Generally, for “low-risk” patients, treatment with topical steroids will prevent rejection as indicated above. Daily application of steroid drops plays a major role in local control of the host immune system by preventing the invasion of IL-1 and IL-6 producing macrophages and subsequent initiation of adaptive T cell responses^[103]. However, topical steroids alone are not sufficient in preventing rejection in “high-risk” recipients due to much stronger immune response generated by unfavourable microenvironment of the graft bed^[103]. Though clinical studies have shown improvement of graft outcome by administering systemic (oral) steroids, steroid treatment alone is not advised in the long-term due to side effects^[104-106]. Further studies have shown that use of systemic immunosuppressive therapy with either cyclosporine A (CsA) or mycophenolate mofetil (MMF) is successful in preventing corneal allograft rejection, but MMF has shown greater success than CsA^[104,107-109]. Intraocular delivery of immunosuppressants has been shown to prevent “high-risk” graft rejection in rabbits while topical treatment did not show any significant effect^[110,111].

Biologics, the novel immunosuppressive agents, comprised mainly of recombinant antibodies and fusion proteins, bind to receptors and block immune cells; similarly inhibitors of mediators of corneal inflammation and vascularization like IL-2 receptor (IL-2R), TNF- α , vascular endothelial growth factor (VEGF)

and CCL2, all of which are involved in allograft rejection may be effective^[112]. Local anti-VEGF treatment is a proficient strategy to reduce corneal angiogenesis and lymphangiogenesis and this may reduce the incidence of rejection especially in “high-risk” recipients^[113-116]. Some biologics like anti-VEGF, anti-TNF- α or anti-IL-2R are already in use to inhibit “high-risk” graft rejection while potent blockers of TNF receptors are currently being evaluated in clinical trials^[112].

Corneal allograft survival would be greatly improved if, in addition to tissue matching and topical steroids, an appropriate low dose immunosuppressant was also used^[98]. However, alternative therapies should also be considered as discussed below.

PROMOTION OF IMMUNOLOGICAL TOLERANCE - CELL-BASED THERAPIES

Currently, cell-based therapies such as stem cells, tolerogenic DC or Treg are proposed as alternative treatments especially for “high-risk” corneal grafts and they function by promoting immune tolerance.

Stem cells

Stem cells are undifferentiated cells which give rise to two daughter cells comprising one self-renewing and one differentiating progenitor generated by asymmetric cell division^[117]. Stem cells include embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) and mesenchymal stem cells (MSC) and they have been investigated as a therapeutic strategy in promoting transplant tolerance^[118] and in ocular surface reconstruction^[119].

ESC and iPSC: The most fascinating breakthrough of the last decade is the generation of iPSC from adult somatic cells. This is a novel method of generating stem cell which ensures a continuous supply of self-renewing PSC. The process of reprogramming somatic cells *ex vivo* by transmitting the signalling cues through four well-defined transcription factors such as Oct3/4, Sox2, c-Myc, and Klf4 has opened the way for a wide range of clinical applications^[120,121]. Like ESC, iPSC are also capable of trans-differentiating into cells of different lineages. Several *in vitro*, *in vivo* studies and even phase I clinical trials were initiated using ESC and iPSC to treat sequelae of sight threatening intraocular inflammation or retinal degenerative diseases^[122-126].

In the context of corneal reconstruction and repair, *in vitro* studies have shown the feasibility of differentiating ESC and iPSC into corneal epithelial, keratocytes and endothelial cells individually as an option to treat corneal scarring, stromal opacity and malfunctioned endothelial cells^[127-130]. Furthermore, *ex vivo* transplantation of ESC derived cells onto partially de-epithelialized cornea led to regeneration of normal stratified layers of the corneal

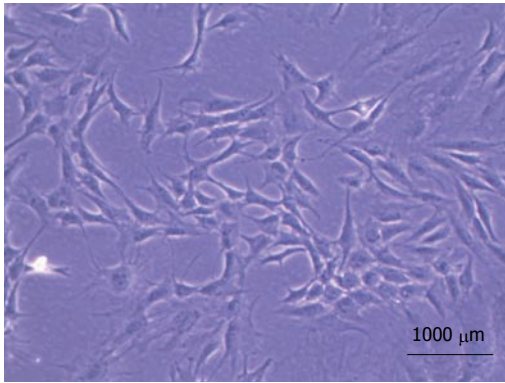


Figure 2 Spindle shaped morphology characteristic of multipotent mesenchymal stem cells. Figure shows passage 4 mesenchymal stem cells derived from the non-haematopoietic sub-population of bone marrow harvested from 6-8 wk old Balb/c mice.

epithelium^[131]. iPSC are able to differentiate into limbal stem cells (LSC) *in vitro*, confirmed by expression of LSC markers ABCG-2 and p63 α at both cellular and molecular levels^[119]. The successful engraftment of a differentiated LSC-seeded scaffold demonstrated significant reconstruction of the ocular surface with functional re-epithelization, minimal corneal scars and corneal vascularization in an experimental model of alkali burn in rabbits^[132]. Hence, PSC could potentially be used to replace damaged LSC which is a characteristic feature found in many "high-risk" ocular pathologies^[119,132].

Though there is much to be explored, the therapeutic impact of PSC is remarkable. The advantages of PSC are they do not induce allogeneity and related immune rejection^[126]. However, problems with insufficient supply of cells as well as the possibility of differentiating into the malignant cells still remain^[133,134].

LSC: LSC play a vital role in maintaining corneal integrity and renewal of epithelial cells. The limbus, reservoir of LSC, is responsible for homeostasis of the corneal epithelium^[135,136]. Damage to LSC occurs during severe burns, injury or infection to the ocular surface and results in a "high-risk" cornea with limbal stem cell deficiency (LSCD) features such as chronic inflammation, severe corneal vascularization, persistent epithelial defects, conjunctivalization of the cornea and increased risk of corneal perforation^[137].

Autologous transplantation of limbal epithelial sheets is considered a long-term effective clinical solution for unilateral corneal stem cell deficiency; and for bilateral deficiency, LSC from deceased donors is a possible option but raises the problems of matching and increased chance of rejection^[138-140]. In addition, autologous limbal transplantation was shown to be performed in a 2 step approach, with PK performed at a later date. However, the outcome of these procedures were not satisfactory in bilaterally

deficient patients with severe ocular damage^[139,140]. Nevertheless, a large clinical study reported that autologous LSC transplantation was effective even in "high-risk" patients post alkali burn or with previously failed corneal graft where the outcome was restoration of a stable ocular surface and vision^[141].

Currently, LSC therapy is a promising strategy clinically to improve the chance of normalization of ocular surface and later acceptance of "high-risk" corneal grafts^[142,143]. However, there are still considerable obstacles to overcome such as methods to isolate/prepare cells, expand the cells in culture and avoiding damaging cells due to the surgical procedure and immune reaction. As such, the procedure is limited to clinics that have a specialized laboratory for cell expansion, operating at a level conforming to guidelines for good manufacturing practice. A new simpler method that has been recently developed, termed simple limbal epithelial transplantation combines existing know-how but allows for the entire grafting procedure to be performed in the operating room^[144].

MSC: MSC are multipotent stem cells mainly isolated from bone marrow amongst other sources^[145-151] (Figure 2). These cells are being tested currently in repairing tissue defects by attenuating scar formation and in immunomodulation^[152]. MSC have the capability of differentiating into cells of mesenchymal and non-mesenchymal origin induced by paracrine and autocrine signals according to the local microenvironment^[153]. Several *in vitro* studies have shown MSC capable of reducing T cell immune responses by promoting the activation of Treg and production of IL-10, TGF- β , prostaglandin E2 and thrombospondin-1^[154,155]. Likewise, *in vivo* studies of different solid organ transplantation models also suggested significant reduction of adaptive immune response and promotion of immune tolerance in the presence of MSC^[156-159].

Initial studies demonstrated that MSC are promising candidates to treat corneal blindness by restoring corneal transparency in a congenital keratocyte dysfunction model^[160] and differentiating into keratocytes in corneal stroma, thereby facilitating tissue repair^[161]. Based on these studies, MSC therapy has been promoted in many acquired corneal disease and injury models. Recent studies have shown that systemic injection of MSC prolonged corneal allograft survival by homing into the inflamed graft site and DLN and suppressing APC function thus inhibiting allosensitization^[162-165]. Local administration of MSC was also able to induce anti-inflammatory and anti-angiogenic effects and prevent LSCD in models of acute alkali burn^[166,167].

Despite relative scarcity and difficulties with isolation and expansion, MSC are safer than PSC for treatment in pre-clinical studies as no adverse effects such as a tumour formation (teratoma), have so far been observed^[168].

Immune cell therapy: Dendritic cells and T regulatory cells

DC possess both immunogenic and tolerogenic functions^[169]. Activated mature immunogenic DC have been used in cancer immunotherapy for more than a decade and found to be efficacious. In this setting, DC are used as natural adjuvants carrying tumour specific peptides and induce antigen specific T cells in the DLN with subsequent tumour lysis^[170,171]. DC based immunotherapy can also be used as vaccination to protect against tumours by promoting tumour antigen specific immunity and prevent cancer recurrence^[172,173].

However, in contrast to their immunogenicity when activated, DC mainly maintain immune homeostasis by immune regulatory action against self-antigen specific T effector cells and so prevent autoimmunity^[174]. This tolerogenic feature of DC presents them as a possible candidate for treatment in autoimmune disease and allograft rejection^[175]. Phenotypically immature DC remain tolerogenic as they fail to deliver an adequate costimulatory signal required for specific T cell activation. These non-activated or partially activated T cells undergo optimally low proliferation, cell death, anergy or develop the phenotype of Treg^[176,177]. *In vitro* manipulation of DC by exposing them to an antigen at a sub-optimal level or treating them with anti-inflammatory cytokines such as IL-10 and TGF- β leads to alternatively activated DC which are poor stimulators of the alloimmune response but promote immune tolerance^[174,176]. The *in vitro* manipulated immature DC have been shown to impair CD4+ effector T cell induction and enrich CD4+CD25+Foxp3+ Treg by inducing hyporesponsiveness of the DC to the antigenic stimuli through toll-like receptors^[178].

This phenomenon of inducing or restoring tolerance by DC therapy has been applied in transplantation models in an attempt to enhance allograft survival^[179]. A number of pre-clinical studies on rodents and non-human primate transplantation models have shown long-term survival and function of allograft by administering *ex vivo* manipulated DC^[175,177,180]. The efficacy of donor derived DC based therapy was tested in a pre-clinical "high-risk" corneal transplantation model and was reportedly effective by significant reduction in IFN- γ and increased production of Foxp3+ Treg^[181,182].

Treg are crucial in maintaining self-tolerance and their absence leads to autoimmune diseases^[183,184]. The *in vitro* generation, phenotype and immunosuppressive function of Treg have been reviewed in detail previously^[185]. *In vitro* manipulated donor-derived CD8+Foxp3+ Treg were infused and found to induce CD4+CD25+Foxp3+ to provide donor specific tolerance to allografts and protect from aggressive host immune rejection in a fully mismatched skin graft murine model^[186]. Similarly, production of Treg is critical for the survival of corneal allografts^[44] (as discussed above) and interestingly, even the local administration of naïve Treg prolongs corneal allograft

survival in infant rats^[187].

DC and Treg are recognised as promising candidates for the clinical application of immunosuppressive therapy to promote corneal graft survival. It has been demonstrated that autologous DC are safe with no toxic or immunogenic effects^[188,189] while graft versus host disease (GVHD) was not observed when allogeneic cells were used^[173,190]. Instead, they were shown to inhibit GVHD after bone marrow transplantation in pre-clinical and clinical studies of leukemia^[173,190]. Though already in clinical trials, efficient isolation without manipulation of their phenotype and function is still under development for potential application, especially in "high-risk" grafts.

ALTERNATIVES TO NORMAL CORNEAL TISSUE - ARTIFICIAL CORNEAS

The use of artificial corneas is an exciting option, which would overcome the problems with shortage of donors and frequent graft rejection in "high-risk" hosts^[191,192]. Two approaches have been used to replace the damaged corneal tissue so far: (1) keratoprosthesis; and (2) bioengineered scaffolds that serve as templates for promoting corneal regeneration^[193].

Keratoprosthesis

Keratoprostheses are synthetically generated corneas made of artificial materials which are not fully biocompatible and "only" provide central vision, yet are a viable option for patients who are at the end stage of severe corneal disease where grafting a donor cornea is almost certain to fail^[194-196]. The Boston Keratoprosthesis (BKPro) is the most commonly used artificial cornea in clinical practice. Though the device is made of synthetic material, a donor cornea still has to be used as the carrier of the central optical device^[197,198]. Patients with "high-risk" herpetic keratitis transplanted with BKPro were shown to have better outcomes than transplanted allografts only^[199]. Nevertheless, several postoperative complications including keratolysis (corneal melt), tissue necrosis which may result in corneal perforation in both host and donor cornea, and retro-prosthetic membrane formation have been reported^[197,200,201]. In addition, lack of bio-integration of the prosthesis seems to be the major reason for BKPro extrusion, instability and ultimate failure^[195,197]. The other type of prosthesis known as the osteo-odonto-keratoprosthesis (OOKP) was designed with an autologous tooth that forms the frame for central transparent optical cylinder^[196]. This is a complicated procedure, and an end stage choice for patients with severe dry eye disease. Retro-prosthetic membrane is not a significant complication in OOKP unlike BKPro^[202] but, the osteo-dental lamina resorption is a specific problem of OOKP as it compromises integrity of the eye^[202] while glaucoma and retinal detachment are the secondary complications of both types^[203].

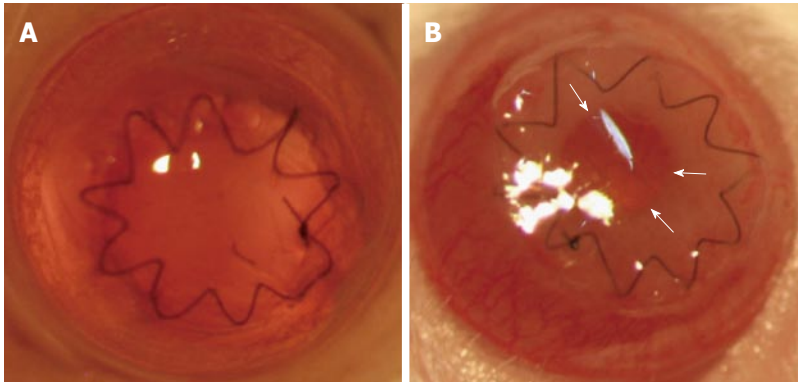


Figure 3 Clinical images of tissue engineered collagen-based hydrogels transplanted by full-thickness keratoplasty into naïve Balb/c mice at different time points post grafting. A: Clear hydrogel 1 d post transplantation; B: Hydrogel clarity is reduced 9 d post transplantation due to retro-hydrogel membrane formation (from periphery towards central cornea as indicated by arrows).

The persisting problem of stable integration of corneal implants with host and implant extrusion may be better addressed by developing tissue engineered biomimetic collagen-based corneal equivalents as discussed below.

Bioengineered corneal equivalents

Bioengineered equivalents of the corneal stromal extracellular matrix have also been tested clinically. These biosynthetic implants are based on chemically crosslinked collagen designed as regeneration templates^[204-206].

Pre-clinical studies were performed in a murine full-thickness orthotopic corneal transplantation model using porcine collagen and recombinant human collagen (RHC) (Figure 3), the latter of which, by using fully biologically synthetic material, reduces the risk of transmission of disease across species as well as reducing the chance of inducing adaptive immune responses^[207,208]. Studies show a strong local innate immune response associated with excessive fibrin production and deposition in the AC. This may represent an exaggerated tissue repair/wound healing response^[207]. Interestingly, only minimal or no activation of APC or CD4+ and CD8+ T lymphocytes in eye-DLN as well as a minimal systemic humoral response was detected^[204,207]. Thus, the main problem seems to be the generation of a retro-hydrogel membrane (Figure 3, arrows), which ultimately reduces the clarity of the graft. Surprisingly, neither an immune response to the hydrogel nor retro-hydrogel membrane formation was detected in a guinea pig model of PK^[209]. Additionally, regeneration of endogenous corneal layers and functional corneal nerves were also determined in the collagen matrix^[209]. Similar findings were demonstrated when the structurally reinforced collagen-based hydrogels were transplanted in a "high-risk" graft model of ocular alkali burn in rabbits^[210]. Furthermore, additional advancements were made in the fabrication of biomimetic, acellular, corneal implants by incorporating biocompatible silica (SiO₂) nanoparticle (NP) carriers

for sustained release of anti-viral drugs such as acyclovir and LL-37 for use in "high-risk" grafts due to herpetic keratitis to prevent re-activation/re-infection of virus and this was supported by low viral copy numbers in *in vitro* experiments^[211,212].

Hydrogel implants have also had their premiere in clinical medicine. A phase I human clinical study using the biosynthetically designed corneal hydrogel substitutes made of RHC which were shown to mirror the natural cornea structurally, mechanistically and functionally by promoting active regeneration of endogenous corneal epithelial and stromal cells has been reported^[213]. In addition, recent outcomes of the 4-year follow-up clinical study show high acceptance/adaptation of the hydrogel to the ocular surface with improved visual acuity and sensory nerve ingrowth^[214]. A most recent clinical observation (case report) in three patients with severe corneal ulcers and recurrent erosions suggests that RHCIII hydrogels reinforced with phosphorylcholine polymer networks potentially withstand the "high-risk" environment (Figure 4) and is a safe and efficient alternative to donor corneal allografts in emergency situations where a corneal allograft is not available, as the corneal integrity can be well maintained in recipients^[215].

Instead of fully *in vitro* generated hydrogel matrixes, decellularized corneas have also been tested in a clinical study^[216]. This study showed promising clinical results in "high-risk" fungal keratitis patients where the implanted decellularized porcine corneas caused regression of corneal vascularization and improved corneal clarity. Although no safety problems were demonstrated, immunogenicity still could be a problem and so further studies addressing this issue may be required^[216].

Thus, bioengineered collagen-based corneal equivalents have shown to be a promising alternative to keratoprosthesis. Though collagen hydrogels show promise in the clinic, this applies mainly to lamellar keratoplasty, which is a partial thickness replacement of damaged cornea, where host endothelium is intact. Thus, the complications observed in experimental

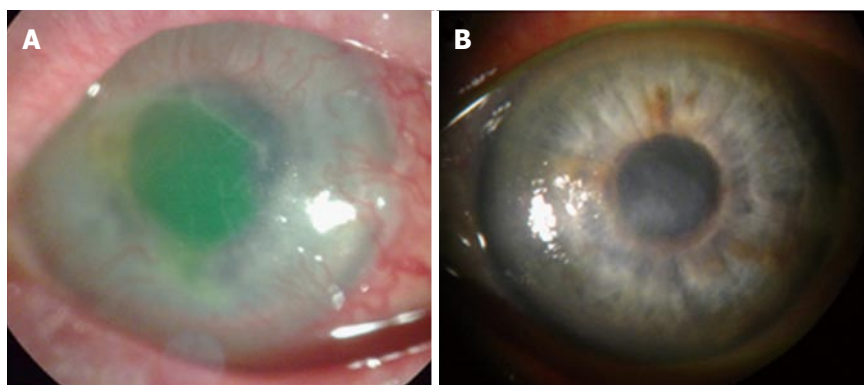


Figure 4 Clinical images of a “high-risk” cornea from a patient with ocular surface disease transplanted with a recombinant human collagen-based hydrogel. A: Corneal graft bed showing fluorescein stained epithelial erosion/ulcer (green/yellow staining) and vascularization before transplantation; B: Relatively clear cornea with clinically visible regression of peripheral corneal vessels 12 mo post-surgery^[215].

models - fibrin deposition and retro-hydrogel membranes formation are eliminated as the integrity of the anterior segment microenvironment is preserved. For PK, the “holy grail” of full-thickness artificial cornea remains the ultimate aim of current research.

CONCLUSION AND FUTURE DIRECTIONS

In full-thickness corneal transplantation in “low-risk” settings - the balance between the strength of alloimmune response and regulatory mechanisms dictates the outcome of the graft, whereas in “high-risk” settings heightened innate and adaptive immune responses significantly tilt the balance to favour graft rejection. Though highly debated, tissue matching with long-term immunosuppression is recommended to reduce the rejection of “high-risk” grafts. Meanwhile, alternative approaches are being explored to avoid the side effects of prolonged use of systemic immunosuppressants. Such approaches including cell-based therapies and development of collagen-based corneal equivalents appear to be promising. Research continues to refine the available therapies for the betterment of the clinical outcomes. The recent surgical advances made in endothelial and stromal lamellar keratoplasty would be a potential realistic option to increase the success rates of some “high-risk” grafts. Manipulation of immunomodulatory molecules like TGF- β and IL-10 in the donor corneal layers by gene therapy might facilitate weakening the aggravated host immune response in “high-risk” grafts. The combined approach of cell or gene therapy along with allograft transplantation might render a better preventive measure for “high-risk” corneal graft rejection.

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Animals in Vision and Ophthalmic Research and Animal License Act (United Kingdom).

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Proteomics for rejection diagnosis in renal transplant patients: Where are we now?

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Abstract

Rejection is one of the key factors that determine the long-term allograft function and survival in renal transplant patients. Reliable and timely diagnosis is important to treat rejection as early as possible. Allograft biopsies are not suitable for continuous monitoring of rejection. Thus, there is an unmet need for non-invasive methods to diagnose acute and chronic rejection. Proteomics in urine and blood samples has been explored for this purpose in 29 studies conducted since 2003. This review describes the different proteomic approaches and summarizes the results from the studies that examined proteomics for the rejection diagnoses. The potential limitations and open questions in establishing proteomic markers for rejection are discussed, including ongoing trials and future challenges to this topic.

Key words: Kidney transplantation; Acute rejection; Chronic rejection; T cell-mediated rejection; Antibody-mediated rejection; Long-term outcome; Graft failure; Biopsy; Non-invasive markers; Proteome; Proteomics; Mass spectrometry; Diagnostic marker; Study design; Diagnostic trial

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Core tip: Timely detection and treatment of acute and chronic rejection is important to maintain the allograft function in renal transplant patients. Allograft biopsies are unsuitable for continuous monitoring for rejection. This review summarizes the past experience with proteomic approaches to diagnose rejection non-invasively. Potential limitations and open questions

in establishing proteomic markers for rejection are discussed, including ongoing trials and future challenges to this topic.

Gwinner W, Metzger J, Husi H, Marx D. Proteomics for rejection diagnosis in renal transplant patients: Where are we now? *World J Transplant* 2016; 6(1): 28-41 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/28.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.28>

INTRODUCTION

Since 2003, proteomics in blood and urine has been explored for non-invasive rejection diagnosis in renal transplant patients. In this review, we summarize and discuss the approaches and results of previous proteomic studies on the background of the heterogeneous and complex condition "allograft rejection". Ongoing studies on this topic are reported and future challenges in establishing proteomic markers for rejection are discussed.

IMPORTANCE OF REJECTION FOR THE LONG-TERM ALLOGRAFT OUTCOME

Despite all improvements in immunosuppressive protocols and patient surveillance after kidney transplantation, allograft rejection remains a significant adverse factor for the long-term allograft survival. In a previous study, both T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) were reported as leading causes of graft failure in a substantial proportion of patients^[1]. Acute TCMR is most prevalent in the first year after transplantation and has been suggested as a trigger for subsequent development of ABMR^[2]. ABMR often evolves over prolonged time and may become chronic, with appearance of donor-specific antibodies first, followed by acute injury of peritubular and glomerular capillaries which in the later course leads to transplant glomerulopathy and tubulointerstitial scarring^[3]. Some patients may also present with concomitant findings of TCMR and ABMR (*i.e.*, mixed rejection)^[4]. Consequently, early recognition of rejection is important during the entire post-transplant course on a continuous basis to treat the rejection timely and to adjust the maintenance immunosuppression in order to prevent further rejection episodes and chronification of the rejection.

Monitoring for rejection is a challenge and has not been satisfactorily solved. Regular measurement of serum creatinine or cystatin C to detect declining allograft function (which then triggers an allograft biopsy) is insensitive and is a late indicator when tissue injury has already taken place^[5]. Some patients may present with increased proteinuria but similar to declining graft function, this can only indicate

established injury and is non-specific as to the cause of injury^[6]. In the case of ABMR, monitoring for donor specific antibodies may identify patients at risk; however, in our experience full-blown histopathologic features of ABMR can be present without detectable antibodies using currently available assays. Many transplant centres have turned to protocol biopsies to evaluate the course of the allograft. Protocol biopsies may give valuable information, *e.g.*, on silent and early rejection processes, toxicity of medical treatments, BK virus infection and development of chronic scarring processes^[5]. However, continuous monitoring for rejection over the entire post-transplant course would require performing biopsies unrealistically often.

Due to this diagnostic dilemma, there is clearly a need for sensitive, non-invasive methods to monitor for rejection and to detect rejection at an early stage. Such tests could be performed regularly to identify those patients who need further workup by an allograft biopsy. Several molecules in blood and urine have been evaluated (either as a single marker or as a combination of markers) based on the hypothesis that blood and urine can reflect the molecular processes in the allograft. In theory, testing for markers of rejection in blood and urine could even outperform the diagnosis by biopsy, which is prone to sampling errors and inter-observer variability. However, none of these tests has gained widespread clinical use^[5].

RATIONALE FOR A MULTI-MARKER APPROACH TO DIAGNOSE REJECTION

Rejection is a heterogeneous process^[7-9] and therefore it is unlikely that a single marker or small number of markers can reflect all facets of rejection reliably. Heterogeneity refers to the entities of T cell- and antibody-mediated rejection but also to the sites of immunological attack and to the morphological severity as specified by the Banff classification^[7] and shown in Figure 1. Also, as a reflection of the severity the rejection may be subclinical, *i.e.*, without a concomitant decline in allograft function or clinical with accompanying graft dysfunction^[10]. As outlined in Figure 1, rejection is a disease process that extends from the activation of the immune system to the scarring of injured renal structures. This implies that time-dependent features may also be important to consider in terms of early and later stages of rejection. Given these facts, the hypothesis of multi-marker approaches is that a panel of molecules is better suited to detect the diverse aspects of rejection than a single molecular marker. In fact, gene expression analysis of allograft biopsies has demonstrated that different types of rejection present with distinct molecular phenotypes, containing a wide array of chemokines, cytokines and other regulatory molecules^[11]. Some of these phenotypic signatures should be detectable in blood and urine and usable for the rejection diagnosis.

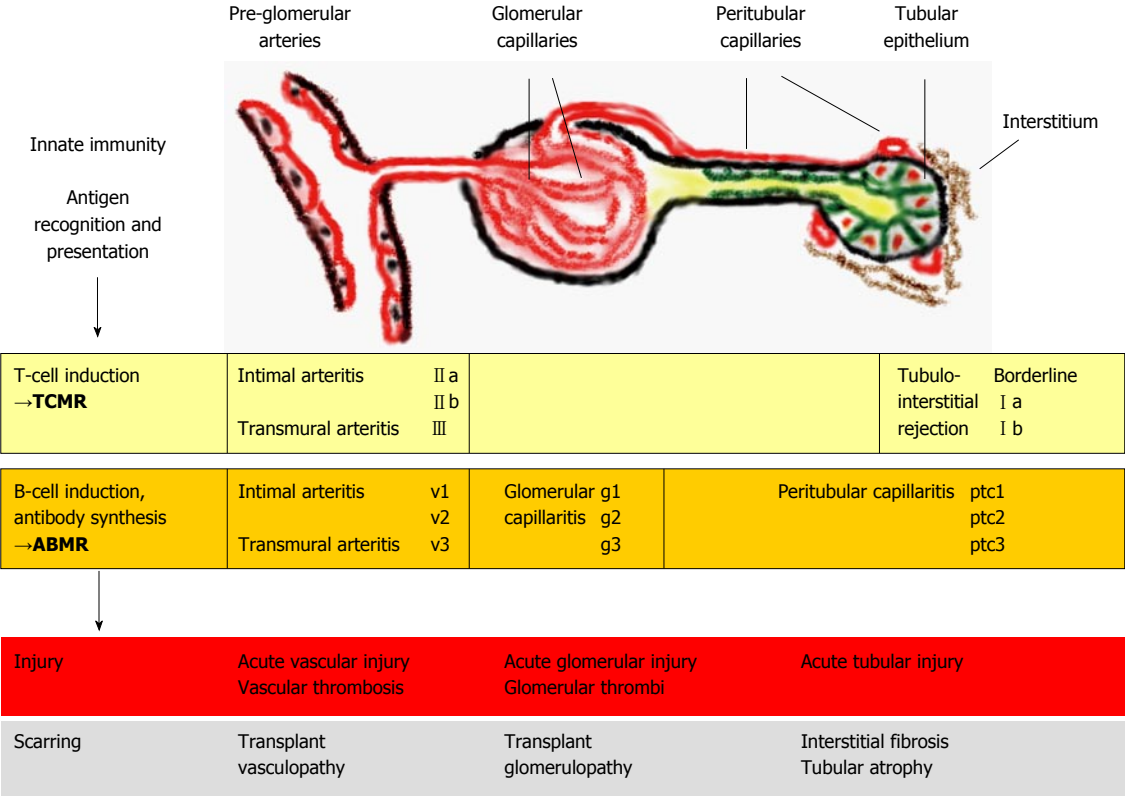


Figure 1 Kidney allograft rejection types, histological sites of injury and underlying mechanisms. TCMR includes recognition and presentation of donor antigens by antigen-presenting cells to T cells, which become activated and undergo proliferation. Activated T-cells invade vascular, tubular and interstitial structures. Vascular rejection often presents with some degree of tubulointerstitial inflammation; however pure cases of vascular rejection ("v-only") can be observed^[6]. In ABMR, activated T cells induce B cells to undergo plasma cell proliferation resulting in the production of donor-specific antibodies. Antibody-mediated injury to pre-glomerular arteries, glomerular and peritubular capillaries is mediated by local activation of complement factors however, non-complement-fixing antibodies may also play a role in some cases^[9]. Isolated findings of glomerular and peritubular capillaritis or pre-glomerular arteritis may be present or a combination of these features^[7]. TCMR and ABMR can occur simultaneously (*i.e.*, mixed rejection)^[4]. The rejection processes can lead to different histological forms of injury and if not successfully treated, to scarring. The Banff classification^[7] associates the elementary lesions of glomerular (g) and peritubular capillaries (ptc) and pre-glomerular vessels (v) to ABMR. TCMR includes tubulointerstitial infiltration (Borderline, I) and arteritis of pre-glomerular vessels (II - III). Banff grades (a-b, II - III, v1-3, g1-3, ptc1-3) denote different severities of the lesions. TCMR: T cell-mediated rejection; ABMR: Antibody-mediated rejection.

It is important to note that the rejection process induces host responses like repair and healing mechanisms including scarring processes which contribute to molecular signatures^[12] (Figure 1). On theoretical grounds, marker sets for the diagnosis of rejection should be distinct from those signatures as they rather reflect the sequel of rejection instead of depicting specifics of the rejection process itself. As an example, urinary β 2-microglobulin or fragments of it have been reported as potential indicators of rejection^[13,14]. Further analysis however showed that increased urinary β 2-microglobuline-derived peptides are similarly present in pure cases of acute tubular injury^[15] and in cases with tubular atrophy and interstitial fibrosis^[16,17], without any evidence of rejection.

To date, several approaches have been employed to establish multi-marker models for the non-invasive diagnosis of rejection. Gene expression, RNA analysis and proteomics are the commonest whereas fewer studies concentrated on microRNA analysis^[18], metabolomics^[19] and lipidomics. This review focuses on proteomics in blood and urine of kidney transplant patients to diagnose rejection.

PROTEOME ANALYSIS

The proteome is the whole set of proteins present in an organism or in one of its functional or structural units at a given state. Compared to the transcriptome or the metabolome, the proteome is the most functional compartment and is subject to continuous and dynamic changes either in response to external stimuli or alterations in homeostasis^[20]. In recent years, clinical research mainly focused on the detection of single proteins by immunological techniques. This hypothesis-driven approach requires precedent knowledge on the functional characteristics of a specific protein target. Proteome analysis in contrast is hypothesis-free since it explores a biological sample in its proteomic entirety. Therefore, by comparison of the proteomic content at two or more distinct conditions (*e.g.*, diseased and non-diseased) all differently expressed proteins may be captured as potential differentiating markers. Technically, proteomic technologies rely on the physicochemical properties of the proteins instead of immunological properties, which are required for antibody-mediated analyte detection.

Biomarker research by proteomics is based on the hypothesis that at least one of the following conditions is true: (1) Proteins are differentially expressed from their genes during a disease process; (2) Proteins are subject to differential post-translational modifications due to disease-specific changes in the activity of enzymes; and (3) Proteins are detectable in different amounts due to altered production, degradation or release from cells by the disease process.

Sample matrix

In biomarker research, easily accessible sample matrices like blood or urine are preferred because procurement of tissue relies on invasive methods. Blood has a high dynamic range of protein concentrations, necessitating depletion of the most abundant proteins to improve detection of low abundant protein markers. It is also characterized by lower stability due to high proteolytic activity. Urine on the other hand, has a higher stability and lower complexity than blood. However, urine is in contact with the genital-urinary tract and thus, prone to bacterial contamination. Moreover, the proteomic compounds in urine originate from different sources, namely from the systemic circulation *via* glomerular filtration, from the kidney, and from the urinary tract. The exact contribution by these sources is unknown and may change in disease conditions.

Proteomic workflow

The proteomic workflow includes the preparation of the sample to clear the proteomic content from other compounds, followed by complexity-reducing separation and physicochemical detection methods.

Sample preparation: Before proteomic analysis, a sample usually needs processing to remove insoluble materials like cell debris and interfering salt and lipids. It is however important to note that such preparation steps introduce bias and add variability, and therefore should be restricted to the absolute requirements^[21]. Because proteins can be degraded by proteases, heat, bacteria and pH changes, the integrity of the samples should be maintained by applying standardized collection protocols and immediate freezing.

Protein separation: Historically, 2-D gel electrophoresis used to be the principal proteomic separation method^[22]. This is now largely replaced by the non-gel based separation methods liquid chromatography (LC) and capillary electrophoresis (CE), which have a higher resolving capacity. Using LC and CE, small proteins and peptides can be directly subjected to mass spectrometry analysis whereas larger proteins have to be cleaved by trypsin before separation and mass detection^[23].

Protein ionization: There are many different mass spectrometry methods but they all have in common

that proteins and peptides are transferred into ions, which are then subjected to an electric or magnetic field. The subsequent characterization of each ion is based on its mass over charge ratio (m/z). Electron spray ionization, matrix-assisted laser desorption/ionization and surface enhanced laser desorption/ionization are the main ionization techniques used in clinical proteomic studies.

Protein mass detection: The desolvatized ions in the electric or magnetic field are then collected by the mass detector. Many different concepts exist, mostly in respect to how an ionic signal is amplified. "Time of flight", Orbitrap and Triple Quadrupoles are the most commonly used detectors in biomarker research.

Protein quantification

Normally, only relative quantification is possible with mass spectrometry (MS) techniques, based on an approximate proportionality between signal intensity and the relative protein/peptide abundance in a sample. Advanced methods have been developed like "isobaric tags for relative and absolute quantification"^[24]. And "multiple reaction monitoring"^[25] to compare the protein/peptide abundance between different samples.

Protein sequence identification

In its simple one-dimensional form, mass spectrometry gives mass over charge ratios of peptides and proteins but no information on the amino acid sequence. This may be sufficient to identify and detect proteomic markers for disease conditions simply by their physicochemical characteristics. Nevertheless, identification of the proteins and peptides may be desirable, *e.g.*, to understand pathophysiologic pathways or to transfer the discovered markers to another platform (*e.g.*, ELISA). With tandem mass spectrometry (MS/MS), a MS-detected peptide can be isolated in the first MS dimension and then forced into multiple rounds of collisions in the second MS dimension to generate an ordered fragment ion spectrum^[26].

Construction of multi-marker diagnostic models

Although average levels of single proteins or peptides may be significantly different between case and control groups large overlap of values is often observed when individual samples are compared with each other^[27]. To construct classifiers with as little overlap as possible between case and control groups, biomarkers are often combined into multi-marker sets^[28]. This strategy can compensate for analytical variances and biological variability like heterogeneity of the disease process, time-dependent changes, or confounding conditions. The integration of proteins/peptides into a multi-marker set can range from a few individual molecules up to whole "fingerprints" (chromatograms, spectra), depending on the requirements for sensitivity and

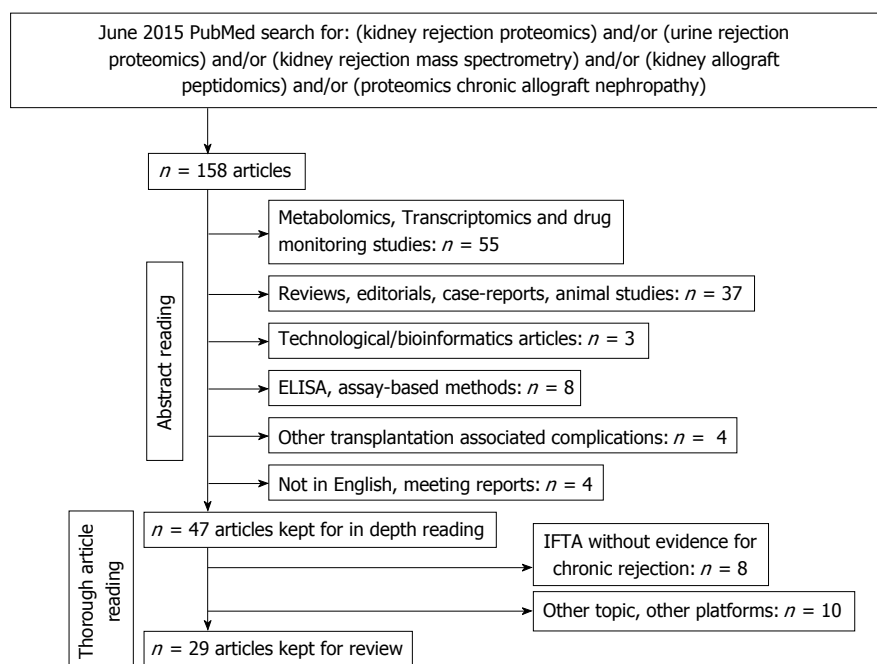


Figure 2 Search strategy for proteomic studies in the field of renal allograft rejection. IFTA: Interstitial fibrosis and tubular atrophy.

specificity and on the complexity of the disease of interest.

Methods to integrate multiple discriminative proteins into a biomarker model can be divided into "linear" and "high dimensional" algorithms, the latter tending to have better results due to a weighted combination of the markers according to the degree of their correlation. Here, the most frequently used algorithms are "support vector machine", adaptive boosting, random forest and neural networks.

PROTEOMIC STUDIES ON RENAL ALLOGRAFT REJECTION

The literature search was done in PubMed using the keywords "kidney, rejection, proteomics, urine mass spectrometry, allograft, peptidomics, chronic allograft nephropathy" in different combinations (Figure 2). Of the 158 publications, 111 were excluded after reviewing title and abstract of each publication. The remaining 47 articles were kept for in depth study. Ten articles were excluded because they concentrated only on technical aspects ($n = 4$), did not use shotgun proteomic methods ($n = 5$), or did not examine rejection patients ($n = 1$).

Examination of patients with chronic rejection/chronic allograft nephropathy was reported in eight studies^[16,17,29-34]. However, evaluation of the histomorphological reporting revealed that patients in these studies had merely interstitial fibrosis and tubular atrophy (IFTA; Banff category 5) according to the latest update of the Banff classification^[7], without any evidence of acute or chronic rejection. This mistaking is explained by the historical definition of "chronic allograft nephropathy", which does not

differentiate between patients with non-specific chronic lesions (IFTA) and patients with signs of chronic rejection. Hence, these studies were considered as non-relevant for the topic "rejection" and excluded from the reporting in Table 1.

The remaining 29 studies^[13-15,35-60] are listed in Table 1. Five studies reported a prospective study design^[37,41,45,46,57], with assumable random or consecutive sample selection. In the remaining studies, samples seemed to be drawn from a biobank/sample archive not specifically established for the proteome study, without giving details to selection process and randomness of the samples. Most studies were cross-sectional. Nine studies described longitudinal aspects with regard to sample collection^[39], profiling of sequential samples or comparison of proteome patterns before and after rejection^[13,35,37,41,45,53,60] and to the assessment of graft survival^[59].

One third of the study performed proteomic analysis on an independent validation set of samples to confirm the discovered markers. Validation on independent samples was also performed by ELISA assays for the discovered markers^[50,51,53,60].

Urine was clearly the diagnostic matrix of choice, with 23 studies compared to the six studies that examined blood samples. In the study of Ling *et al.*^[40] mRNA expression in biopsies was examined in parallel to the urinary proteome. O'Riordan *et al.*^[45] stained biopsies to confirm the identified urinary proteomic marker β -defensin-1.

In approximately half of the studies, patients with TCMR were examined, as evident from the reported Banff grades. Patients with ABMR were included in six studies^[35,47,48,51,58]; in one study^[46] a few patients were reported to have mixed rejection (TCMR + ABMR). In

Table 1 Proteomic studies on renal allograft rejection

Ref.	B/U	Training set	n	Validation set	n	Proteomic method	Performance	Identified molecules	Remarks
Akkina <i>et al</i> ^[35]	U	C (bx) BL II a aABMR	13 1 1 1	None		iTRAQ-MALDI-MS/MS	NR	None	Study included healthy individuals. Study concentrates on longitudinal stability of peptides in rejecting and non-rejecting patients
Clarke <i>et al</i> ^[36]	U	C (st) AR	15 15	None		SELDI-TOF-MS	Accuracy 91% Sensitivity 83% Specificity 100% (2-marker classifier)	None	
Freue <i>et al</i> ^[37]	B	C (bx) I a I b II a	21 7 1 3	None		iTRAQ-MALDI-MS/MS	AUC 0.86 Sensitivity 80% specificity 90% (4-marker classifier)	Up-regulated: TTN, LBP, PI16, CFD, MBL2, SERPINA10, B2M Down-regulated: KNG1, AFM, SERPINA5, LCAT, SHBG	ELISA was performed on 4 of the identified markers (coagulation factor IX, SHBG, CFD, LCAT) in blood
Günther <i>et al</i> ^[38]	B	C (st) AR	13 13	C (st) AR	7 7	iTRAQ-MALDI-MS/MS	AUC 0.76 Sensitivity 57% specificity 86%	21 peptides	Different statistical approaches to integrate proteomics and transcriptomic results are presented
Jahnukainen <i>et al</i> ^[39]	U	C (st) I a- II b BKV	29 28 21	None		SELDI-TOF-MS	Sensitivity 81% Specificity 84% (100-marker classifier)	None	21 of the 28 rejection samples showed also signs of chronic rejection Article concentrates on differentiation of AR and BKV-NP
Ling <i>et al</i> ^[40]	U	C (bx) AR BKV	10 10 10	C (bx) AR BKV	10 10 4	LC-MALDI-TOF-MS LC-MS/MS	AUC 0.96 (40-marker classifier)	COL1A2, COL3A1, UMOD, MMP-7, SERPING1, TIMP1	Study included healthy individuals and patients with native kidney disease (nephrotic syndrome). Results of proteomic analysis are related to mRNA expression profiling of corresponding biopsies
Loftheim <i>et al</i> ^[41]	U	C (st) BL I a II a	6 1 4 1	None		2D LC-MS/MS	NR	Up-regulated: IGFBP7, VASN, EGF, LGALS3BP	Study collected sequential urines from the beginning after Tx. Analysed samples for rejection patterns were taken 7-11 d before biopsy
Mao <i>et al</i> ^[42]	U	C (bx) TCMR	22 27	C (bx) TCMR	14 10	SELDI-TOF-MS	Sensitivity 90% Specificity 71% (4-marker classifier)	None	All TCMR cases were subclinical rejections with grades \geq I a
Metzger <i>et al</i> ^[43]	U	C (bx) I a I b	23 13 3	C (bx) I a I b	36 23 5	CE-MS LC-MS/MS	AUC 0.91 Sensitivity 93% Specificity 78% (14-marker classifier)	3 fragments of COL1A1, 1 fragment of COL3A1	Rejections in the training set were all subclinical. The validation set contained 10 clinical and 18 subclinical rejection cases. Confounder like ATI in biopsies, urinary tract infection and CMV infection were considered
O'Riordan <i>et al</i> ^[44]	U	C (st) AR	22 23	None		SELDI-TOF-MS	AUC 0.91 Sensitivity 91% Specificity 77% (2-marker classifier)	Up-regulated: SERPINA3 Downregulated: DEFB1	Study included healthy individuals
O'Riordan <i>et al</i> ^[45]	U	C (st) BL I a I b II a II b	22 3 6 4 7 3	None		SELDI-TOF MS LC-MS/MS	AUC 0.91 Sensitivity 91% Specificity 77% (2-marker classifier)	Up-regulated: SERPINA3 Downregulated: DEFB1	
Pisitkun <i>et al</i> ^[46]	U	C (bx) I a I b II a ATI	2 4 1 2 7	None		LC-MS/MS	NR	Numerous molecules	
Quintana <i>et al</i> ^[47]	U	C (st) a/cABMR IFTA	8 10 8	a/cABMR IFTA	8 6	MALDI-TOF-MS	IFTA <i>vs</i> cABMR AUC 1.0 Sensitivity 100% Specificity 100% (6-marker classifier)	None	Study included healthy individuals

Quintana <i>et al</i> ^[48]	U	C (st) a/cABMR IFTA	5 10 8	C (st) a/cABMR IFTA	9 11 8	LC-MS/MS	C <i>vs</i> IFTA/ABMR: AUC 0.82 IFTA <i>vs</i> ABMR 100% correct IFTA, 90% correct ABMR (2-markers)	Down-regulated: UMOD Differentiation between controls and IFTA/ABMR: KNG1	Study included healthy individuals Two unidentified peptides could differentiate between IFTA and ABMR, based on quantitative differences of the peptides (higher in ABMR)
Reichelt <i>et al</i> ^[49]	U	C (bx) I a I b II a II b	10 7 3 1 2	None		SELDI- TOF-MS	SAX2 protein chip: Sensitivity 90% Specificity 80% CM10 protein chip: Sensitivity 92% Specificity 85% (2-marker classifier)	None	
Schaub <i>et al</i> ^[13]	U	C (bx) I a I b II a ATI GL	22 7 8 3 5 5	None		SELDI- TOF-MS	Sensitivity 94% Specificity 82% (3-marker classifier)	Cleaved B2M Cleaved B2M	Study included healthy individuals. The clinical confounder CMV viremia was assessed. Longitudinal evaluation of urine proteome patterns differentiated between patients with stable course and rejection
Schaub <i>et al</i> ^[15]	U	C (bx) I a I b II a ATI GL	22 7 8 3 5 5	None		SELDI- TOF-MS, LC-MALDI- MS	NR		Study included healthy individuals. Study concentrated on cleavage mechanisms for b2-microglobulin
Sigdel <i>et al</i> ^[14]	U	C (bx) AR	10 10	None		LC-MALDI- MS/MS	NR	List of 73 candidates, incl. fragments of collagens, UMOD, B2M, PTGDS	Study included healthy individuals
Sigdel <i>et al</i> ^[50]	U	C (bx) AR	10 10	None		LC-MS/MS	AUC 0.84-0.97 for 3 single molecules (by ELISA)	Upregulated: SERPINF1 Down-regulated: UMOD, CD44	Study included healthy individuals and patients with native kidney disease (proteinuria)
Sigdel <i>et al</i> ^[51]	U	C (bx) I a- II b aABMR IFTA BKV	30 30 2 30 18	None		iTRAQ- LC-MS/MS	AUC 0.8 for 3 single molecules (by ELISA)	HLA-DRB1, KRT14, HIST1H4B, FGG, ACTB, FGB, FGA, KRT17, DPP4, cleaved B2M	In ELISA studies, FGG could also segregate AR from BKV- nephropathy Validation set for detection of FGG, HLA DRB1, FGB by ELISA included 44 stable transplant patients and 44 patients with rejection
Sigdel <i>et al</i> ^[52]	U	C (bx) ≥ I a	20 20	None		iTRAQ- LC-MS/MS	NR	Enriched in exosomal fraction in AR: A2M, APOA2, APOM, CD5L, CLCA1, FGA, FGB, IGHM, DEFA5, PROS1, KIAA0753 Exclusively in the exosomal fraction in AR: CLCA1, PROS1, KIAA0753	Study concentrated on differences between the whole proteome in urine (non-fractionated) and the exosomal fraction
Stubendorff <i>et al</i> ^[53]	U	C (st) AR	16 16	C (st) AR	16 16	SELDI- TOF MS	Sensitivity 94% Specificity 44% (4-marker classifier) Sensitivity 80% Specificity 81% for 2 molecules (by ELISA)	Up-regulated: A1MG, HP	Results on longitudinally collected samples suggest that alpha-1- microglobulin and haptoglobin indicate upcoming AR early
Sui <i>et al</i> ^[54]	B	C (bx) AR CR	12 12 12	None		MALDI- TOF-MS	Recognition capability for AR 90%	None	Study included healthy individuals. Sample clean-up was performed with magnetic beads

Wang <i>et al</i> ^[55]	B	C (bx) ≥ I a TCMR ATI	19 14 28 10	C (bx) ≥ I a	10 10	SELDI- TOF-MS	C <i>vs</i> subclinical a Sensitivity 100% Specificity 90% (3-marker classifier) C <i>vs</i> TCMR Sensitivity 90% Specificity 90% (7-marker classifier) AR <i>vs</i> subclinical Sensitivity 100% Specificity 100% (4-marker classifier)	None	≥ I a refers to subclinical rejections only. All (non-graded) TCMR cases were clinical rejections
Wittke <i>et al</i> ^[56]	U	C (bx) I a I b II a II b UTI	29 11 6 1 1 10	C (bx) I a I b UTI	10 6 3 7	CE-MS, LC-MS/MS	Sensitivity 67% Specificity 80% (17-marker classifier)	COL4A5	Transplant patients with urinary tract infection were included, with biopsy-confirmed absence of rejection. Of the rejection cases, 13 were subclinical and 6 clinical
Wu <i>et al</i> ^[57]	B	C (st) I b II a II b III	8 1 2 1 1	None		iTRAQ- 2D LC- MS/MS	NR	Numerous molecules belonging to different pathways: <i>e.g.</i> , inflammatory response, complement, defence response, protein maturation and processing, humoral immune response	
Yang <i>et al</i> ^[58]	U	C (bx) TCMR aABMR ATI	36 30 25 10	C (bx) TCMR aABMR	14 10 10	SELDI- TOF-MS	C <i>vs</i> TCMR/ABMR Sensitivity 100% Specificity 78% (3-marker classifier) ABMR <i>vs</i> TCMR Sensitivity 80% Specificity 95% (5-marker classifier)	None	
Zhang <i>et al</i> ^[59]	U	C (bx) CR/(AR)	41 90	None		MALDI- TOF-MS MALDI- MS/MS	Different classifier combinations: Sensitivity 73%-88% Specificity 53%-62%	Up-regulated: B2M, SERPINA1. Down-regulated: PSAP	Study included healthy individuals and patients with native kidney disease (nephrotic syndrome). Saposin B was high in transplant patients with stable course over 280 d and low in patients with subsequent graft failure
Ziegler <i>et al</i> ^[60]	B	C I a I b	48 10 7	None		SELDI- TOF-MS MALDI- MS/MS	Sensitivity 100% Specificity 94% for 2 molecules (by ELISA)	Out of 22 candidates decreased: APOA1, SERPINA3	Two patients with TCMR had also signs of additional ABMR. The 2 markers for rejection were not informative in samples collected a few days before the rejection

Patient group definitions: C (bx): Control patients with biopsy-confirmed absence of rejection; C (st): Control patients without biopsy to exclude rejection; AR: Acute rejection without further histologic grading; CR: Chronic rejection without further histologic grading; TCMR: T cell-mediated rejection without further histologic grading; ABMR: Antibody-mediated rejection with prefix "a" (acute) and "c" (chronic); BL: Borderline rejection (suspicious for rejection); IFTA: Interstitial fibrosis and tubular atrophy; BKV: BK virus nephropathy; ATI: Acute tubular injury; GL: *De novo* or recurrent glomerulopathy; UTI: Urinary tract infection with biopsy-confirmed absence of rejection; I a, I b: T cell-mediated tubulointerstitial rejection specified as "mild" (a) and "severe" (b); II a, II b: T cell-mediated vascular rejection specified as "mild" (a) and "severe" (b); III: T cell-mediated vascular rejection with transmural arteritis; CMV: Cytomegalovirus; AUC: Area under the curve; CE: Capillary electrophoresis; iTRAQ: Isobaric Tags for Relative and Absolute Quantification; LC: Liquid chromatography; MALDI: Matrix-assisted laser desorption ionization; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; SELDI: Surface-enhanced laser desorption ionization; TOF: Time of flight; B/U: Examined matrix (blood: B, urine: U); *n*: Number of patients in each category; NR: Not reported.

the remaining studies, no clear Banff descriptors were provided leaving it open whether TCMR or ABMR was present and which severity grades and subtypes of rejection were observed. Apparently, almost all studies concentrated on acute rejection. Cases with chronic TCMR were included in the study of Jahnukainen *et*

al^[39], patients with chronic active ABMR were reported by Quintana *et al*^[47,48]. One study examined chronic rejection without detailed scoring with regard to TCMR and ABMR^[59].

In any proteomic marker discovery study the selection of appropriate comparators (controls) is an

important issue because definition of proteome patterns specific for the disease condition - in this case rejection - is deduced by comparison to samples without the disease condition. Thirteen studies used samples from clinically stable transplant patients without confirming absence of rejection by biopsy. This implies that these patients could have had subclinical rejection (*i.e.*, typical histological rejection findings without concomitant impaired allograft function). It has been shown that subclinical rejection produces proteomic patterns which are similar to clinical rejection and three studies have examined subclinical TCMR so far^[42,43,56].

Another important point to consider is the delimitation of confounding conditions. For example, it is well known that acute tubular injury is present in a substantial proportion of patients with acute rejection^[43]. If no measures are taken to differentiate the proteomic signature of rejection from acute tubular injury, the proteomic profile for rejection might lack specificity as tubular injury is a non-specific finding which is also related to drug-toxicity and ischemic/reperfusion injury. In fact, some of the studies included control samples with acute tubular injury^[13,15,46,55,58]. Likewise, infection could be a confounder, as inflammatory pathways are activated in both, infection and rejection. To this end, BK virus nephropathy, urinary tract infection and CMV have been taken into account in some studies^[13,39,43,51]. Another important confounder may be concurrent IFTA present in biopsies with ABMR as compared to biopsies showing IFTA without rejection which was addressed in the studies from Quintana *et al.*^[47,48].

Sample size numbers varied considerably in the studies, with two to ninety rejection samples for the trainings set, and with seven to twenty-eight for the validation of the discovered proteomic markers. There is certainly no simple rule of thumb to determine the necessary sample size. As discussed in the second chapter, rejection is a heterogeneous condition. Variability can probably be reduced by applying stringent histomorphological and clinical criteria to define the disease condition, nevertheless training sets for rejection should be large enough to cover the whole spectrum of the rejection type studied. In addition, controls/comparator groups without rejection should be of sufficient size to cover the whole spectrum of confounding conditions. Eventually, measures like area under the curve (AUC), sensitivity, specificity, negative and positive predictive values will give information about the performance of the defined marker set for rejection. Some of the studies reported exceptionally optimistic performance values, however, performance derived from cross-validation within the training set inherently carries overfitting of proteomics data and validation with external samples can correct for this limitation.

Various molecules have been discovered in the different studies and only a few were independently

reported by different research groups, like fragments of collagens, β 2-microglobulin, alpha-1-antichymotrypsin and uromodulin. The large variability in the reported markers for rejection is probably not primarily related to differences in the rejection characteristics of the examined patients. As outlined in chapter III, "proteome analysis", the use of different MS methods will inevitably result in capturing diverse peptides and proteins. This issue is certainly relevant once efforts are undertaken to implement such tests into the clinical routine.

An important aspect is the biological significance of the identified molecules and the identification of the modulated processes which are involved. Combining all proteins from the studies mentioned above resulted in eighty-nine non-redundant molecules. These were subjected to a systematic analysis of biological contextualization using the pathway- and enzyme reaction-related Reactome information resource (Figure 3). Based on the known molecular associations a physical interaction graph was constructed (Figure 4). The analyses were performed without prior knowledge of disease areas or other information that might lead to bias. Reactome analysis using ClueGO (PMID: 19237447) showed processes related to platelet degranulation, keratan sulfate degradation, lipid digestion, mobilization and transport, antigen presentation and interferon gamma signalling to be directly associated with the input proteins. If the molecules involved worked in a synchronized manner some degree of physical association should be expected. To test this, the proteins were clustered using MiMI (PMID: 18812364), which connects molecules based on prior knowledge observed in other studies such as protein-protein interactions. This analysis allows expanding the molecular network to connect a maximum number of input proteins using gap-filling, or bridging, proteins. What is evident from the analysis (Figure 4) is that indeed a majority of molecules form a large network that is bound together by an additional 35 entries, which can serve as an entry point for further investigations. To this end, several of these gene ontology pathways have also been deduced from microarray analysis of transplant biopsies with rejection^[61].

CONCLUSION

In summary, the studies published so far convincingly show that proteomics is capable of discovering molecular mechanisms of renal allograft rejection and of defining molecular markers which can aid to detect rejection early and reliably. To bring proteomics further forward into clinical application in kidney transplantation the limitations of previous studies should be used as challenges for future trials in the discovery and/or validation of rejection markers. Points to consider include but are not limited to:

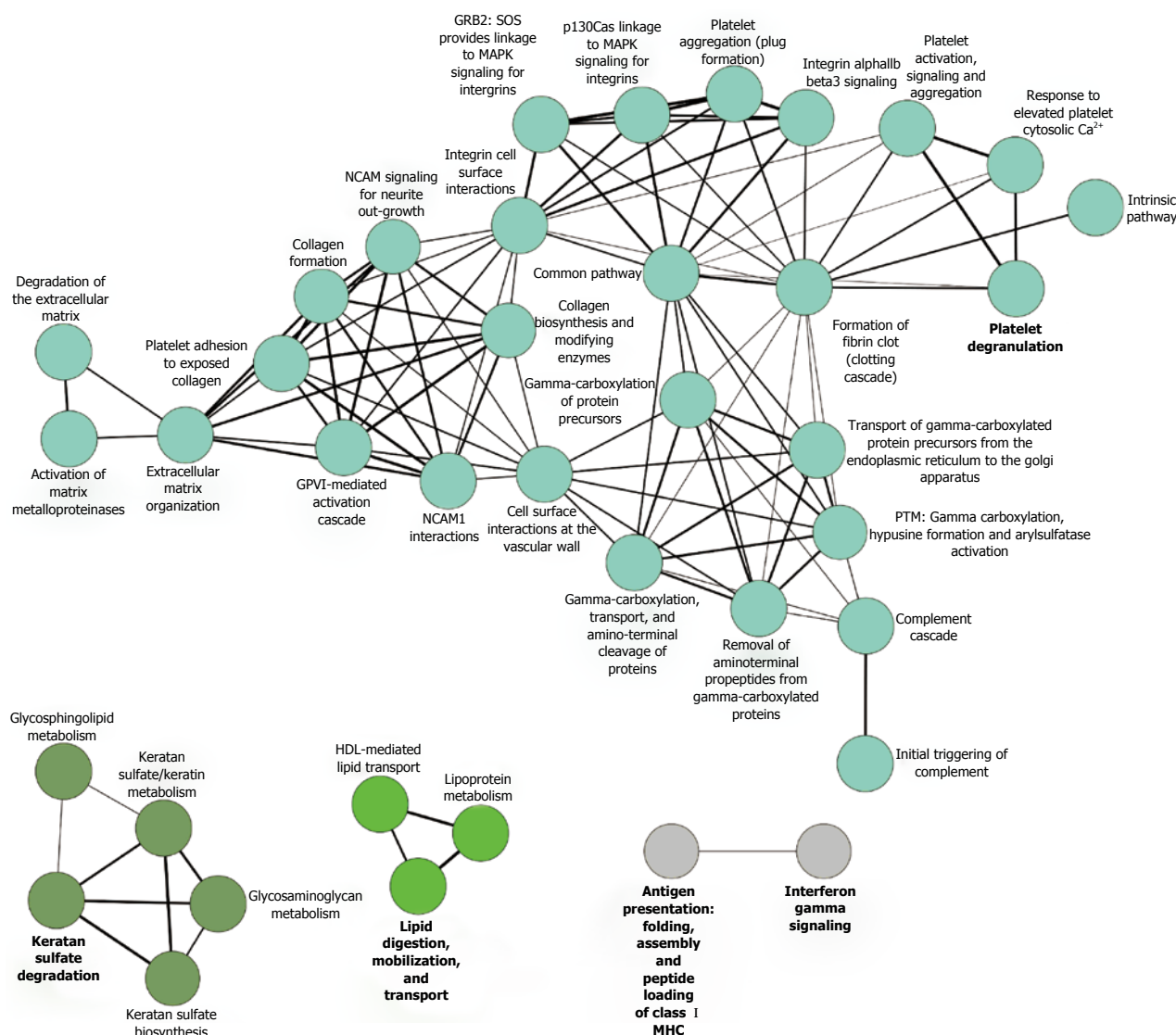


Figure 3 Reactome graph, showing the functional association of renal allograft rejection molecules. Literature-derived proteins associated with acute and chronic rejection ($n = 89$, concatenated from the proteomic studies listed in Table 1) were analyzed by functional Reactome group-clustering using CytoScape's ClueGO plug-in (CytoScape v2.8.3, ClueGO v1.5). Enriched Reactome-terms are represented as circles, and lines denote the relationship between these terms as functional groups. Line thickness and font-size are directly correlated with the statistical significance of terms and relationships (all with $P < 0.05$ after Bonferroni-adjustment for multiple testing correction). MAPK: Mitogen-activated protein kinase; GRB2: Growth factor receptor-bound protein 2; NCAM: Neural cell adhesion molecule.

Study design: (1) Sufficient number of patients with biopsy-confirmed absence of rejection, representing the whole spectrum of transplanted patients; (2) Rigorous histological and serological classification of patients with rejection, with a sufficient number of cases for each rejection type; (3) Inclusion of important and frequent confounding conditions which may be concurrently present in patients with and without rejection (either in the biopsy or clinically); and (4) Besides validation on selected samples as done so far in some studies, prospective in-place validation under everyday clinical conditions to determine the practical value of non-invasive tests for rejection.

Endpoints: (1) Emphasis on early markers which can detect incipient, subclinical stages of rejection (this will require longitudinal sample collections); (2) Development of markers which can indicate response

to the rejection therapy (this will require longitudinal observation); and (3) Prospective, randomized studies with and without non-invasive monitoring to determine the costs and benefits.

Technical aspects: (1) Uniform sample collection protocols, sample preparation and analyses, especially if proteomic markers should find wide application; (2) Development of simplified test systems which can be applied outside highly specialized laboratories (provided the number of proteomic markers is not too high); (3) Reliable measures for the test system (AUC, sensitivity, specificity, negative and positive predictive values, thresholds of the test), all derived from independent validation studies and measures for reproducibility/variability; and (4) Identification of confounders that reduce the sensitivity or specificity of the proteome markers.

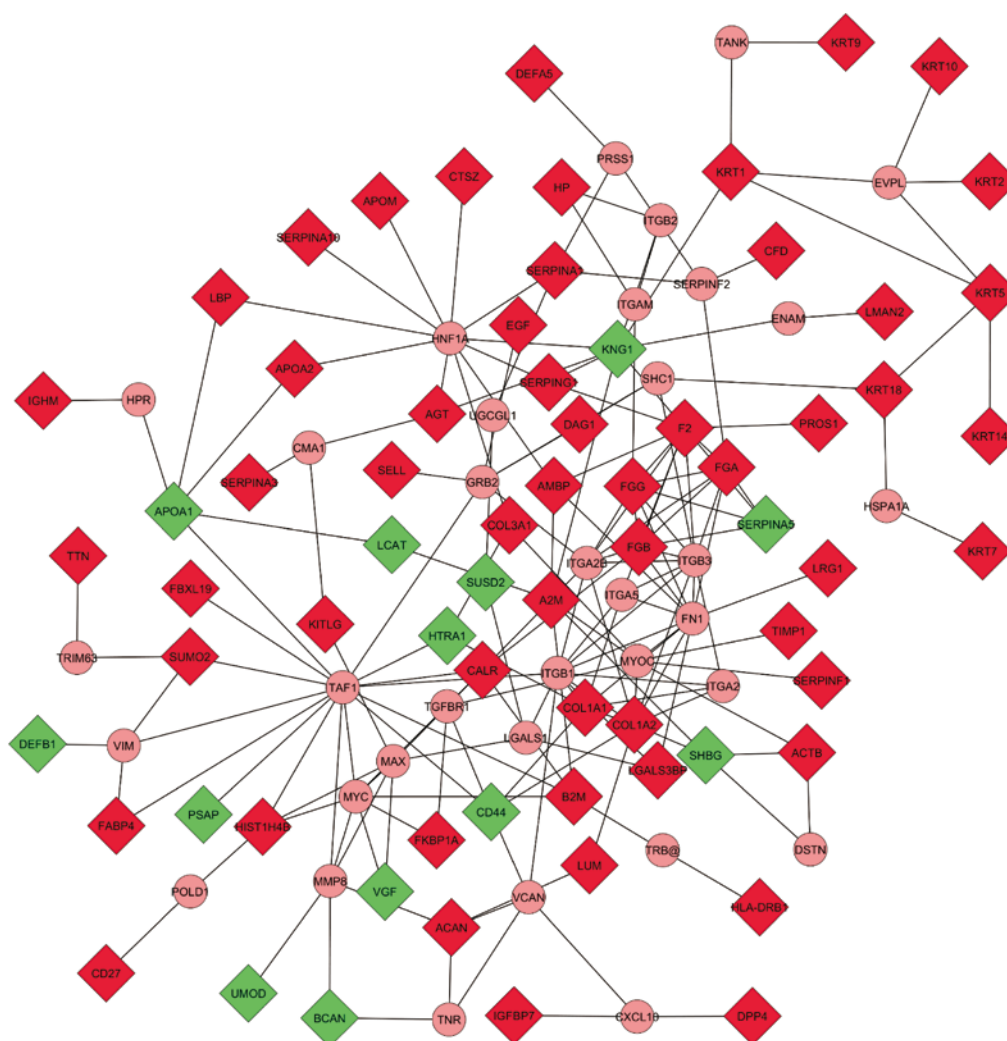


Figure 4 Expanded molecular interaction model. Physical interaction representation of molecules involved in renal allograft rejection. The concatenated list of literature-derived proteins associated with acute and chronic rejection was subjected to interactome-mapping using CytoScape's Michigan Molecular Interactor (MiMI) plug-in (CytoScape v2.8.2, MiMI v3.1). Known protein-protein interactions with up to two additional bridging molecules to maximize the interconnectivity were used to generate the map shown, which contains 68 of the 89 differentially expressed molecules and 35 additional bridging proteins. Input molecules are depicted as rectangles, and bridging molecules as circles. Each line between proteins represents a direct known association. Included literature-derived proteins associated with acute and chronic renal allograft rejection in the network (Rectangles; Green: Down-regulated; Red: Up-regulated; $n = 68$); Included additional bridging proteins for maximum interconnectivity (circles; $n = 35$); Excluded literature-derived proteins associated with acute and chronic renal allograft rejection not connected to the network (not shown; $n = 21$). A2M: Alpha-2-macroglobulin; ACAN: Aggrecan core protein; ACTB: Actin, cytoplasmic 1; AGT: Angiotensinogen; AMBP: Alpha-1-microglobulin; APOA1: Apolipoprotein A1; APOA2: Apolipoprotein A-2; APOM: Apolipoprotein M; B2M: Beta-2-microglobulin; BCAN: Brevican core protein; CALR: Calreticulin-3; CD27: CD27 antigen; CFD: Complement factor D; COL1A1: Collagen alpha-1(I) chain; COL1A2: Collagen alpha-2(I) chain; COL3A1: Collagen alpha-1(III) chain; CTSZ: Cathepsin Z; DAG1: Dystroglycan; DEFA5: Defensin-5; DEFB1: β -defensin 1; DPP4: Dipeptidyl peptidase 4; EGF: Pro-epidermal growth factor; F2: Prothrombin; FABP4: Fatty acid-binding protein, adipocyte; FBXL19: F-box/LRR-repeat protein 19; FGA: Fibrinogen alpha chain; FGB: Fibrinogen beta chain; FGG: Fibrinogen gamma chain; FKBP1A: Peptidyl-prolyl cis-trans isomerase FKBP1A; HIST1H4B: Histone H4; HLA-DRB1: HLA-DRB1 protein; HP: Haptoglobin; HTRA1: Serine protease HTRA1; IGFBP7: Insulin-like growth factor-binding protein 7; IGHM: Ig mu chain C region; KITLG: Kit ligand; KNG1: Kininogen-1; KRT: Keratin, type II cytoskeletal; KRT9: Keratin, type I cytoskeletal 9; LBP: LPS-binding protein; LCAT: Phosphatidylcholine-sterol acyltransferase; LGALS3BP: Galectin-3-binding protein; LMAN2: Vesicular integral-membrane protein VIP36; LRG1: Leucine-rich alpha-2-glycoprotein; LUM: Lumican; PROS1: Vitamin K-dependent protein S; PSAP: Saposin B; SELL: L-selectin; SERPINA1: Alpha-1-antitrypsin; SERPINA10: Protein Z-dependent protease; SERPINA3: Alpha-1-anti-chymotrypsin; SERPINA5: Serine protease inhibitor; SERPINF1: Pigment epithelium-derived factor; SERPING1: Plasma protease C1 inhibitor; SHBG: Sex hormone-binding globulin; SUMO2: Small ubiquitin-related modifier 2; SUS2: Sushi domain-containing protein 2; TIMP1: Metalloproteinase inhibitor 1; TTN: Titin; UMOD: Uromodulin; VGF: Neurosecretory protein VGF; CMA1: Chymase; CXCL10: C-X-C motif chemokine 10; DSTN: Destrin; ENAM: Enamelin; EVPL: Envoplakin; FN1: Fibronectin; GRB2: Growth factor receptor-bound protein 2; HNF1A: Hepatocyte nuclear factor 1-alpha; HPR: Haptoglobin-related protein; HSPA1A: Heat shock 70 kDa protein 1A; ITGA2: Integrin alpha-2; ITGA2B: Integrin alpha-II b; ITGA5: Integrin alpha-5; ITGAM: Integrin alpha-M; ITGB1: Integrin beta-1; ITGB2: Integrin beta-2; ITGB3: Integrin beta-3; LGALS1: Galectin-1; MAX: Protein max; MMP8: Neutrophil collagenase; MYC: Myc proto-oncogene protein; MYOC: Myocilin; POLD1: DNA polymerase delta catalytic subunit; PRSS1: Trypsin-1; SERPINF2: Alpha-2-antiplasmin; SHC1: SHC-transforming protein 1; TAF1: Transcription initiation factor TFIID subunit 1; TANK: TRAF family member-associated NF-kappa-B activator; TGFBR1: TGF-beta receptor type-1; TNR: Tenascin-R; TRB@: T-cell receptor beta; TRIM63: E3 ubiquitin-protein ligase TRIM63; UGCG1: UDP-glucose:glycoprotein glucosyltransferase 1; VCAN: Versican core protein; VIM: Vimentin; AFM: Afamin; CD5L: CD5 antigen-like; CLCA1: Calcium-activated chloride channel regulator 1; CLEC14A: C-type lectin domain family 14 member A; DPEP1: Dipeptidase; FAM151A: Protein FAM151A; FAM3C: Protein FAM3C; GGT6: Gamma-glutamyltransferase 6; GLB1: Beta-galactosidase; HAVCR2: Hepatitis A virus cellular receptor 2; KIAA0753: Uncharacterized protein KIAA0753; LGALS9B: Galectin-9B; MBL: Mannose-binding lectin; MMP-7: Matrilysin; MRC2: C-type mannose receptor 2; PGA4: Pepsin A-4; PI16: Peptidase inhibitor 16; RTN4RL2: Reticulon-4 receptor-like 2; SERPINA2P: Putative alpha-1-antitrypsin-related protein; SHISA5: Protein shisa-5; VASN: Vasorin.

Table 2 Ongoing proteomic studies on rejection in renal transplant patients

Study identifier and title	Aim	Institution/PI	Single/ multi-centre	Patients	Study start	Estimated primary completion	Status of the study
NCT01515605 Molecular biological and molecular genetic monitoring of therapy after kidney transplantation	Analysis of GATA3, GATA4, GAPDH, TRPC3, TRPC6, granzyme B, perforin, FOXP3, ISG15, Mx1, MMP-3, MMP-9 in blood cells, proteomic analysis of urine, tissue analysis in a longitudinal fashion. Correlation of these parameters to the outcome	Odense University Hospital, Denmark	NR	1000	January 2011	March 2014	Unknown
NCT01315067 Non-invasive diagnosis of acute rejection in renal transplant patients using mass spectrometry of urine samples - a multicentre diagnostic phase III trial ^[62]	Phase III in-place validation of a pre-defined, published urinary peptide panel for acute TCMR against the current standard allograft biopsy ^[43]	Hannover Medical School, Germany	Multi	600	October 2011	December 2015	Active, not recruiting
NCT01531257 Proteogenomic monitoring and assessment of kidney transplant recipients	Validation of a set of candidate molecules by urine proteomics, gene expression analysis of blood cells and graft biopsies in a longitudinal fashion with respect to AR and IFTA	Northwestern University, Chicago, Illinois, United States	Single	250	April 2010	April 2016	Recruiting
NCT01289717 Discovery and validation of proteogenomic biomarker panels in a prospective serial blood and urine monitoring study of kidney transplant recipients - transplant proteogenomics	Discovery and validation of candidate molecules by urine proteomics, gene expression analysis of blood cells and allograft biopsies in a longitudinal fashion with respect to AR and IFTA	National Institute of Allergy and Infectious Diseases; Northwestern University, Chicago, Illinois, United States	Multi	307	March 2011	June 2016	Active, not recruiting
NCT02463253 Correlation of molecular biomarkers with biopsy findings and outcomes in renal transplant recipients	Analysis of proteogenomic and proteomic biomarkers in relation to the biopsy diagnosis of acute rejection in a longitudinal fashion	University of California, Sacramento, California, United States	Single	50	April 2015	December 2016	Recruiting

All studies are prospective, observational cohort studies in adult patients. Preliminary reports have not been published yet. Except study NCT 01315067, all studies collect samples in a longitudinal fashion and examine additional markers obtained by genomic analysis of blood cells. PI: Principal investigator site; AR: Acute rejection; IFTA: Interstitial fibrosis and tubular atrophy; NR: Not reported.

Some of these goals may be not too far away on the horizon. Currently, a few ongoing studies might address some of the discussed issues (Table 2). All studies are prospective, observational cohort studies and all except one collect samples in a longitudinal fashion. Results are expected in 2015 and 2016. These studies will hopefully clarify which role proteomic markers for rejection might have in the future care of kidney transplant patients.

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Immunological aspects of liver cell transplantation

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Abstract

Within the field of regenerative medicine, the liver is of major interest for adoption of regenerative strategies due to its well-known and unique regenerative capacity. Whereas therapeutic strategies such as liver resection and orthotopic liver transplantation (OLT) can be considered standards of care for the treatment of a variety of liver diseases, the concept of liver cell transplantation (LCTx) still awaits clinical breakthrough. Success of LCTx is hampered by insufficient engraftment/long-term acceptance of cellular allografts mainly due to rejection of transplanted cells. This is in contrast to the results achieved for OLT where long-term graft survival is observed on a regular basis and, hence, the liver has been deemed an immune-privileged organ. Immune responses induced by isolated hepatocytes apparently differ considerably from those observed following transplantation of solid organs and, thus, LCTx requires refined immunological strategies to improve its clinical outcome. In addition, clinical usage of LCTx but also related basic research efforts are hindered by the limited availability of high quality liver cells, strongly emphasizing the need for alternative cell sources. This review focuses on the various immunological aspects of LCTx summarizing data available not only for hepatocyte transplantation but also for transplantation of non-parenchymal liver cells and liver stem cells.

Key words: Liver cell transplantation; Cell-based therapy; Hepatocyte transplantation; Transplant immunology; Regenerative medicine

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Core tip: Failure of durable engraftment of transplanted hepatocytes despite application of immunosuppression is mainly attributed to the remaining recipient's immune responses against these allogenic grafts. Immune responses significantly differ from those observed for transplantation of whole livers and other solid organs. Innate immunity in combination with adaptive immune responses by T- and B-cells have to be taken into account for liver cell transplantation-specific immunosuppressive strategies. Possible clinical solutions to these obstacles will involve new combinations of novel and established immunosuppressive and anti-inflammatory drugs, co-transplantation of other liver cell types or regulatory immune cells. In the future, also (syngenic) liver stem cells will be an option.

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INTRODUCTION

Liver cell transplantation (LCTx) constitutes a promising approach for the treatment of various acute and chronic liver diseases^[1,2] as well as surgically induced small-for-size syndrome^[3]. In addition, LCTx also offers the option for cell therapeutic intervention using genetically modified liver cells with repair functions introduced^[4].

Mature hepatocytes were regarded the most obvious cell type to be applied in LCTx since the hepatocyte itself has been identified as a central functional unit of the liver. Albeit established in many small animal models, state-of-the-art protocols for LCTx in humans still have not resulted in the expected clinical successes^[5,6]. Failure of durable engraftment of transplanted hepatocytes mainly can be attributed to the recipient's immune responses against these allogenic cells^[7] and the severe competition with fully integrated organ-resident cells in a non-preconditioned environment^[8]. Furthermore, despite of using immunosuppression, long-term graft acceptance after LCTx has not yet been achieved in humans^[9]. This is in contrast to established small animal models (mice and rats) for LCTx that often rely on the use of genetically modified animals^[10,11] and/or hepatotoxic damaging^[12] of the recipient liver for pre-conditioning but cannot be transferred to the clinics. The broad clinical use of LCTx is further hampered by limited proliferative capacities of currently applied primary human hepatocytes (PHH), and cells suitable for transplantation purposes under GMP compliant production procedures remain scarce^[13].

Consequently, considerable research efforts are ongoing to optimize clinical protocols for LCTx as well as to identify reliable sources of liver cells suitable for LCTx. Use of alternative cell types such as stem cells or

stem cell derived hepatocytes might not only solve the problem of shortage in donor organs for hepatocyte isolation but - also by including options for autologous cell transfer - could overcome the existing hurdle of graft rejection by the recipient's immune system.

Hepatocyte rejection has been an underestimated problem, since from experiences with whole liver transplantations, the liver is considered an immune-privileged organ: Animal studies demonstrated long-term survival of liver allografts without the need for immunosuppression in strain combinations that would rapidly reject kidney or cardiac allografts^[14,15]. In addition, patients usually require smaller doses of immunosuppressive drugs after orthotopic liver transplantation (OLT) compared to other solid organs^[16]. Thus, the initial assumption was that transplantation of allogenic hepatocytes would also profit from this immune-privilege defined as low alloreactivity against liver grafts. However, allogenic hepatocyte transplants were not "invisible" or resistant to the recipient's immune system since *in vivo* a rapid rejection of purified transplanted allogenic hepatocytes is observed^[17]. This discrepancy between a potentially tolerogenic organ, *i.e.*, the liver, and isolated hepatocytes implies that either other hepatic cells like stellate cells or liver sinusoid endothelial cells (LSEC) contribute to this liver-specific tolerance^[18] or that singularized hepatocytes lose their tolerogenic potential in an allogenic environment accompanied by an inflammatory process.

Therefore, detailed knowledge of the immune responses induced by transplanted liver cells is instrumental for an improvement of cell engraftment and long-term acceptance of liver cell grafts. Nevertheless, to date there is still only limited literature available on these issues. This review aims at summarizing the *in vitro* and *in vivo* data addressing the immunological aspects of LCTx.

CLINICAL APPLICATION AND OUTCOME

The experience with clinical application of hepatocyte transplantation in humans is still limited to about 140 cases^[19]. Hepatocyte transplantation has been performed as an alternative to OLT to treat inborn errors of liver metabolism, chronic or acute liver failure or to maintain liver function as a bridge to OLT^[20]. In the former case, most pediatric patients suffered from urea cycle defects like Ornithine transcarbamylase deficiency or Citrullinemia. Clinical observation of these transplanted individuals demonstrated the safety of this procedure and patients showed clinical improvement and/or partial correction of the underlying metabolic disease. However, in the majority of the cases sustainable and significant benefits were not observed, and so far there is no report about a patient with a metabolic disease which has been completely cured^[21]. In patients with acute liver failure clinical improvement such as a reduction of ammonia and bilirubin levels

were observed, but the clinical outcome in the course of cell transplantation still was not significantly affected. In few individuals hepatocyte transplantation has been applied to treat patients with chronic liver disease: Here the outcomes likewise were very heterogeneous and overall comparable to the results reported for pediatric patients^[20]. Major hurdles hampering the success of hepatocyte transplantation seem to be rejection of transplanted cells by the recipient's immune system as well as insufficient engraftment of the donor cells within the recipient's liver.

TRANSPLANTATION OF PRIMARY HEPATOCYTES

Rejection of hepatocytes by the innate immune system

The innate immune system plays a critical role in the early immune response after hepatocyte transplantation. Both syngenic and allogenic transplanted liver cells have been shown to be targeted by the innate immune system in *in vitro* experiments^[22,23]. For further characterization of these immune responses experiments have been performed in mouse models showing that cells of the innate immune system such as granulocytes and macrophages cells infiltrate areas surrounding the transplanted hepatocytes in an early phase after transplantation (1-3 d)^[24]. Overall, it has been reported that up to 70% of transplanted hepatocytes may be eliminated by this initial innate immune response^[24]. Most interestingly, there were no differences in quantity or quality of infiltrating immune cells when comparing transplantation of allogenic vs syngenic hepatocytes, suggesting that stimulation by alloantigen does not seem to be a prerequisite for induction of an innate immune reaction. At present, three major mechanisms have been proposed which might explain this distinct phenomenon:

The first molecular mechanism postulated by Olszewski *et al.*^[25] suggests that uncovered intercellular surface adhesion molecules (cadherins) are recognized as "non-self" by granulocytes and monocytes/macrophages and subsequently provoke lysis of the transplanted cells. These adhesion molecules are hidden in the hepatic trabeculae and, thus, normally are inaccessible for immune cells in healthy liver tissue. However, they become exposed during the process of liver cell isolation applying collagenase for enzymatic digestion of the liver tissue and can subsequently be recognized by immune cells which, in turn, initiate the cytotoxic process leading to elimination of transplanted cells. Blocking of these molecules with monoclonal antibodies (mAb) resolved the effect in this experimental setting.

Bennet *et al.*^[26] described an additional mechanism termed "instant blood-mediated inflammatory reaction" (IBMIR), a reaction which has also been shown following islet cell transplantation^[26]. In their study with

fresh hepatocytes, they showed that PHH exposed to human blood induced a rapid loss of platelets from the blood, an extensive generation of thrombin-antithrombin complexes and a concomitant increase in the complement component C3a, followed by a drop in the polymorphonuclear leukocyte (PMN) count^[27]. Examination of the clots by confocal microscopy revealed infiltrating PMNs and platelets surrounding the PHH. This inflammatory reaction might explain why Kupffer cells are rapidly surrounding the transplanted cells after LCTx^[28]. Overall, this reaction with its main features resembled the IBMIR originally defined in clinical islet cell transplantation^[26].

The third mechanism was described by Gupta *et al.*^[24] assuming that portal occlusion by cell emboli of transplanted hepatocytes may induce perfusion-reperfusion injury, oxidative stress and impairment of cell viability. This, in turn, results in recruitment of inflammatory cells and eventually depletion of transplanted hepatocytes^[24]. This mechanism mainly leads to an activation of non-parenchymal cells such as Kupffer and stellate cells. In consequence, the survival of transplanted hepatocytes could be considerably increased *in vivo* by pretreatment of graft recipients with gadolinium chloride, known to significantly impair the function of Kupffer cells^[28].

Natural killer (NK) cells represent another key player of innate immunity. In the context of organ transplantation, NK cells were suggested for a long time to belong primarily to the first line of innate defence against pathogens and this pro-inflammatory effector concept was also applied for allograft rejection^[29]. NK cells have the potential of allo-specific recognition of transplanted cells by the so-called "missing self concept"^[30] which is based on the presence of inhibitory receptors specific for self-MHC that protect autologous tissue. In case of missing self-MHC molecules either in allogeneic situations or down-regulation of MHC by pathogens, the lack of protective inhibitory signals results in NK cell activation, *i.e.*, cytotoxicity and cytokine secretion. Despite this capacity of allorecognition, NK cells have not yet been investigated in hepatocyte transplantation and, therefore, their potential involvement in rejection of transplanted PHH remains to be defined.

More information is available for whole organ liver transplantation focusing rather on consequences of liver transplantation on NK cell repertoire and function than on a potential tolerogenic effect of PHH or other hepatic cells on NK cell alloreactivity. For example, alterations of the peripheral NK cell repertoire were observed in pediatric liver transplant recipients^[31]. A special role of the liver in NK cell generation was demonstrated by the observation of an infiltration of peripheral c-kit-positive NK cell precursors into the liver and the local development of an hepatic NK cell repertoire^[32]. Furthermore, donor NK cells derived from the grafted liver, *i.e.*, passenger leukocytes, were

detected in the periphery of pediatric liver recipients during the first month after transplantation^[33]. In a rat model of allogenic liver transplantation, no direct evidence for an involvement of donor-derived NK cells in liver transplant tolerance could be demonstrated^[34]. In addition, expression profiling of peripheral blood derived from tolerant liver transplant recipients revealed NK cell-related signatures in addition to other iron metabolism signatures^[35-37], suggesting that NK cells may rather be involved in an establishment of tolerance than in rejection of allogenic tissue. This differential view on the role of NK cells in organ and, especially in hepatocyte transplantation, demonstrates the need for further investigations of these innate immune cells in transplantation.

Rejection of hepatocytes by the adaptive immune system

In addition to the innate immune response, transplanted hepatocytes also face rejection mediated by the adaptive immune system, *i.e.*, T- and B-cells. Bumgardner *et al.*^[38] developed an animal model of hepatocyte transplantation to analyze rejection of transplanted cells *in vivo*. Hepatocytes of a transgenic mouse line expressing the human α -1-antitrypsin (*hA1AT*) gene were transplanted into the recipient by intrasplenic injection and the survival of the transgenic hepatocytes was determined by detection of secreted hA1AT protein in the recipient's serum. This group performed a series of experiments to characterize the rejection of allogenic hepatocytes: First, hepatocytes were transplanted into completely T-cell, selectively CD4⁺ or CD8⁺ T-cell, or B-cell deficient mice. Only recipients deficient of T-cells showed long-term survival of transplanted hepatocytes (> 16 wk). Transplantation of allogenic hepatocytes into recipients deficient of B-cells, CD4⁺ or CD8⁺ T-cells alone resulted in a loss of hA1AT by day 10 after transplantation^[38], demonstrating that immunologic rejection of allogenic hepatocytes is mediated primarily by T-cells.

T-cell mediated rejection and more specifically CD4⁺ T-cell mediated rejection is well known from transplantation of allogenic hearts and pancreatic islet allografts. Heart and islet allograft survival was significantly prolonged by treatment with anti-CD4-mAbs^[39,40], whereas the outcome of hepatocyte transplantation was not improved in this setting. When hepatocytes and heart allografts were transplanted simultaneously with a short-term medication of anti-CD4-mAbs, hepatocytes were destroyed by day 10 post-transplantation while most hearts survived more than 60 d^[41], further underlining the different intensity of graft rejection between solid organs and allogeneic hepatocytes.

To further dissect this T-cell response, allogenic hepatocytes were transplanted into mice pretreated with mAb against CD4, CD8 or the combination of both. The median survival time of hepatocytes in graft

recipients only pretreated with a single mAb against CD4 or CD8 showed a mean survival of only 10 and 14 d (10 d in the untreated control group), respectively. In recipients treated with the combination of anti-CD4-mAb and anti-CD8-mAb, hepatocyte survival was prolonged to approximately 35 d. This study confirmed that hepatocytes can be highly immunogenic and stimulate a strong cell-mediated immune response by both CD4⁺ and CD8⁺ T-cells^[42].

Also, when allogenic hepatocytes were transplanted into CD4 knock-out (KO) or CD8 KO mice without any further treatment, the mean survival time of transplanted cells were 10 and 14 d, respectively. However, when CD4 KO mice were treated with anti-CD8-mAb and CD8 KO mice with anti-CD4-mAb, respectively, hepatocellular allografts survived for 35 d in both groups. The reported studies collectively demonstrate that both CD4⁺ and CD8⁺ T-cells can independently promote hepatocyte rejection^[43].

As mentioned above, the importance of CD4⁺ T-cell mediated rejection is well known from other solid organ transplantation models^[39]. However, rejection of hepatocytes may also be initiated solely by CD8⁺ T-cells due to MHC class I -specific alloreactivity. When both CD4- and CD8-dependent pathways are available, the latter pathway seems to predominate, suggesting that direct MHC class I - and indirect MHC class II -specific T-cell activities may cooperate in hepatocyte rejection.

In concordance with these observations, Allen *et al.*^[44] reported about a patient with Crigler-Najjar syndrome type 1 undergoing hepatocyte transplantation. Despite initial successful engraftment of transplanted allogenic liver cells, there was a continuous loss of graft function due to strong CD8⁺ T-cell alloreactivity, predominately directed against a particular HLA class I alloantigen. Hence, in the absence of any evidence for humoral rejection, the authors concluded that cell-mediated rejection was the most likely cause of graft loss in this patient.

Bumgardner *et al.*^[17] summarized their experimental data to three possible mechanisms of hepatocyte allograft rejection. The first is a CD4⁺ T-cell dependent CD8⁺ T-cell mediated hepatocyte rejection. In this case, CD4⁺ T-cells become activated by host APCs and produce pro-inflammatory cytokines which permit activation and maturation of CD8⁺ precursor cytolytic effector T-cells (pCTL). These recognize MHC class I molecules on donor hepatocytes, become activated and target hepatocytes for apoptotic cell death *via* Fas/FasL, granzyme/perforin, TNF or other cytotoxic effector molecules.

The second mechanism is also CD8⁺ T-cell-mediated but CD4⁺ T-cell independent. CD8⁺ cytolytic T-cells directly recognize allogenic MHC molecules on donor hepatocytes. In a CD40-dependent process as substitute for CD4⁺ T-cell help, allospecific cytolytic T-cells are activated and target donor cells for apoptotic cell death also *via* the same mediators

mentioned above such as Fas/FasL, granzyme/perforin or TNF.

The third mechanism is CD8⁺ T-cell-independent CD4⁺ T-cell-mediated hepatocyte rejection. Donor hepatocyte MHC class I alloantigens are shed and subsequently scavenged by both host APC and host B-cells, which cross-present allogenic peptides *via* host MHC class II to host CD4⁺ T-cells in a B7 (CD80)- and CD40-dependent manner. CD4⁺ T-cells become activated and produce pro-inflammatory cytokines stimulating the activation and maturation of B-cells to produce alloantibodies that finally mediate the various mechanisms involved in antibody-mediated rejection.

Apart from T-cell mediated rejection, some data also suggest an involvement of humoral components, *i.e.*, antibodies, in rejection of allogenic hepatocytes. Horne *et al.*^[45] studied the acute damage of allogenic liver parenchymal cells by the CD4-dependent pathway and showed that this pathway is mediated by alloantibodies. This alloantibody-mediated acute rejection is targeting transplanted allogenic hepatocytes *via* macrophage-mediated cytotoxic immune damage^[46]. However, donor-reactive alloantibodies were only produced in significant quantities in hepatocyte recipients with lack of CD8⁺ T-cells or impaired cytotoxic effector mechanisms^[45].

Zimmerer *et al.*^[47] showed that CD4⁺ T-cells significantly upregulate IL-4 and downregulate IFN- γ in the absence of CD8⁺ T-cells. When CD4⁺ T-cells are transferred into CD8-depleted IL-4 KO mice that cannot produce any post-transplant alloantibodies on their own, high antibody levels are observed following hepatocyte transplantation, suggesting that IL-4-producing CD4⁺ T-cells are critical for post-transplant alloantibody production. In addition, CD8⁺ T-cells have the ability to reverse this IL-4-dominated cytokine profile by upregulating IFN- γ and, therefore, they can negatively regulate alloantibody production^[47]. Moreover, CD8⁺ T-cells also appear to directly downregulate alloantibody production by eliminating alloprimed B-cells through perforin- and FasL-mediated cytotoxicity^[48]. These data suggest that there might be a distinct subset of CD8⁺ cytotoxic T-cells that recognize primed B-cells and inhibit humoral rejection, which is an interesting paradox due to the previously reported CD8⁺ T-cell mediated rejection *via* the same cytotoxic molecules.

Horne *et al.*^[49] conclude that when hepatocytes activate both CD4- and CD8- dependent immune responses, the CD8-dependent response predominates CD4-dependent and B-cell-dependent immune pathways.

Role of co-stimulatory signals for rejection of allogenic hepatocytes

Effective T-cell activation on one hand requires antigen-specific signals to the T-cell receptor by the MHC/peptide complex on APCs and, on the other hand, depends on non-antigen-specific co-stimulatory signals to T-cells. The CD28/B7 (CD80) and CD40L/

CD40 co-stimulation pathways play critical roles in the activation of T-cells after allogenic transplantation of solid organs, kidney in particular, and their inhibition can lead to prolonged allograft survival^[50,51]. In kidney transplantation, costimulation blockade by a mutated fusion protein of CTLA-4-Ig (Belatacept/Nulojix[®]) was clinically approved with remarkable improved long-term outcome regarding kidney function^[52,53]. To determine the role of these co-stimulation pathways for the rejection of allogenic hepatocytes, mice were treated with either anti-CD40L-mAb or CTLA4-Ig to block CD40L/CD40 or CD28/B7 signaling, respectively. Administration of anti-CD40L-mAb caused significant prolongation of hepatocyte allograft survival whereas the application of CTLA4-Ig showed no significant effects. Thus, the CD40L/CD40 system plays a critical part in T-cell mediated rejection of allogenic hepatocytes, whereas the CD28/B7 co-stimulatory pathway may just play a subsidiary role^[54].

Gao *et al.*^[55] further studied the role of these co-stimulatory pathways in CD4 KO and CD8 KO mice and showed unexpectedly that treatment with CTLA4-Ig, ineffective in wildtype C57BL/6 mice, significantly prolonged the survival of allogenic hepatocytes in CD8 KO mice. These data implicate that both CD8⁺ and CD4⁺ T-cells may utilize the CD40L/CD40 co-stimulation pathway during hepatocyte rejection, but only the CD4⁺ T-cells also can promote rejection of hepatocytes *via* the CD28/B7 pathway^[55].

However, even the combination of CD28/B7 and CD40L/CD40 co-stimulatory pathway inhibition leads to only slightly prolonged survival of allogenic hepatocytes, while being capable of inducing immunologic tolerance to heart and pancreatic islet cell allografts. CD4⁺ and in particular CD8⁺ T-cells can still reject hepatocytes in absence of CD40L/CD40 signaling^[55], indicating that further co-stimulatory pathways may be involved in T-cell mediated rejection of hepatocytes.

One example for alternative co-stimulation pathways could be the blockade of LFA-1/ICAM-1 interaction that has been reported to prolong survival of several allografts and allogenic hepatocytes expressing ICAM-1. This adhesion molecule promoted the development of allospecific cytolytic effector T-cells (CTL) *in vitro* and *in vivo*, which could be inhibited by the application of anti-ICAM-1-mAb^[56,57].

Wang *et al.*^[58] demonstrated the importance of the LFA-1-mediated co-stimulatory pathway showing that 70% of the hepatocytes survived more than 60 d when transplanted into a CD4 KO mice with simultaneous suppression of LFA-1 signaling, pointing towards the importance of LFA-1 co-stimulation on CD8-dependent rejection. Moreover, targeting both the LFA-1/ICAM-1 pathway and CD40L/CD40 co-stimulation resulted in synergistic effects, thus, survival of hepatocytes could be achieved for more than 60 d in 100% of mice in both CD4- and CD8-dependent T-cell rejection^[58].

TRANSPLANTATION OF NON-PARENCHYMAL LIVER CELLS

The role of hepatic non-parenchymal cells for the induction of rejection or tolerance

As described above, hepatocytes can be acutely rejected *via* the innate and adaptive immune system, but at least in animal models, solid liver allografts are spontaneously accepted in many species without immunosuppression^[16]. This might suggest that liver non-parenchymal cells such as stellate cells, Kupffer cells and liver endothelial cells also could play an important role protecting allogenic hepatocytes from rejection.

Hepatic stellate cells

Hepatic stellate cells (HSC) are known to possess the ability to differentiate into myofibroblasts for the production of extracellular matrix leading to hepatic fibrosis^[59]. However, HSC have also demonstrated a strong T-cell inhibitory activity in *in vitro* and *in vivo* studies:

Charles *et al.*^[60] demonstrated *in vitro* that IFN- γ stimulated HSCs express B7-H1 (PD-L1), in a dose-dependent manner as well as produce the suppressive cytokines IL-10 and TGF- β . The formation of PD-1/PD-L1 complexes transmits an inhibitory signal which reduces the proliferation of CD8⁺ T-cells. Hence, HSCs can markedly inhibit T-cell responses elicited by either allogenic APCs or CD3/CD28-beads, which was associated with an increase in activated CD4⁺ and CD8⁺ T-cell apoptosis. In addition, the B7-H1-blocking antibody significantly reversed the inhibitory effect suggesting that inhibition *via* the PD-1/PD-L1 pathway plays an important role for the immunosuppressive effect of stellate cells^[60]. However, PD-L1 might not be the only relevant protein in this context, since neutralization of the latter by anti-B7-H1-mAb only partially reverses HSC-induced inhibition of T-cell proliferation^[60].

Yang *et al.*^[61] analyzed several death molecules in HSC by qPCR finding that only the TNF-related apoptosis-inducing ligand (TRAIL) was upregulated following IFN- γ stimulation. Moreover, they showed that HSCs from TRAIL KO mice largely lost their capacity to protect co-transplanted islet cell allografts. Thus, TRAIL might be involved in the immune-regulatory function of HSCs, which is likely mediated by TRAIL receptor-triggered death of activated T-cells^[61].

In addition, in a mouse model of islet cell transplantation, co-transplanted HSCs were seen to protect islet allografts from rejection^[62]. The underlying mechanism for this immunomodulatory effect seems to include not only elimination of activated specific CD8⁺ T-cells as shown by the *in vitro* studies stated above, but also expansion of regulatory T-cells (T_{reg}). The expansion of T_{reg} due to HSC co-transplantation cannot finally be explained by this study, but the

authors postulate that HSC influence APCs that process alloantigens from islet cells and induce T_{reg} in the draining lymphnodes^[63].

Recently, Dusabineza *et al.*^[64] showed that HSC can improve engraftment of PHH in a mouse model of transplantation of hepatocytes co-cultured with HSC into immunodeficient SCID mice. Due to the lack of T- and B-cells, adaptive immune responses have no influence in this setting. Nevertheless, co-transplantation of hepatocytes with HSC did not generate fibrosis but significantly improved hepatocyte engraftment, probably by supporting hepatocytes to cross the sinusoidal-endothelial barrier. The authors state that HSCs may protect hepatocytes from dying while entrapped in the sinusoidal network or promote adhesion to the endothelial wall. A further explanation could be that HSCs produce vasoactive peptides that may increase endothelial permeability and improve crossing and homing of hepatocytes^[64].

Kupffer cells

Kupffer cells are the largest population of tissue-resident macrophages and play an important role as tolerogenic APCs shown to induce tolerance after liver transplantation^[65,66]. However, from our knowledge, no data exists on the administration of allogenic Kupffer cells and the resulting immunological effects. Nevertheless, when Kupffer cells function as APCs, they have been described to either promote tolerogenic effects *via* IL-10 and TGF- β production and proliferation of T_{reg} or to enhance pro-inflammatory effects through the activation of NK T-cells *via* CD1-dependent antigen presentation^[67-70].

Furthermore, Kupffer cells are of special interest in the setting of ischemia/reperfusion injury after liver transplantation. In several studies, depletion of Kupffer cells was shown to worsen the transplantation outcome compared to control groups. This effect seems to correlate with the secretion of the potent anti-inflammatory cytokine IL-10 by Kupffer cells, which is necessary to balance the cytokine milieu towards Th₂-mediated protection^[71,72].

A possible role of Kupffer cells in LCTx thus needs to be evaluated in future studies.

LSEC

In a hemophilia KO mouse model (hemophilia A), Follenzi *et al.*^[73] demonstrated that LSEC have the capability to repopulate the livers of mice with healthy endothelial cells and to rehabilitate plasma factor VIII activity with correction of the bleeding phenotype. This study shows that transplantation of LSEC can be safely performed in a mouse model and that transplanted cells may integrate and function in the recipient's liver.

Multiple studies have shown an immunoregulatory effect of LSEC when functioning as APCs, for example during liver transplantation^[74]. *In vitro* studies have shown that allogenic LSEC possess an

immunoregulatory effect due to induction of allospecific T-cell hyporesponsiveness^[74,75]. Banshodani *et al.*^[76] also recently published *in vivo* experiments showing that LSEC also have immunoregulatory effects *via* the PD-1/PD-L1 pathway in a mouse model of LSEC transplantation.

In conclusion, many studies describe immunoregulatory effects of non-parenchymal liver cells, most often in the context of whole liver transplantation and chronic liver inflammation. In general, tissue based immunomodulation is a widespread property of many tissues. However, there are only few studies that analyzed the effect of allogenic transplanted non-parenchymal liver cells on the immune system with further studies urgently required.

TRANSPLANTATION OF STEM CELLS AND HEPATOCYTE-LIKE CELLS

Liver stem cells (LSC) can be seen as the optimal future source for LCTxs. On one hand, they would be capable to proliferate *in vitro*, thus, provide an unlimited cell source. On the other hand, if derived from patient's own liver biopsies and propagated *in vitro*, autologous liver stem cell transplantation could become a therapeutic option for a number of indications where the patients are not in acute need for cell and gene therapy - without any immunological complications as opposed to allogenic cell transplantation. Thus, intense research for (human) LSC are ongoing worldwide for more than 30 years without clinically useful definitions of a liver-specific stem cell phenotype. Also, numerous attempts are being made to derive transplantable, functional hepatocyte-like cells from other unlimited sources like embryonic stem (ES) or induced pluripotent stem (iPS) cells, so far with only moderate success^[77].

Recently, considerable progress was made regarding the transplantation of murine^[78] and the generation of potential human LSC^[79], own unpublished data). So far, only murine^[78] and rat^[80] LSC were successfully transplanted, albeit in autologous settings. Thus, no data exist so far regarding immunogenicity of allogenic LSC. However, some findings from allogenic stem cell transplantations in combination with other organ systems such as bone^[81], retinal epithelium^[82] and endothelium^[83] indicate at least immune-privileged properties of stem cells compared to mature tissue cells upon transplantation. At first thought, the reduced immunogenicity of transplanted stem cells appears to delay but not to prevent the onset of immune-recognition. The importance of the immature state is underlined by the observation that cell maturation during engraftment towards terminally differentiated cells is associated with a loss of their immune-privileged state. However, there is some evidence that tolerance, developed towards transplanted allogenic stem cells, extends later to their differentiated progeny^[84]. Furthermore, for epithelial tissue types

like the liver, transplanted cells might be immune-privileged initially during tissue repair (associated with full immune exposure), whereas later immunogenic properties on the surfaces of matured engrafted cells maybe partially invisible to the immune system within the fully reformed tissue.

Taken together, little is known about the potential effects of LSC transplantations with respect to immunological aspects and liver regeneration. Nevertheless, one can safely assume that allogenic LSC transplantation will certainly be associated with reduced immunological consequences as compared to transplantation of mature hepatocytes.

IMMUNOSUPPRESSION/IMMUNOMODULATION

Conventional immunosuppressive drugs

Most centers performing hepatocyte transplantation simply adapted protocols used for OLT, consisting of steroids and calcineurin-inhibitors (CNI) (Tacrolimus/Cyclosporin). Continuous and effective immunosuppression with CNI seems to be of particular importance since patients with low levels of Cyclosporin displayed acute rejection of transplanted hepatocytes^[85]. Several studies have demonstrated that CNI improve hepatic regeneration^[86,87] and the administration of Cyclosporin or Tacrolimus increased the mitotic index in the regenerating liver of adult rats^[88]. These effects seem to be even more important after hepatocyte transplantation as compared to OLT, since engraftment and proliferation of liver cells are fundamental for the success of LCTx. Immunosuppressive regimens without steroids or with low doses of CNI have been recommended, especially in patients affected by urea cycle disorders, because of their catabolic effects^[85]. The complete removal of CNI has been achieved by the addition of drugs such as mycophenolate mofetil (MMF) or mTOR-inhibitors such as Rapamycin. However, some data suggest that Rapamycin is associated with an increased risk of graft loss, death and sepsis after OLT when compared to the use of conventional-dose Tacrolimus alone^[89]. Furthermore, mTOR-inhibitors might inhibit liver regeneration^[90] and, therefore, could potentially delay hepatocyte engraftment and proliferation.

Wu *et al.*^[91] compared Tacrolimus, Rapamycin and MMF in a rat hepatocyte transplantation model and showed that mTOR-inhibition could be beneficial during the phase of engraftment of transplanted cells. However, it may be advisable to avoid Rapamycin or other mTOR-inhibitors during the anticipated period of transplanted cell proliferation. CNI and MMF could serve as alternatives during this phase of transplantation. Later, when proliferation of transplanted cells has been completed, Rapamycin could possibly be used again if required^[91].

As mentioned before, the co-stimulation blockade

has been clinically approved for kidney transplantation but not for other solid organ transplantations. Belatacept is a high affinity fusion protein that binds to B7.1 (CD80) and B7.2 (CD86) on human APCs. Regarding a possible tolerogenic effect of co-stimulation blockade using Belatacept for the use in OLT, no association with operational tolerance was observed^[92]. Since in animal experiments a beneficial effect of CTLA-4-Ig on CD4⁺ T-cell mediated rejection of hepatocytes *via* the CD28/CD80 (B7) pathway was found^[55], Belatacept, nevertheless, might be of interest for the use in LCTx and should be investigated in the future.

Novel anti-inflammatory drugs

After delivery of transplanted hepatic cells to liver sinusoids, several steps follow before cells are fully integrated in to the tissue architecture. During these steps, including entry into sinusoids and passage into the liver parenchyma, 70%-80% of initially transplanted cells are destroyed mainly due to sinusoidal effects, oxidative stress and cytokine-mediated toxicity^[13]. Novel strategies, hence, have been developed to optimize engraftment and minimize early hepatocyte cell loss early after transplantation. The majority of these strategies aims at pre-treating recipients prior to cell transplantation to either minimize the vascular and inflammatory changes induced by transplanted cells or to reduce the endothelial barrier between liver sinusoids and parenchyma or to activate HSC to release beneficial substances: The COX-2-specific inhibitors Naproxen and Celecoxib were shown to increase the number of engrafted hepatocytes by activation of HSC. These drugs induce HSC to express cytoprotective genes, vascular endothelial and hepatocyte growth factor, matrix-type metalloproteinases and tissue inhibitor of metalloproteinase-1, which regulate hepatic remodeling^[93].

Furthermore, transplanted hepatocytes promote IBMIR and, therefore, the treatment with anti-inflammatory drugs like the TNF antagonist Etanercept seems to downregulate this IBMIR. In a rat model of hepatocyte transplantation, Etanercept showed beneficial effects by blocking the synthesis of inflammatory cytokines, chemokines as well as their appropriate receptors leading to enhanced cell survival and engraftment of transplanted cells into the recipient's liver^[94]. Similar to Etanercept, the dual endothelin-1 receptor blocker Bosentan improves cell engraftment, independently of hepatic ischemia or inflammation, but without improving liver repopulation. However, incubation of hepatocytes with Bosentan protected cells from cytokine toxicity *in vitro* and produced superior cell engraftment and proliferation *in vivo*^[95].

Immunomodulation with Treg

To prevent rejection in hepatocyte transplantation currently continuous treatment with immunosuppressive medication is needed, which may be harmful due to

nephrotoxicity, increased risk of infections and cancer just to name the most important ones. Furthermore, despite the use of potent immunosuppressive agents, acute rejection remains the major cause of early allograft loss not only in solid organ transplantation but also in hepatocyte transplantation. An immunomodulatory regimen which improves patient and allograft survival and reduces the need for immunosuppressive drugs would be optimal and cell therapeutic approaches may be able to fulfill these requests. There are a number of lymphoid cell types with regulatory capacity that can promote tolerance induction in animal models of transplantation^[96]. Treg are the most widely studied and applied lymphoid cells for an immunomodulatory regimen. CD4⁺CD25⁺FoxP3⁺ Treg could be proven to control autoimmunity, inhibit graft versus host disease (GVHD) and prevent or delay allograft rejection in animal models^[97,98]. However, there are no studies concerning the effect of Treg in the context of hepatocyte transplantation. The only data available come from solid liver transplant studies in animals and human patients.

In a liver transplant rat model, Pu *et al.*^[99] could show that the adoptive transfusion of *ex vivo* donor alloantigen-stimulated CD4⁺CD25⁺ Treg combined with short-term Tacrolimus treatment prolonged the survival of liver allografts.

In humans, the frequency of circulating Treg is significantly decreased during acute rejection of liver allografts^[100]. Pediatric patients who achieved operational tolerance after liver transplantation showed increased levels of circulating Treg compared to patients who received immunosuppression^[101]. Therefore, an increased level of circulating Treg may be beneficial in particular for liver allograft survival. Yamashita *et al.*^[102] just recently conducted a clinical trial applying the infusion of donor antigen-driven Treg in 10 patients undergoing living donor liver transplantation. In 6 patients, immunosuppression was successfully withdrawn without causing allograft rejection and graft function was well maintained which may represent a landmark study for clinical application of cell therapy with Treg^[102].

In conclusion, the data from liver transplanted patients emphasizes that Treg could also have immunomodulatory potentials in hepatocyte transplantation.

CONCLUSION

Despite current hurdles concerning the engraftment and long-term acceptance of cellular allografts, LCTx still represents a very promising tool for the treatment of various liver diseases in the near future. Deeper knowledge of the immunological responses induced by transplanted cells though is a prerequisite for the success of this therapeutic approach. The available data clearly demonstrate that rejection of liver cell allografts is by far more complex than initially assumed and, most importantly, differs considerably from those

immune reactions observed following solid organ transplantation. Further immunological investigations *in vivo* and *in vitro* are desperately required - especially human data are still scarce.

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Update on the treatment of focal segmental glomerulosclerosis in renal transplantation

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Abstract

Focal segmental glomerulosclerosis (FSGS) represents one of the most severe glomerular diseases, with frequent progression to end-stage renal disease and a high rate of recurrence in renal allografts (30%-50%). Recurrent FSGS portends a negative outcome, with the hazard ratio of graft failure being two-fold higher than that of other glomerulonephritis. Two patterns of clinical presentations are observed: Early recurrence, which is characterized by massive proteinuria within hours to days after implantation of the renal graft, and late recurrence, which occurs several months or years after the transplantation. Many clinical conditions have been recognized as risk factors for recurrence, including younger age, rapid progression of the disease to end-stage renal disease on native kidneys, and loss of previous renal allografts due to recurrence. However, much less is known about the incidence and risk factors of the so-called "*de novo*" type of FSGS, for which sufferers are transplanted patients without disease on native kidneys; but, rapid development of allograft failure is frequently observed. Management of both forms is challenging, and none of the approaches proposed to date have been demonstrated as consistently beneficial or effective. In the present review we report an update on the available therapeutic strategies for FSGS in renal transplantation within the context of a critical overview of the current literature.

Key words: Focal segmental glomerulosclerosis; Kidney transplantation; Permeability factors; Plasma exchange; Rituximab

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Core tip: Focal segmental glomerulosclerosis (FSGS) presents as a histological pattern of kidney damage

with different, multifactorial, and often undefined pathogenesis. Primary FGSS represents one of the most severe glomerular diseases, with frequent progression to end-stage renal failure and a high rate of recurrence in renal allografts. FSGS recurrence also portends a negative outcome. Despite the proposal of multiple therapeutic approaches, none has emerged as the resolutive option. This review provides an update on the currently available therapeutic strategies for FSGS in renal transplantation, along with a critical overview of the related literature.

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INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) presents as a histological pattern of kidney damage with different, multifactorial, and frequently undefined pathogenesis. FSGS represents one of the most serious glomerular diseases, with frequent progression to end-stage renal disease and a high rate of recurrence in renal allografts. Clinical classification includes the following five forms^[1]: Primary or idiopathic FSGS, the etiology of which is largely unknown; secondary or adaptive FSGS, which commonly refers to an adaptive response to glomerular hypertrophy/hyperfiltration and which presents a nonspecific pattern of scarring due to a previous injury; genetic FSGS; drug-induced FSGS; virus-associated FSGS.

In renal transplanted patients, both primary and secondary FSGS are observed. For the primary form, recurrent and *de novo* types are more severe. Obtaining an accurate estimation of *de novo* FSGS occurrence, however, is challenging because of the high rate of renal diseases of unknown cause in native kidneys (15.6% and 18.2% in the OPTN-SRTR annual report and ERA-EDTA registry, respectively)^[2,3]. FSGS recurrence occurs frequently after transplantation, with reported rates ranging from 30% to 50%^[4-6]. The risk of recurrence is substantially higher (up to nearly 100%) in patients who lost their first graft due to a recurrence^[7]. Recurrent FSGS portends a negative outcome, with the hazard ratio (HR) of kidney failure being 2.03 compared to other kinds of recurrent glomerulonephritis^[8]. Two patterns of clinical presentations are observed: Early recurrence, which is most commonly encountered in pediatric patients and characterized by a massive proteinuria that occurs within hours to days after implantation of the new kidney; late recurrence, which often develops insidiously at several months to years after the transplantation^[9].

Many clinical conditions have been recognized as

risk factors for recurrence^[4,8,10], including younger age (particularly in children who were > 6-year-old at FSGS onset), mesangial proliferation in the native kidneys, rapid progression of the disease to end-stage renal disease (ESRD; < 3 years from onset) for native kidneys, pre-transplant bilateral nephrectomy, non-African race, specific genetic background, heavy proteinuria before transplantation, and, as cited above, loss of previous allografts due to recurrence.

Update on pathogenetic mechanisms

Several lines of evidence have suggested that proteinuria and glomerular histologic alterations can be mediated by the direct activity of a circulating factor. These data were obtained from *ex vivo* analysis of glomerular changes after incubation with serum from patients with FSGS, as firstly described by Sharma *et al*^[11] in 1999, as well as from analysis of animal models in which kidneys from a specific line of affected mice showed recovery from FSGS after transplantation into normal mice^[12]. The most striking data, however, was obtained from a study of a kidney with FSGS recurrence that had been re-grafted from a patient to another and led to total regression of the disease^[13]. However, identification of the responsible factor(s) is still a matter of investigation, although some different molecules are considered likely candidates.

In recent years research interest has focused on the soluble form of the urokinase type plasminogen activator receptor (suPAR). suPAR appears to be able to cause podocyte foot effacement in mice^[14], and suPAR levels observed in patients with FSGS are higher than those in patients with other glomerulopathies^[15]. Nevertheless, the specific involvement of suPAR in glomerulonephritis has not been confirmed by other studies, which showed increased (plasma) suPAR levels in other pathological situations (*i.e.*, bacterial and viral infections, sepsis, and cancer)^[16]. Rather, increased suPAR levels were observed primarily in patients with reduced glomerular filtration rate (GFR), suggesting that an elevation of suPAR levels may merely be an indicator of reduced GFR^[17]. Finally, the usefulness of suPAR to distinguish between FSGS and non-FSGS glomerulonephritis has been questioned by Bock *et al*^[18], who showed similar (plasma) suPAR levels among FSGS patients, non-FSGS controls, and healthy volunteers.

Other circulating factors, such as cardiotropin-like cytokine 1 (CLC-1), vasodilator-stimulated phosphoprotein and apolipoprotein A-I, have also been proposed as effectors in the glomerular permeability process, but their clinical and pathological roles remain unknown^[19]. Recently, detection of a panel of serum antibodies directed towards podocyte antigens was found to be associated with a high percentage of relapses in FSGS (predictive recurrence value of 92%)^[20]. The most prominent of these antigens is CD40; the expression of which is up-regulated in podocytes in FSGS, supporting the hypothesis of

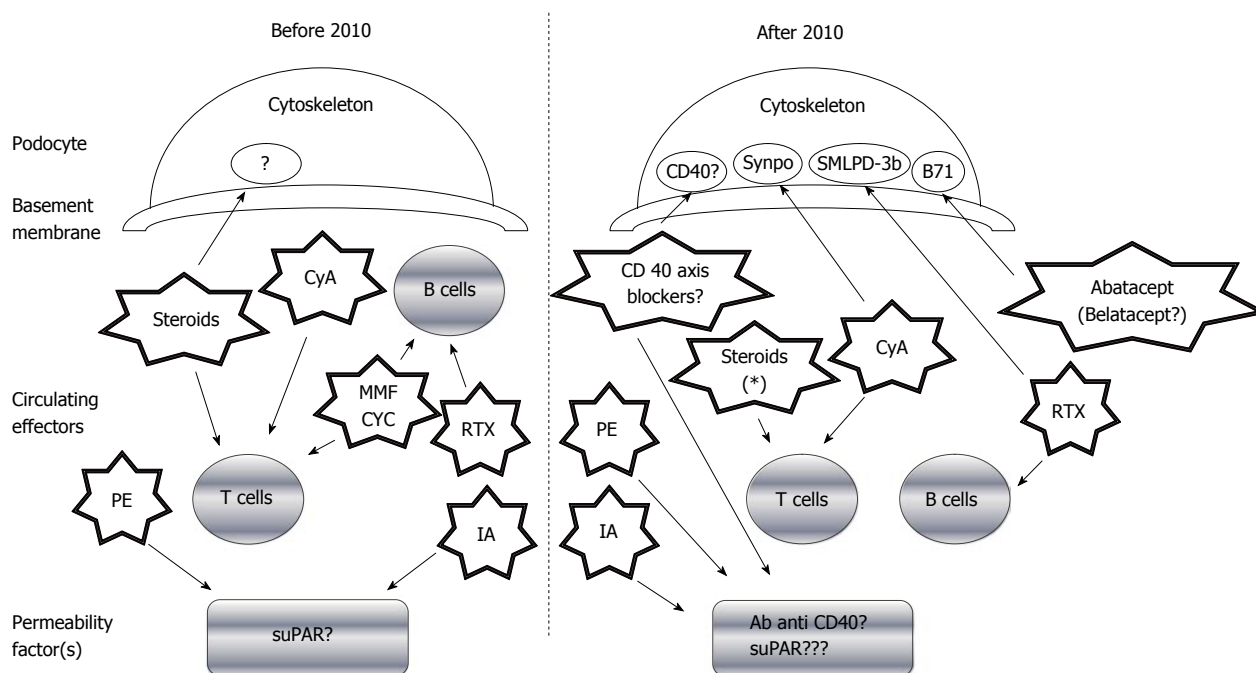


Figure 1 Evolution of the therapeutic approaches for focal segmental glomerulosclerosis recurrence and related recent perspectives. The various treatments and their mechanisms are represented by the star shapes. CyA: Cyclosporine; PE: Plasma exchange; MMF: Mycophenolate mofetil; suPAR: Soluble form of the urokinase type plasminogen activator receptor; CYC: Cyclophosphamide; RTX: Rituximab®; IA: Immuno-adsorption; Synpo: Synaptopodine; SMLPD-3b: Sphingomyelin-phosphodiesterase-acid-like-3b protein. Note: Steroids also regulate the podocyte activity of stabilizing the actin cytoskeleton, preserving glomerular permselectivity, and directly reducing apoptosis via the PI3K/Akt signaling pathway.

a potential direct pathogenetic effect of anti-CD40 antibodies.

Demonstration of the precise permeability factor(s) remains elusive. Yet, recent findings have confirmed the critical role played by podocytes in FSGS development, and different podocyte antigens/cellular pathways have been associated with the disease course and medical treatment response (Figure 1). For example, it has been postulated that the B71 and sphingomyelin-phosphodiesterase-acid-like-3b (SMLPD-3b) proteins (both of which are expressed on the podocyte membrane) may directly interact with the cytoskeleton-inducing foot process effacement in response to a permeability factor^[21,22]; interestingly, this effect could be antagonized by some drugs recently adopted in FSGS treatment [abatacept (Orencia®)/belatacept (Nulojix®)] for B71 and Rituximab® for SMLPD-3b, in particular), as outlined below in the therapeutic section.

Drug-induced or genetic-related alterations of the podocyte metabolic pathways may also lead to a maladaptive response to cell injury, defining a “pro-FSGS” phenotype, as has been observed in some patients with specific donor APOL1 polymorphisms^[23] or in animal models with inhibition of the mTOR/Akt axis^[24].

Another step forward in defining this disease may be achieved upon increasing our knowledge of the influence of micro (mi)RNAs on podocyte activity. In a mouse model, Gebeshuber *et al.*^[25] observed that transgenic expression of miR-193a (a down-regulator of WT1, itself a crucial effector in podocyte

homeostasis) rapidly induces FSGS and observed up-regulated expression of miR-193a in isolated glomeruli from individuals with FSGS, as compared to kidney levels in healthy individuals or individuals with other glomerular diseases.

In addition to the probably pivotal role of podocytes in the disease process, it is also likely that T and B cells of the immune system contribute to FSGS development. A Th2 phenotype is commonly observed in patients with idiopathic nephrotic syndrome (NS)^[26], and overexpression of IL-13, a characteristic Th2 cytokine, is associated with significant proteinuria in Wistar rats^[27]. An indirect confirmation of B cell involvement derives from evidence showing a selective Rituximab®-induced depletion is correlated to disease remission^[28]. This association has recently been questioned, however, so the role of B cells in FSGS pathogenesis is still not well defined.

OVERVIEW OF CURRENT FSGS TREATMENTS

FSGS treatment in renal transplantation, both for recurrent and *de novo* types, is a significant clinical challenge. Unfortunately, most of the reports consist of few cases or even a single case. Studies of the available strategies are few and have shown unclear and conflicting results for each, possibly due to their retrospective nature, uncontrolled design and limited number of enrolled patients or short follow-up periods. Consequently, while experimental studies have pro-

vided major advancements in our knowledge of the pathophysiology of FSGS, the treatment remains largely empirical. Some interesting preliminary data about the use of novel therapies are emerging, but they need further evaluation and validation. Therapeutic indications for *de novo* idiopathic and non-idiopathic FSGS are even more elusive^[29].

Here, we summarize the most frequently reported available strategies for the management of recurrent and *de novo* FSGS, and suggest the potential benefit of these emerging therapies (summarized in Table 1).

Plasma exchange

The adoption of plasma exchange (PE) for treatment of FSGS recurrence has been based on the hypothesis of the presence of circulating factor(s) that could be removed in order to treat or prevent the disease. Despite research into this causative factor remaining in a status of "cold case", PE is still a cornerstone in FSGS recurrence treatment, since the 1985 report of its first positive application by Zimmerman^[30]. A systematic review by Ponticelli^[4] showed that PE promotes partial or complete remission in 70% of children and 63% of adults with FSGS recurrence. Most of the analyzed studies, however, are limited by their retrospective or uncontrolled design.

Adoption of PE in a pre-emptive protocol to reduce FSGS recurrence has been described by Gohh *et al.*^[31] in one of the few prospective studies in the literature. Ten transplanted patients with FSGS and at high risk of recurrence (both children and adults, including 5 transplants from living donors and 5 from deceased donors) were treated with a course of 8 PE sessions in the peri-operative period. Seven of the patients (including all 4 who received first grafts and 3 out of 6 who had prior recurrence) were free of recurrence at the end of follow-up (range of 238-1258 d). The use of pre-emptive PE in a high risk pediatric patient who underwent a second living kidney transplantation (the first kidney was lost due to recurrence) was more recently described by Chikamoto *et al.*^[32]. The patient had also received a 2-wk course of Rituximab® (375 mg/m²; 2 doses), methylprednisolone (1 mg/kg per day), tacrolimus (10 ng/mL) and mycophenolate mofetil (MMF) (600 mg/m² per day) before transplantation; at 12 d before transplantation, 4 PE sessions were performed. No sign of recurrence was found in protocol biopsies at 8 mo after transplantation.

Canaud *et al.*^[33] described positive outcome (complete remission at 3 mo after diagnosis) for 10 patients with FSGS recurrence that had been treated with a 9-mo course of intravenous cyclosporine (CyA; C₀ levels at 200-400 ng/mL), followed by oral CyA (C₂ levels at 1200-1400 ng/mL), high dose oral steroids (1 mg/kg per day for the first 4 wk, then progressively tapered) and a course of PE sessions. The only patient who experienced recurrence of

proteinuria after post-transplant year 1, concurrent to PE frequency reduction, had been successfully treated with Rituximab® (2 doses) and PE sessions bimonthly, obtaining a complete proteinuria remission in the 34 ± 6.7 mo of follow-up.

A positive effect is also described for plasma absorption in some papers^[34-37], but further studies are needed to define the potential additive benefit in comparison with PE.

Glucocorticoids

KDIGO guidelines suggest for FSGS on native kidneys a 4-wk to 16-wk course of prednisone (1 mg/kg per day, with a maximum of 80 mg and a slow tapering in the 6 mo after remission)^[38]. Glucocorticoids may act to stabilize the actin cytoskeleton, thereby preserving glomerular permselectivity^[39] and directly reducing podocyte apoptosis *via* the PI3K/Akt signal pathway^[40]. Efficacy of steroid treatment in recurrent/*de novo* FSGS has never been evaluated in a randomized trial; on the other hand, considering its pivotal therapeutic role in FSGS on native kidneys, many different regimens have included steroids in post-transplantation FSGS treatment.

Apart from the paper by Canaud *et al.*^[33], who described a combined treatment of CyA in association with high dose steroids and PE, Shishido *et al.*^[41] also reported a favorable outcome (7/10 complete remission) for pediatric patients with FSGS recurrence in response to a combined treatment with methylprednisolone pulses (20 mg/kg after diagnosis on 3 consecutive days in weeks 1, 3 and 5) and an increase in CyA target levels (area under the curve₀₋₄ 4500-5500 ng/h per milliliter for the first month, 4000 ng/h per milliliter for the next 2 mo, and 3000 ng/h per milliliter thereafter).

Cyclosporine

CyA is commonly applied for the treatment of several immune-mediated diseases and as a second-line therapy for steroid-resistant/dependent FSGS on native kidneys^[38]. Conversely, CyA does not appear to prevent post-transplant FSGS recurrence when given as a part of the initial immunosuppressive regimen^[42,43]; although, this potential has not been evaluated in more recent studies. Standard oral doses of CyA have not been associated with reduced incidence of recurrent FSGS. Nonetheless, higher intravenous doses have been associated with remission of proteinuria for the first time since reported by Ingulli *et al.*^[44] 25 years ago.

Overall, limited evidence has supported the administration of high dose CyA to achieve remission of FSGS recurrence with a persistent effect^[45,46]. Salomon *et al.*^[45] reported a remission of recurrent proteinuria in 14/17 (82%) of children following administration of intravenous CyA (mean period of 21 d; range of 250-350 ng/mL); after 4 years, 11/17 (64%) patients

Table 1 Therapeutic strategies for focal segmental glomerulosclerosis in renal transplantation

	Treatment schedule	Patients	Outcome	Adjunctive information
Plasma exchange Ponticelli <i>et al</i> ^[4]	Analysis of PE response in 22 studies	144 patients (70 < 18 yr, 77 ≥ 18 yr)	Partial/complete remission of proteinuria in 49/70 (70%) children and 49/77 (63%) adults	Analysis also includes Canaud <i>et al</i> ^[33] 10 patients
Gohh <i>et al</i> ^[31]	Prophylactic course of 8 PE sessions in the peri-operative period in patients at high risk of recurrence	10 patients (1 < 18 yr, 9 ≥ 18 yr)	7/10 free of recurrence	
Chikamoto <i>et al</i> ^[32]	Prophylactic course of 4 PE sessions 12 d before transplantation in a high risk patient	1 patient (< 18 yr)	No recurrence after 8 mo	Patient also received Rituximab® (375 mg/m ² ; 2 doses), methylprednisolone (1 mg/kg per day), tacrolimus (10 ng/mL) and mycophenolate mofetil (600 mg/m ² per day) 2 wk before transplantation
Glucocorticoids Shishido <i>et al</i> ^[41]	Methylprednisolone pulses (20 mg/kg on three consecutive days in weeks 1, 3 and 5) and increasing CyA target levels	10 patients (8 < 18 yr, 2 ≥ 18 yr)	Complete remission in 7/10	
CyA Canaud <i>et al</i> ^[33]	Intravenous CyA (C0 levels between 200-400 ng/mL), followed by oral CyA (C2 levels 1200-1400 ng/mL), high dose oral steroids (1 mg/kg per day for the first 4 wk, then progressively tapered) and a course of PE sessions for 9 mo	10 patients (≥ 18 yr)	Complete remission of proteinuria in 10/10; proteinuria relapse in 1/10 successfully treated with Rituximab® (2 doses)	
Ingulli <i>et al</i> ^[44]	Progressive up-titration of CyA oral doses	2 patients (< 18 yr)	Complete remission in 1; partial remission in 1	
Salomon <i>et al</i> ^[45]	Intravenous CyA (through levels: 250-350 ng/mL)	16 patients (< 18 yr; 1 re-grafted with a subsequent recurrence)	Complete remission in 14/17 (82%); partial remission in 2/17 (12%)	
Raafat <i>et al</i> ^[46]	Progressive up-titration of CyA oral doses until proteinuria reduction/serum creatinine elevation (CyA doses from 6 to 25 mg/kg per day)	16 patients (< 18 yr)	Complete remission in 11/16 (69%); partial remission in 2/16 (12%)	
CYC/MMF Kershaw <i>et al</i> ^[53]	CYC (1-2 mg/kg per day, adjusted for white blood cell count) for 8-12 wk	3 patients (< 18 yr)	Complete remission in 2/3; partial remission in 1/3	
Cheong <i>et al</i> ^[54]	CYC (2 mg/kg per day) + PE (10 sessions over 2 wk followed by one session per week for 2 mo)	6 patients (< 18 yr)	Complete remission in 3/6; partial remission in 3/6	
Dall'Amico <i>et al</i> ^[55]	CYC (2-mo course, 2 mg/kg per day) and PE sessions	11 patients (< 18 yr)	Complete remission in 9/11 (persistent remission in 7/9)	
Gipson <i>et al</i> ^[57]	12-mo course of CYC vs MMF + dexamethasone	138 patients [93/168 (67%) < 18 yr]	CyA arm: complete remission in 14/72 (19%), partial remission in 19/72 (26%) MMF + dexamethasone arm: complete remission in 6/66 (9%), partial remission in 16/66 (24%)	
Renin angiotensin system blockers Freiberger <i>et al</i> ^[62]	Ramipril (10 mg) + candesartan (64 mg) + aliskiren (300 mg)	1 patient (≥ 18 yr)	Partial remission	Patient was previously treated with Rituximab® (375 mg/m ² ; 3 doses) and PE without response
Galactose Jhaveri <i>et al</i> ^[64]	High galactose diet + supplemental powder galactose (0.2 g/kg orally 2 times per day) one month later	1 patient (≥ 18 yr)	Complete remission	No apparent response with previous treatments including Rituximab® (1 g, 2 doses), PE (15 sessions) and IgEv (2 doses)

Robson <i>et al</i> ^[65]	High galactose diet (14 g twice daily in patient 1, 10 g twice daily in patient 2)	2 patients (≥ 18 yr)	Complete remission in 1; partial remission in 1	
Sgambat <i>et al</i> ^[66]	High galactose diet (0.2 g/kg per dose twice daily orally)	7 patients (< 18 yr) with steroid-resistant nephrotic syndrome (2/7 with recurrent FSGS)	Reduction in permeability factor without effect on proteinuria values	
Anti-TNF- α agents Leroy <i>et al</i> ^[69]	Infliximab (3 mg/kg twice monthly)	1 patient (< 18 yr)	Complete remission	No apparent response with previous treatments including reinforced immunosuppression, CyA (5 mg/kg per day in continuous <i>i.v.</i> perfusion) followed by oral high dose CyA (10 mg/kg per day), methylprednisolone pulses followed by high dose oral prednisone (60 mg/1.73 m ² per day), MMF (600 mg/1.73 m ² per day) switch to cyclophosphamide (100 mg/d, interrupted for hematologic toxicity) and PE (15 sessions within 1 mo)
Bitzan <i>et al</i> ^[70]	Etanercept (twice weekly)	1 patient (< 18 yr)	Partial remission	
Rituximab [®] Pescovitz <i>et al</i> ^[28]	Rituximab [®] (6 doses, 375 mg/m ²)	1 patient (< 18 yr)	Complete remission	
Hristea <i>et al</i> ^[74]	Rituximab [®] (2 doses, 375 mg/m ²)	1 patient (≥ 18 yr)	Complete remission	Patient also received a short course of oral cyclophosphamide (100 mg/d, days 22-40) and 3 additional PE sessions (days 34, 39, 49)
Gossmann <i>et al</i> ^[75] Fornoni <i>et al</i> ^[21]	Rituximab [®] (2 doses, 375 mg/m ²) Rituximab [®] within 24 h after surgery (1 dose, 375 mg/m ²) in patients at high risk of recurrence	1 patient (≥ 18 yr) 41 patients (14 controls <i>vs</i> 27 treated)	Complete remission Nephrotic proteinuria within 1 mo in 7/27 patients in Rituximab [®] group <i>vs</i> 9/14 patients in control group ($P < 0.005$)	Patient mean age: 12.3 \pm 5.2 yr (control group), 15.0 \pm 5.5 yr (Rituximab [®] group)
Audard <i>et al</i> ^[76]	Rituximab [®] induction in patients at high risk of recurrence (first graft lost due to recurrence)	4 patients (≥ 18 yr)	No evidence of significant proteinuria at the end of follow-up	Single dose of 75 mg/m ² in 2/4 patients, repeated dose of 375 mg/m ² on day 7 in the remaining 2 patients; associated PE sessions (6 and 15, respectively) in 2/4 patients
Hickson <i>et al</i> ^[77]	Rituximab [®] (375 mg/m ² ; 2-4 doses) + PE	4 patients (3 < 18 yr, 1 ≥ 18 yr)	Complete remissions in 4/4 patients	Early Rituximab [®] treatment in 3/4 (7-63 d post-transplantation), late treatment in 1/4 (982 d post-transplantation during a prolonged PE-dependent remission)
Dello Strologo <i>et al</i> ^[78]	Rituximab [®] (375 mg/m ² ; 1-4 doses) + PE	6 patients (4 < 18 yr; 2 ≥ 18 yr)	Complete remission in 3; partial remission in 2; no response in 1	1/7 patients received one dose, 4/7 patients received 2 doses, and 1/7 received 4 doses; 1/7 patients experienced a severe reaction during first infusion and was excluded from the analysis
Tsagalis <i>et al</i> ^[79]	Rituximab [®] (1 g, 2 doses) + PE	4 patients (2 < 18 yr; 2 ≥ 18 yr)	Complete remission in 2; partial remission in 2	
Cho <i>et al</i> ^[80] Yabu <i>et al</i> ^[87]	Rituximab [®] (100 mg, 1 dose) Rituximab [®] + PE	1 patient (≥ 18 yr) 4 patients (≥ 18 yr)	Complete remission No response or proteinuria relapse after Rituximab [®]	Rituximab [®] schedule: 1 g, 2 doses in 1/4; 375 mg/m ² , 4 doses in 1/4; 375 mg/m ² , 6 doses in 2/4
Kumar <i>et al</i> ^[117]	Rituximab [®] + PE	8 patients (< 18 yr)	Complete remission in 2/8; partial remission in 4/8; no response in 2/8	Rituximab [®] schedule: 375 mg/m ² , 4 doses in 4/8; 375 mg/m ² , 1 doses in 1/8; 375 mg/m ² , 3 doses in 1/8; 375 mg/m ² , 8 doses in 1/8; 375 mg/m ² , 10 doses in 1/8

Park <i>et al</i> ^[88]	Rituximab® (375 mg/m ² , 1 or 2 doses) before transplantation with or without PE	9 patients PE ± Rituximab® treated (Rituximab® group) vs 18 patients (control group)	No statistical difference in the prevention of recurrence between PE ± Rituximab® group (2/9, 22%) vs control group (5/18, 28%)	Rituximab® schedule: 375 mg/m ² , 1 dose for desensitization in high risk patients; 375 mg/m ² , 2 doses in ABO-incompatible transplantation; data not shown for recurrence prevention
Kamar <i>et al</i> ^[89]	Rituximab® (2-4 doses, 375 mg/m ²)	2 patients (≥ 18 yr)	Complete remission in 1 patient; no response in 1 patient	Rituximab® schedule: 75 mg/m ² , 2 doses in the first patient (a supplemental dose was repeated after proteinuria relapse in association with PE sessions, achieving a new complete remission); 375 mg/m ² , 4 doses in the second patient
El-Firjani <i>et al</i> ^[90]	Rituximab® (6 doses, 375 mg/m ²)	1 patient (≥ 18 yr)	No response	Proteinuria relapse in 1/3 patients with complete remission response to PE sessions intensification + an adjunctive dose of Rituximab®
Apeland <i>et al</i> ^[81]	Rituximab® (3 doses, 375 mg/m ²)	1 patient (≥ 18 yr)	Complete remission	
Grenda <i>et al</i> ^[82]	Rituximab® (4 doses, 375 mg/m ²)	1 patient (< 18 yr)	Complete remission	
Sethna <i>et al</i> ^[83]	Rituximab® (4 doses, 375 mg/m ²) + PE	4 patients (< 18 yr)	Complete remission in 3/4; partial and unsustained response in 1/4	
Prytula <i>et al</i> ^[91]	Rituximab® (1-5 doses, 375 mg/m ²)	14 patients (< 18 yr)	Complete remission in 6/14; partial remission in 3/14; no response in 5/14	Treatment was adopted after a diagnosis of post-transplant lymphoproliferative disorder. One patient received a single dose; the other patient, after achieving a complete remission with the first dose, experienced a proteinuria relapse and rapidly responded to a second Rituximab® dose
Stewart <i>et al</i> ^[92]	Rituximab® (4 doses, 375 mg/m ²)	1 patient (< 18 yr)	Complete remission	
Nozu <i>et al</i> ^[84]	Rituximab® (4 doses, 375 mg/m ²)	1 patient (< 18 yr)	Complete remission	
Nakayama <i>et al</i> ^[85]	Rituximab® (1-2 doses, 375 mg/m ²)	2 patients (< 18 yr)	Complete remission in 2 patients	Patients 1 and 2 received a single dose; patients 3 and 4 received 2 doses; patient 5 (the only one with FSGS on native kidneys) received 3 doses (days 1, 15, 30) and a dose monthly thereafter
Marks and McGraw ^[93]	Rituximab® (4 doses, 375 mg/m ² in one case; 2 doses 750 mg/m ² in the other one)	2 patients (< 18 yr)	No response	
Bayrakci <i>et al</i> ^[86]	Rituximab® (4 doses, 375 mg/m ²)	1 patient (< 18 yr)	Complete remission	
Rodríguez-Ferrero <i>et al</i> ^[94]	Rituximab® (4 doses, 375 mg/m ²)	3 patients (≥ 18 yr)	Partial remission in 2/3; no response in 1/3	
CTLA4-Ig (considered as the prevalent treatment) Yu <i>et al</i> ^[103]	Abatacept	4 patients (2/4 < 18 yr, 2/4 ≥ 18 yr) with FSGS recurrence; 1 patient (≥ 18 yr) with FSGS on native kidneys	Complete remission in 2/5; partial remission in 3/5	Patients 1, 2 and 4 received 2 abatacept doses; patient 3 received 1 abatacept dose; patient 5 was treated with belatacept
Alachkar <i>et al</i> ^[104]	Abatacept (1 dose; 10 mg/kg) in patient 1; belatacept (3 doses 10 mg/kg or continuative treatment) in patients 2-5	5 patients (≥ 18 yr)	No response	
Garin <i>et al</i> ^[105]	Abatacept (1 or 2 doses; 10 mg/kg) or belatacept (16 doses 5 mg/kg)	5 patients (2/5 < 18 yr with minimal change in disease or FSGS on native kidneys; 3/5 with FSGS recurrence (1/3 < 18 yr, 2/3 ≥ 18 yr))	Partial response in minimal change disease patient; no response in primary FSGS patient; partial remission in 1/3 with FSGS recurrence (abatacept treated); no response in 2/3 (abatacept/ belatacept treated respectively)	
Alkandari <i>et al</i> ^[106]	Abatacept (3 doses; 10 mg/kg)	1 patient (< 18 yr)	No response	Partial response in 2/5; no response in 3/5 (no worsening in proteinuria values pre- and post-belatacept therapy in 1/3)
Grellier <i>et al</i> ^[107]	Belatacept (days 1, 15, 30 and monthly thereafter, 5 mg/kg)	5 patients (≥ 18 yr)	Partial response in 2/5; no response in 3/5 (no worsening in proteinuria values pre- and post-belatacept therapy in 1/3)	

PE: Plasma exchange; CyA: Cyclosporine; CYC: Cyclophosphamide; FSGS: Focal segmental glomerulosclerosis; TNF-α: Tumor necrosis factor-alpha; MMF: Mycophenolate mofetil.

had achieved sustained remission. In a second series, remission was induced in 13/16 patients (81%), which also included PE sessions for 4 of the cases; CyA doses were from 6 to 25 mg/kg per day^[46]. At the latest follow-up (range of 10 mo to 12 years), 11/13 (84%) patients had a functioning allograft. It is noteworthy to mention that in this study, as in the studies by Canaud *et al.*^[33] and Chikamoto *et al.*^[32], the CyA treatment was combined with PE sessions.

The mechanism by which CyA might decrease proteinuria has been elucidated recently. Briefly, CyA has been shown to act by means of a direct effect on the cytoskeleton *via* dephosphorylation of synaptopodin, a crucial stabilizer of podocyte actin cytoskeleton, rather than through an immunosuppressive activity such as inhibition of T cells^[47,48]. According to these clinical evidence, it was postulated that the anti-proteinuric effect had been observed only with high dose CyA because the hypercholestorelemic state induced by NS limits the CyA active fraction^[49].

Currently, the option of CyA therapy in FSGS is more frequently used in combined-therapy regimens. The long-term safety/efficacy ratio of such a therapy, however, remains to be confirmed by study, which is of particular importance in light of the severe toxicities associated with high dose CyA.

Cyclophosphamide and mycophenolate mofetil

Cyclophosphamide (CYC) is an alkylating agent that inhibits cell DNA duplication, leading to cell death. It is active both on resting and dividing lymphocytes^[50]. Anecdotal experiences with CYC therapy (2 mg/kg per day) reported achievement of partial or complete remission in patients with FSGS on native kidneys and also in steroid-dependent patients; however, no benefit was found in steroid-resistant patients^[51,52].

In FSGS recurrence, Kershaw *et al.*^[53] treated 3 pediatric patients with CYC (1-2 mg/kg per day, adjusted for white blood cell count) for 8-12 wk and obtained two complete remissions and one partial; the patient with the longest follow-up (125 mo) experienced two additional relapses, each of which were treated successfully with pulse intravenous steroids. A more recent report described a series of 6 patients with FSGS recurrence all of whom were treated with a combination of CYC and PE (10 sessions over 2 wk, followed by 1 session per week for 2 mo), with complete remission being achieved in 3 of the patients and partial remission in the other 3^[54]. A second case series described 11 pediatric patients with FSGS recurrence who were treated with a 2-mo course of CYC (2 mg/kg per day) and PE sessions, with initial remission being achieved in 9/11 and with 7/9 being free of disease at the last follow-up (32 ± 15 mo)^[55].

MMF inhibits the inosine monophosphate dehydrogenase-mediated reduction of T and B lymphocyte proliferation. Gbadegesin *et al.*^[56] suggested MMF for treatment of steroid-dependent/resistant FSGS on

native kidneys. Subsequently, a randomized controlled trial including 138 patients (both children and adults) with primary FSGS compared CyA and MMF plus dexamethasone, but no difference was observed in complete or partial remission rates after 52 wk of follow-up and both groups showed poor outcome (remission in 46% vs 33%, respectively)^[57]. At the present time, as reported by Lau *et al.*^[58], no randomized controlled trial has yet to demonstrate the efficacy of MMF in association with other therapies or as a single agent in FSGS treatment on native or transplanted kidneys.

Renin angiotensin system blockers

Renin angiotensin system (RAS) blockers have an important role in blood pressure control, but they also have anti-proteinuric and systemic anti-inflammatory effects^[59]. RAS inhibition represents an important therapeutic strategy in proteinuric glomerular disease as FSGS, for either recurrent or *de novo* types.

Despite some reports having suggested RAS blockers as effective therapeutics for this disease^[60,61], the association of these drugs with other therapies limits a final judgment on their real effect as a single drug. Freiburger *et al.*^[62] reported a favorable outcome after the use of a triple RAS blockage [angiotensin-converting enzyme (ACE) inhibitor, angiotensin receptor (blocker) antagonist (ARB), and renin inhibitor] in a transplanted patient with FSGS recurrence; since PE and Rituximab® treatment produced no apparent benefits in the patient previously, a late response to this treatment may not be excluded "a priori".

It is noteworthy that a close monitoring of serum creatinine and potassium levels is essential in all subjects treated with RAS blockers, especially when all these drugs are prescribed together and even more so when renal function is suboptimal.

ANECDOTAL THERAPIES

Galactose

The potential effect of galactose on glomerular permeability and proteinuria was firstly hypothesized by Savin *et al.*^[63], stating that sucrose binds with high affinity and inactivates the supposed "permeability factor", thereby facilitating its plasma clearance.

Jhaveri *et al.*^[64] described a patient with severe recurrent FSGS (massive proteinuria of 37 g/d at day 2 after transplantation) who had been previously treated with PE, intravenous immunoglobulin and Rituximab®, and achieved complete remission of proteinuria after receipt of a high galactose diet and supplemental oral galactose (0.2 g/kg, two times per day). As for other case series mentioned before, the role played by galactose in disease remission vs the role of previous treatment is indistinguishable. More recently, Robson *et al.*^[65] also reported a favorable outcome (1 complete and 1 partial response) in 2 patients with FSGS recurrence treated with high galactose diet. Sgambat

et al.^[66] reported in a recent case series a reduction in permeability factor activity in 7 pediatric patients with steroid-resistant NS (2/7 with recurrent FSGS) treated with high galactose diet (0.2 g/kg, twice daily), without any significant improvement in proteinuria values.

Anti-tumor necrosis factor-alpha agents

The tumor necrosis factor-alpha (TNF- α) signaling pathway is involved in the development of both NS and FSGS, as evidenced by elevated levels of TNF- α detected in plasma and urine obtained from patients with FSGS^[67] and increased glomerular permeability to TNF- α observed *in vitro*^[68].

At the present time, very few cases of FSGS have been treated with anti-TNF- α agents. Leroy *et al.*^[69] reported a favorable outcome (complete remission) for a 15-year-old patient with recurrent FSGS that was presumably resistant to other treatments (increased immunosuppressant dose, PE, intravenous immunoglobulin, high dose steroids, CyA, and CYC) after administration of an anti-TNF- α blocker (firstly infliximab, then etanercept). Bitzan *et al.*^[70] showed that plasmapheresis effluent or fresh plasma (obtained from a child with recurrent FSGS and from two children with primary FSGS) caused cytoskeleton disturbance on podocyte culture. In detail, the plasma from the patient with FSGS recurrence activated β 3 integrin and dispersed focal adhesion complexes, and this effect was reversed by pre-incubation with antibodies against TNF- α or either of the two TNF- α receptors. Following this study's observation, the patient who was plasma resistant was treated firstly with Etanercept and then with Infliximab, which ultimately led to partial remission of the disease.

NOVEL THERAPEUTIC OPTIONS

Rituximab®

Rituximab® is a chimeric monoclonal antibody that recognizes CD20 antigen on B lymphocytes. This agent has several unlabeled applications in the field of kidney transplantation; it has been successfully applied to reduce anti-donor ABO or HLA antibodies^[71] and to treat acute humoral rejection of the graft^[72], post-transplant lymphoproliferative diseases^[73], and also some recurrent/*de novo* glomerulonephritis.

Rituximab® treatment also has a long history of interest in its potential as a therapeutic option for idiopathic NS before and after transplantation. However, after the initial reports about its favorable use in FSGS recurrence were published in 2006 and 2007^[28,74,75], conflicting results were reported by other studies in the literature. Currently, Rituximab® may be adopted as a preventive therapeutic approach to reduce FSGS recurrence rate, or as a treatment of FSGS recurrence.

The use of Rituximab® as a prevention strategy derives from two retrospective studies^[21,76]. In the first,

Fornoni *et al.*^[21] investigated 27 kidney transplanted patients at high risk for FSGS recurrence and showed that use of Rituximab® in the perioperative period (375 mg/m² within 24 h after the kidney transplantation) was associated with a lower incidence of post-transplant proteinuria and with stabilization of GFR at the 12 mo follow-up. This study also demonstrated for the first time that Rituximab® operates in a B cell-independent manner; sera obtained from FSGS recurrent patients caused a down-regulation of SMLPD-3b, a protein involved in regulation of podocyte cytoskeleton, and this phenomenon was prevented by pre-treatment with Rituximab® through direct binding.

Audard *et al.*^[76] observed the absence of a clinical FSGS recurrence (not biopsy proven) in 4 patients who received Rituximab® (375 mg/m²) in their induction protocol for a second kidney transplant (first kidney lost due to a recurrent disease). Nevertheless, the short follow-up (12-54 mo), the difference in Rituximab® schedule (a single administration in 2/4 patients and 2 doses in the other 2 patients), and PE adoption in 2/4 patients partially limit the significance of this uncontrolled study.

To date, Rituximab® has been widely used, alone and in combination protocols, as a treatment for recurrent FSGS in cases of incomplete remission, PE dependence, or as a first-line therapy in specific patient subsets. Despite successful results having been obtained^[77-86], other studies have shown a transient or even absent response to Rituximab®^[62,87-94] (Table 1).

Abatacept

Abatacept is a biologic agent, specifically the CTLA4-Ig recombinant fusion protein derived from the extracellular portion of CTLA4-Ig and genetically fixated to the high and constant portion of the IgG1 immunoglobulin. Its effect is exerted by interfering with lymphocyte co-stimulation^[95,96] upon binding to the APC protein ligands B71 (CD80) or B72 (CD86) and displacing their T cell counterpart or CD28^[97]. In some experimental models of organ transplantation, the systemic administration of CTLA4-Ig effectively dampened the immune response, preventing experimental acute and chronic rejection and resulting in prolonged graft survival and tolerance^[98-100]. On the basis of these findings, different biological T cell co-stimulation blockers became the subject of clinical trials. A high affinity variant of CTLA4-Ig, named LEA29Y (belatacept, Nulojix®; Bristol-Myers Squibb Pharma, Uxbridge, United Kingdom), has been developed and was awarded approval by the Federal Drug Administration (FDA) in 2011 for prophylactic use for organ rejection in adult kidney recipients^[101].

Abatacept was approved by the FDA in 2005 for the treatment of rheumatoid arthritis and active juvenile idiopathic arthritis^[102], and quite recently has been proposed as a new treatment strategy for FSGS recurrence. Yu *et al.*^[103] reported a positive

outcome in 4 patients (2 children) affected by early and Rituximab®-resistant FSGS recurrence and in 1 patient with glucocorticoid-resistant primary FSGS on native kidneys. All these patients received abatacept, at a dose between 250 mg/d and 500 mg/d, the most commonly used dose for rheumatoid arthritis treatment. Before using abatacept, PE sessions were also performed in all 4 patients with FSGS recurrence, while the patient with primary disease on native kidneys received an immunosuppressive treatment composed of prednisone and CyA, with tacrolimus applied as a second line therapy. All patients achieved and maintained a significant proteinuria regression after 10-48 mo of follow-up. The authors suggested that this response was directly correlated with the B71-positive immuno-stained podocytes found in the kidney-biopsy specimens, because B71 may be expressed on the podocyte surface in some proteinuric conditions such as FSGS, thereby altering cytoskeleton organization, a condition that is known to be abrogated by abatacept.

Nevertheless, other studies of patients with FSGS recurrence have shown a slight/absent response after treatment with CTLA4-Ig^[104-107], despite the fact that in some of these cases belatacept (able to bind B71 with an higher affinity than abatacept) was adopted.

Human allogeneic bone marrow mesenchymal stem cells

The use of bone marrow mesenchymal stem cells (BM-MSCs) has been reported to reduce kidney injury in different experimental models of kidney disease^[108-111]. Ma *et al.*^[111] showed in a well-established murine model of FSGS (adriamycin nephropathy) that human umbilical mesenchymal stem cells (HuMSCs) may improve kidney fibrosis and modulate the inflammatory response. Recently, BM-MSCs have been demonstrated as effective treatment for a wide range of immuno-mediated diseases^[112-114].

Belingeri *et al.*^[115] reported successful application of their innovative approach with BM-MSCs in a 13-year-old kidney transplanted patient who had developed an immediate biopsy proven FSGS recurrence after renal transplantation and who was non-responsive to PE and Rituximab® (2 doses). The patient had received allogeneic BM-MSCs infusions (6 doses, according to the most commonly adopted protocol for treatment of graft vs host disease) at months 7, 10 and 14 after transplantation and at month 5 after Rituximab® administration. Remission of proteinuria was achieved after three BM-MSCs infusions, and at the last follow-up (22 mo) both renal function and proteinuria values were stable. The treatment appeared as well tolerated, and no adverse events were noted.

DISCUSSION

In the field of glomerulonephritis, primary FSGS portends one of the most unpredictable and variable

outcomes, carrying one of the highest recurrence rates for transplanted kidneys (from 30% to 50% in patients with a history of primary FSGS on native kidneys)^[4-6]. FSGS recurrence also remains a "clinical drama", with almost 50% of allografts lost at 5 years and having a HR of 2.03 compared to other kinds of recurrent glomerulonephritis^[8]. Despite the proposal of multiple therapeutic approaches over time, none has yet emerged as the resolutive option, either for the recurrent or *de novo* types of FSGS; yet, none has been disproven or ruled out and each has several aspects that still need to be studied.

Indeed, PE is still widely applied as FSGS recurrence treatment and as a pre-emptive strategy, despite the absence of controlled trials. Nevertheless, a course of PE treatment is widely used and recommended, titrated according to the clinical/histological response as proposed by Ponticelli^[4], even if it remains difficult to determine when to start and when to stop and which schedule of PE sessions is best. Interpretation of the literature data for PE is difficult, partially due to the existence of publication bias, in which positive outcomes of some cases may lead to an overestimation of treatment efficacy. In addition, the reports on PE often describe studies in which the therapy is applied as part of a combination regimen that includes other disease-modifying treatments (*i.e.*, corticosteroids, Rituximab®, CyA), complicating the interpretation of results. Besides, few prospective studies are available and none of them used a control group study design.

On the other hand, application of high dose CyA must be carefully considered on the basis of drug-related toxicities, especially nephrotoxicity. Most of the CyA studies have thus far only included pediatric patients or living-related donors, two populations that are more prone to tolerating high dose CyA. To the contrary, when patients are adult recipients of a kidney from a deceased marginal donor, nephrotoxicity from high dose CyA could be a problematic issue. The previous reported considerations for PE regarding its frequent association with other treatments capable of strengthening its effect are also applicable to CyA (see the study by Canaud *et al.*^[33] for an example).

The paucity of data on CYC/MMF adoption for treatment of recurrent FSGS represents another limitation to using the collective literature to draw conclusions about their utility in clinical practice. On the other hand, Rituximab® is one of the most interesting agents proposed to date for treatment of FSGS recurrence; but, again, several limitations lie in the related literature, including the use of a surrogate end-point of disease activity (*i.e.*, clinical/not histological definition of recurrent FSGS in the study by Fornoni *et al.*^[21]), short follow-up^[76,77], and evidence of absence of positive effects^[62,87-94]. Furthermore, the Rituximab® dose is another matter of debate, and the question remains: Should the classic scheme borrowed from hematologic protocols (4 doses of 375 mg/m² each) or a shorter regimen (titrated to the

minimal level necessary to obtain B cell depletion) be adopted? Another first line question involves when the infusion should be performed: As a pre-emptive therapy soon after surgery, in cases at high risk of recurrence, or at the time of recurrence? Although, Rituximab® portends some serious side effects, increasing the risk of opportunistic infections in transplanted patients during the entire time of its blockage of the immune response. Araya *et al.*^[116] reported side effects in about 10% of cases (1 case each of neutropenia, severe anaphylactic reaction, BK virus nephropathy, and severe sepsis). Kumar *et al.*^[117] observed a significant rate of severe complications (3/8 patients), ranging from Rituximab®-associated lung injury, acute tubular necrosis, and central nervous system malignancy.

The ACEs or ARBs should be considered as adjuvant therapy, especially when other therapies have failed or are not applicable. However, their use may be contraindicated by low GFR and risk of hyperkalemia.

Considering the so-called “anecdotal therapies” (galactose, anti-TNF- α agents), their place in the armamentarium for FSGS treatment in renal transplant is very small in current times, but they could be considered for use in rare conditions as a salvage therapy. Considering the more innovative treatments, BM-MSCs represent a promising treatment^[115]. Nevertheless, the results reported in the literature to date need to be evaluated on the basis of the possible influence of previous treatments received by the patients, especially considering a delayed effect of Rituximab® administration, and the natural evolution of the disease, which is often unpredictable.

On the other hand, safety of BM-MSCs remains an open question. On the basis of literature data, auto- and allo-MSCs may interfere with the immune response in a non-defined and unpredictable manner. For example, Reinders *et al.*^[118] found auto-MSCs infusion for the treatment of acute rejection to be associated with opportunistic viral infection in 3/6 patients. Allo-MSCs may also induce the production of anti-donor antibodies, as observed in some animal models^[119]. Nevertheless, a strong limitation to the adoption of cell therapies is the unknown proneoplastic effect, secondary to a direct (but also indirect) MSCs dedifferentiation^[120,121].

A possible way to reduce or abrogate the risk deriving from MSCs infusion is to promote podocyte regeneration. In some experimental models, native parietal epithelial cells (PECs) have been shown to have the potential to migrate to the glomerular tuft after kidney injury, acquiring a phenotype and a morphologic appearance similar to a differentiated podocyte and thereby mitigating the damage^[122,123]. On the other hand, PECs have also been associated with glomerular injury and sclerosis^[124], so a definitive consideration about their role and potential therapeutic applications is far from being defined.

The therapeutic role of co-stimulatory molecule blockades is emerging for some glomerulonephritis

on native kidneys (e.g., lupus nephritis)^[125]. Recently, abatacept was associated with interesting results in proteinuria reduction in a small case series of FSGS recurrent patients^[103]. Nevertheless, a limitation related to the histological findings reported is intrinsically linked with the efficacy, because all positive results were obtained only in patients with positive B71 staining on renal biopsy and the negative outcomes were reported for patients without this staining pattern on renal specimens^[101]. In addition, the absence of response after belatacept use^[99,100,102] (abatacept’s “twin drug” with a higher affinity to the B71 receptor) remains an open issue.

In conclusion, no treatment guideline can be proposed at this time to address FSGS in renal transplantation. In our opinion, waiting for improvement in podocyte biology knowledge and taking the perspective that therapeutic protocols should be tailored to the single patient will help to optimize the risk/benefit balance. Protocol biopsy is a useful strategy chosen during the difficult decision-making process involved in cases possibly needing interruption of on-going targeted therapies (maybe with the only exception of RAS blockers). We suggest, as a first line option, the use of Rituximab® at a single dose of 375 mg/m² (also for induction protocols in patients at high risk of recurrence) with a close monitoring of CD20⁺ count, that will be applied in combination with steroids and a PE course. The initial schedule could be 5-10 sessions on alternating days, followed by tapering to a 1/wk or less schedule according to the patient’s clinical response. The crucial issue is determining the right time to stop PE after proteinuria disappearance.

Therapy for FSGS in renal transplantation remains an unmet clinical need. Randomized-controlled clinical trials are highly important to resolve this issue and necessary to elucidate the correct approach and the real potentiality of the more recently proposed drugs.

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Survival of encapsulated islets: More than a membrane story

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Abstract

At present, proven clinical treatments but no cures are available for diabetes, a global epidemic with a huge economic burden. Transplantation of islets of

Langerhans by their infusion into vascularized organs is an experimental clinical protocol, the first approach to attain cure. However, it is associated with lifelong use of immunosuppressants. To overcome the need for immunosuppression, islets are encapsulated and separated from the host immune system by a permselective membrane. The lead material for this application is alginate which was tested in many animal models and a few clinical trials. This review discusses all aspects related to the function of transplanted encapsulated islets such as the basic requirements from a permselective membrane (*e.g.*, allowable hydrodynamic radii, implications of the thickness of the membrane and relative electrical charge). Another aspect involves adequate oxygen supply, which is essential for survival/performance of transplanted islets, especially when using large retrievable macrocapsules implanted in poorly oxygenated sites like the subcutis. Notably, islets can survive under low oxygen tension and are physiologically active at > 40 Torr. Surprisingly, when densely crowded, islets are fully functional under hyperoxic pressure of up to 500 Torr (> 300% of atmospheric oxygen tension). The review also addresses an additional category of requirements for optimal performance of transplanted islets, named auxiliary technologies. These include control of inflammation, apoptosis, angiogenesis, and the intra-capsular environment. The review highlights that curing diabetes with a functional bio-artificial pancreas requires optimizing all of these aspects, and that significant advances have already been made in many of them.

Key words: Bio-artificial pancreas; Diabetes; Islets of Langerhans; Encapsulation; Oxygen supply; Permselective membrane; Transplantation

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Core tip: Replacing standard insulin therapy for patients with type I diabetics with a cell-based cure is yet to

be achieved. Assuming unlimited supply of beta cells, allogeneic or xenogeneic cells should be separated from the host immune system by a permselective membrane that still allows insulin egress. In addition, a mandatory requirement for such a cure in a poorly oxygenated environment includes adequate oxygen supply. In addition, to optimize islet functionality, control over inflammation, cell apoptosis, angiogenesis, and the close environment of the transplanted cells must be accomplished.

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INTRODUCTION

Diabetes is considered an epidemic with global prevalence of 9% [based on World Health Organization (WHO) data from 01/2015] and a huge economic burden^[1]. Type I diabetes, consists of 10% of the total diabetic population. Prevalence of clinical diabetes is predicted to double in the next 20 years^[2].

Transplantation of cadaveric islets of Langerhans (IOL) by their infusion into vascularized organs, preferentially the liver, is an experimental clinical protocol which was first established in Edmonton in 2000^[3]. Since then, 2000 allogeneic transplantations are estimated to have been performed worldwide. A report published by the Collaborative Islet Transplant Registry at the end of 2013 summarized clinical data from 864 such recipients^[4]. Despite the promise, clinical application of islet transplantation is limited due to short organ supply, inefficient use of organs (approximately 2.5 donors are required per recipient), low reproducibility of quantity and quality of the isolated IOL, and the obligatory use of life-long immunosuppressive therapy. Thus, the current global research focuses on resolving all bottlenecks in the pathway to successful clinical application. These include addressing the limited supply of β -cells by using juvenile/adult porcine IOL^[5-8] and β -cells derived from renewable sources (*e.g.*, stem cells^[9-11]); development of efficient and reproducible protocols for isolating donor IOL^[12-14]; and development of efficient encapsulation technologies in order to allow immunosuppression-free procedures. These encapsulation approaches, which include macro, micro, and nano-encapsulations were tested in animal models and a few clinical trials (for reviews, see^[15-18]). To date, the least developed niche in the IOL transplantation approach is the use of active oxygen supply and auxiliary technologies to provide "friendly microenvironment" to the transplanted islets.

This article reviews the various aspects related to optimizing cell-based curing product for diabetes and

highlights the achievements made to date.

THE IMMUNE BARRIER

For clinical islet transplantation, systemic administration of immunosuppressive drugs has remained the foundation for preventing graft rejection. However, chronic immunosuppressive therapy is associated with loss of islet mass as well as with significant risk for higher rates of malignancies and opportunistic infections. The risk of these serious side effects is inherent, as it is currently impossible to block rejection of foreign tissues without simultaneously suppressing necessary immune functions. Cell encapsulation is an alternative technology. It creates a passive barrier between the implanted graft and the hostile immune system using a permselective membrane. The membrane must be discriminative in terms of molecular diffusivity, allowing for free ingress and egress of low molecular-weight nutrients such as glucose, amino acids, and insulin. Diffusion of small molecules, such as oxygen, glucose, and L-tryptophan, has been shown to be only marginally affected by hydrogel like alginate and agarose^[19-25]. At the same time, the permselective membrane must create impassable barrier for host immune effectors in order to efficiently prevent graft rejection. The immune system uses plethora of mechanisms to reject grafts, most of them are dependent on cell-to-cell contacts and effector macromolecules. Therefore, diffusion resistance constitutes the foundation of all immunoisolation strategies.

The cellular arm of the immune rejection is mediated by cytotoxic T-cells and the process requires direct representation of donor MHC class I molecules to recipient CD8 T cells. This mechanism, however, has only a minor impact on encapsulated grafted cells because the membrane physically separates donor cells from recipient cells^[26].

Humoral rejection does not require cell-to-cell contact and is operable *via* mechanisms activated by the indirect recognition pathway. Antibody-complement mediated rejection is a major contributor to this pathway. A cascade of biochemical reactions, termed the complement cascade, follows the binding of either IgG or IgM paratopes to their matching epitopes. Eventually, this cascade leads to the formation of membrane attack complexes (MAC), which are 100-nm diameter transmembrane channels characterized by a hydrophilic internal surface. MACs are integrated across the cell plasma membrane thus allowing for free 2-way passage of water and molecules. Loss of essential differential concentrations of ions between the intra- and extracellular compartments is fatal and induces necrosis (*e.g.*, as demonstrated by Papadimitriou *et al.*^[27]). With respect to this type of rejection, the merit of inserting a separating membrane between the donor and recipient depends on the permeability indices of the membrane, the dimension of the solutes, and their hydrodynamic radius (R_H). IgG (a pivotal activator of

Table 1 Characteristics of effectors involved in immune rejection of transplanted islets, and of molecular chaperones involved in transporting key nutrients to the transplanted islets

Effector	Molecular weight, kDa	Crystal dimensions, nm	Hydrodynamic radius, nm	Ref.
IgG	150	15 × 6 × 2	5.4	[32-36]
IgM	> 900	30 × 13	12.7	[35,213]
C1q	> 400	30 × 33	12.8	[28-31]
Transferrin	80	5 × 10	3.7	[37,38]

the complement cascade), IgM, C1q (the rate-limiting activator of the complement cascade), and transferrin (a molecular chaperone transporting iron to the graft), vary in their molecular dimensions (Table 1)^[28-38]. In order to concomitantly prevent damage to encapsulated cells and allow essential nutrition, the permselective membrane should permit free diffusion of molecules with $R_H < 4$ nm (*i.e.*, molecular chaperones such as transferrin) while preventing ingress of molecules with $R_H \geq 12$ nm (*i.e.*, IgM, C1q). Notably, even if the intermediate size IgG passes the membrane, it is an inefficient cell killer on its own.

The third path to rejection involves inflammation-type reactions. Surgical incision, preceding any type of graft implantation damages the vascular bed and irritates the tissue, while insertion of any artificial device into an interior site enhances the magnitude of this reaction. The process induces inflammatory responses immediately. These are manifested by cross activation of immune cells of the innate system (neutrophils, basophils, and macrophages^[39]). Once activated, these cells release bioactive cytokines^[40-42] in the vicinity of the graft that aim to heal the wound. However, some of these cytokines are destructive to the grafted cells. Indeed, studies in a model of syngeneic islet transplantation demonstrated that damage to islet grafts is primarily due to nonspecific inflammatory response^[43,44]. This effect is aggravated when allotype or xenotype islets are being transplanted. Although the inflammation lasts less than 2 wk, up to 60% of islet cells may be lost in this timeframe^[45].

The 3 major effectors that damage islets include: Interleukin (IL)-1 β , interferon (INF)- γ , and tumor necrosis factor (TNF)- α ^[46-52]. These cytokines also play a major role in the neutrophils-macrophage activation cascade. Their apparent molecular masses differ (17 kDa for IL-1 β , 47 kDa for dimeric glycosylated INF γ and 52 kDa for trimeric TNF- α); however, their R_H are similar (2.2, 3.1, and 3 nm, respectively)^[53,54]. This range of radii is well below the minimal threshold required for immunoisolating membranes (12 nm), but is close to the R_H value of transferrin. Therefore, reducing the size of membrane pores to approximately 4 nm, and the fact that the pores are geometrically inhomogeneous may attenuate ingress of the pro-inflammatory cytokines TNF- α and INF- γ but at the expense of transferrin. Still, no permselective

membrane can prevent IL-1 β diffusion. In summary, based on pore size only, permselective membranes are effective against cell-mediated and complement-mediated cytotoxicity; however, they are less helpful against harmful cytokines.

Besides pore size, the physical makeup of permselective membranes also affects their permeability properties. In water, diffusion of a solute is a process of random movement of molecules across concentration gradient and is quantitatively portrayed by a diffusion coefficient. In a typical hydrogel, the void volume is > 95%; however, diffusion of a solute across a hydrogel is not determined solely by its diffusion coefficient. Permeability of larger molecules is also controlled by slow transfer across lengthy path of traversing pores, hydrodynamic drag on the moving solute, and polar or hydrophobic interactions between the membrane material and the traversing macromolecule. Crosslinking of acidic alginate polymers by divalent ions creates an "eggs-in-a-box" hydrogel scaffold that is never saturated by the divalent cross-linker. Therefore, under physiological environment (pH = 7.35), alginate hydrogel is negatively charged in its core and even more at the exposed surfaces. Proteins usually have hydrophobic core and hydrophilic surfaces. Therefore, electrical repulsion between negatively-charged domains on protein surfaces and the exterior of the hydrogel is expected^[55] and may play a role in selective permeability of polypeptides. This hypothesis could be tested for IL-1 β , the most devastating interleukin. This cytokine, despite extensive sequence homology and similar biological activity, has a range of isoelectric points (pI) across species. On one side, porcine IL-1 β (NP_001005149.1) has an acidic pI of approximately 5.5, whereas rat IL-1 β (NP_113700) is characterized by a basic pI (> 8.5). Local surface charges may also make a difference. The exposed amino acid shells of human (PDB 9ILB; pI = 5.92) and mouse (PDB 8I1B; pI = 8.30) IL-1 β shown in Figure 1 clearly demonstrate enhanced electronegativity of the human compared with the murine molecule. Therefore, the transfer rate of these cytokines across alginate hydrogels may provide insights into the role of electrical charges in differential permeability, and may help in the design of better protecting membranes.

Concentration of local cytokines is a balance between synthesis and degradation at inflammation sites. Proteolysis of IL-1 β is controlled by a plethora of matrix metalloproteinases (*e.g.*, as described by Ito *et al.*^[56]). In addition, a group of serine proteases (*e.g.*, cathepsin G and elastase) are capable of cleaving nearly all proteins in an unspecific manner. Most cytokines contain many cleavage sites for serine proteases. Activated macrophages and neutrophils, major producers of these proteases, co-localize with inflammatory cytokines at implantation sites. As such, direct restrictive effect of proteases on the lifetime of cytokines is envisaged and was shown for TNF- α which is rapidly degraded by supernatant of activated neutrophils and by

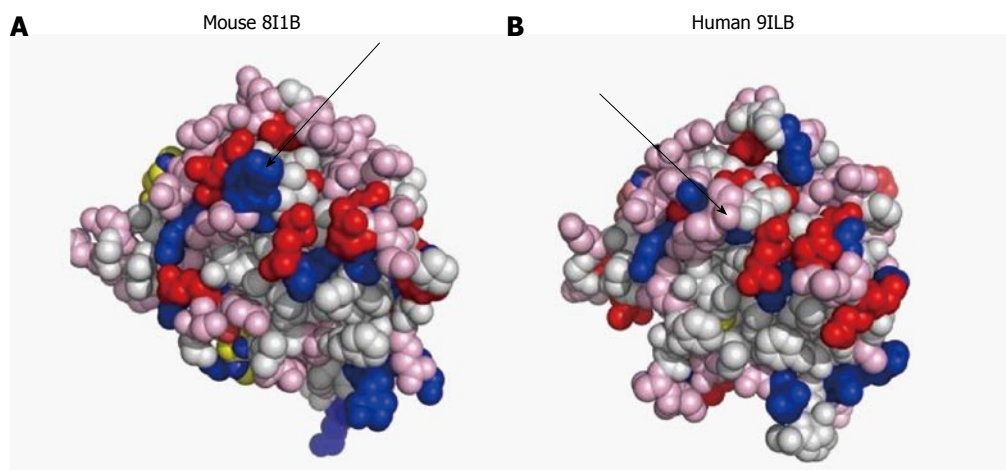


Figure 1 Surface design of mouse (A) and human (B) interleukin-1 β . The proteins are imaged at identical angles. Blue: Positively-charged amino acids; red: Negatively-charged amino acids; pink: Polar amino acids (slightly negative at physiological pH). The arrows point to differences in surface charges between the 2 proteins. Image resolved using ASAview^[214].

elastase^[57,58]. Some membrane design, including those with extended width of the membrane, has been shown to partially protect encapsulated cells against cytokines^[59-62]. Therefore, attenuation of ingress of cytokines may expose them to enhanced degradation by resident proteases thereby reducing the necessity to completely prevent their ingress.

Following islet transplantation, nitric oxide (NO) and reactive oxygen species (ROS) are released by cells of the innate immunity, responding to the insult^[63,64]. Working independently or as effectors of IL-1 β , they contribute to the loss of functionality and viability of encapsulated islets soon after implantation^[65-68]. Likewise, hydrogen peroxide, an abundant ROS, impairs glucose-induced insulin secretion in β -cells^[69,70]. ROS are constantly produced in living systems but are kept by homeostatic mechanisms at relatively low levels. Upon transplantation of IOLs, this balanced state is deranged. Oxidative stress is much enhanced, but is not countered by efficient antioxidant machinery as islets contain ineffective antioxidant protection system. Consequently, transplanted islets are prone to destruction by NO and ROS^[71-74].

Due to their miniaturized molecular dimension, none of the permselective membranes can prevent free passage of NO and ROS. This inherited challenge may be solved using a different approach. It is based on the short half-lives of these molecules (seconds for NO and even shorter for ROS), and consequently their limited radii of effectiveness (approximately 200 μ m for NO and < 100 μ m for ROS)^[75-77]. Thus, increasing the distance between the cells that are generating ROS and NO and the transplanted islets may decrease the deleterious effect of the formers. Figure 2 summarizes proven and putative mechanisms by which permselective membrane protect grafted cells from the host immune system.

In order for the separating membrane to be functional, it should also protect the graft without impacting

the viability/functionality of the grafted cells, be biocompatible to the host, flexible, and mechanically stable. Collectively, immune barrier could replace immunosuppressive therapy only when the size of the graft is small and internal re-vascularization is not mandatory for its proper function (e.g., IOLs).

Several strategies for islet microencapsulation were developed to protect grafted islets from the host immune system. These are described in several excellent review articles^[15,18,45,78-81]. This paper focuses on retrievable devices, for which hollow fiber and flat geometry configurations are practical solutions.

Two major classes of natural polymers are being used for cell encapsulation: Polysaccharides and polypeptides. Polysaccharides gained widespread use because they are simple to use, allow hydrogel formation under mild conditions (gentle heat or presence of divalent cations), and because they do not interfere with cell viability and functional performance. Alginate, the most studied polymer, which was tested in many animal models and even in clinical trials (for example, see Matsumoto *et al.*^[5]), is the leading biomaterial for cell encapsulation. Other polysaccharides are also being used (e.g., chitosan, agarose, and cellulose). Alginate is a natural product mainly extracted from seaweeds. It is chemically composed of two monomers: Guluronic (G) and man-nuronic (M) acid. These form linear polymers with a wide distribution of molecular masses, different ratios of G to M, and various combinations of homo- and hetero-polymer blocks. Therefore, inter-lot variability in the chemical composition of the polymer is inevitable. This variability is an advantage for facilitating selection of an optimal variation of the polymer but once chosen, it presents a disadvantage, as the specific chemical composition of every alginate lot is unique. Currently, no practical method for producing lots with identical chemistry exists. Only 3 variables in the final makeup of an alginate hydrogel are controllable:

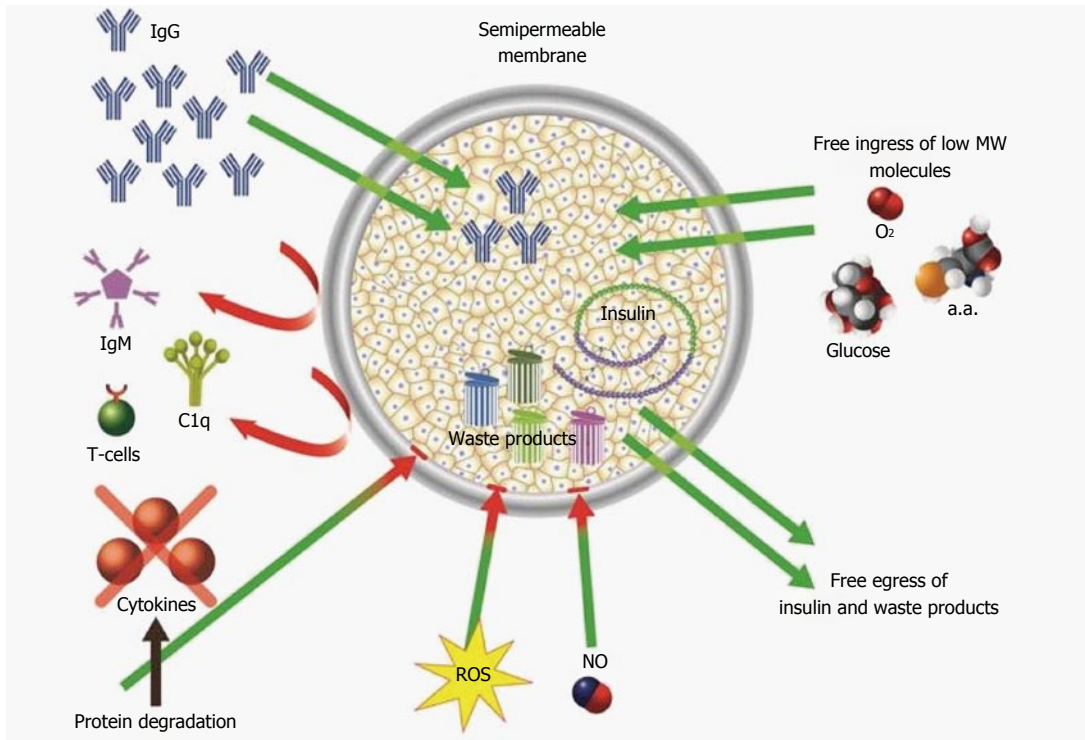


Figure 2 Mechanisms (demonstrated and putative) by which permselective membrane protect grafted cells from the host immune system. The permselective membrane allows free ingress of low molecular weight nutrients (e.g., glucose and amino acids) and egress of insulin and waste products. The membrane separates the grafted cells from the cellular arm of the immune system and prevents humoral rejection by preventing ingress of IgM and C1q (due to their high molecular weight). In addition, the membrane attenuates free diffusion of hazardous cytokines thereby exposing them to proteases, and increases the diffusion distance between reactive oxygen species, nitric oxide, and the grafted cells promoting their thermodynamic degradation.

The G to M ratio, dry matter composition and the type/concentration of the divalent cation used for crosslinking. To a minimal extent, physical parameters of the final hydrogel (e.g., viscosity) can be adjusted by varying these parameters. At present, the field of alginate-based cell encapsulation is in urgent need for an industrialized source of controlled and reproducible raw material. A group of epimerase enzymes^[82-84], converting G to M, thus providing tailor-made alginates form the first step in addressing this critical need.

Agarose has also been tested as an encapsulating hydrogel for cells. Its use for islet encapsulation started in the late 80's^[85,86] and was subsequently broadened^[87-91]. Other natural polysaccharides used for encapsulation of cells/islets include chitosan and cellulose. The data generated for chitosan as an encapsulating hydrogel are limited and chitosan is usually formulated as part of a more complex membrane that also includes alginate or methacrylated glycol^[92-94]. Also, its application is rather limited because it binds crosslinking molecules at acidic pH and does not bind them at physiological pH^[95]. Cellulose was also tested for encapsulation; however, it never reached animal testing^[96,97]. PharmaCyte Biotech, Inc. (Silver Spring, MD) is planning to use cellulose sulfate and polydiallyldimethyl ammonium chloride, known as "Cell-in-a-Box®" as an immune barrier for β -cell transplantation. Chitosan and cellulose were both found to be inferior to agarose and alginate

(reviewed by de Vos *et al.*^[45]).

In 1996, French scientists published a design of a planar bioartificial pancreas (BAP) that used a synthetic membrane developed for dialysis of blood (AN69) to create an immune barrier between grafted islets and the host immune system. Normoglycemia of diabetic mice implanted with this device lasted 30 d^[98]. A variation of this membrane is now a part of a new medical device, MAILPLAN (Defymed; Strasbourg, France), which is scheduled to start clinical trials in 2016. No preclinical data supporting this claim have been published so far. In 2001, Islet Sheet Medical (San Francisco, CA) presented an advanced planar BAP generated by encapsulating donor islets in a thin sheet of alginate^[99]. At a dose of approximately 10000 islet equivalent (IEQ)/kg, a diabetic dog was cured for 84 d. Five years later, a Belgian group reported six-month normoglycemia in diabetic *Cynomolgus* monkeys^[100]. Xenotype islets were encapsulated in a planar monolayer cellular device consisting of 2-sided collagen matrix enveloped in 3% (w/v) high mannuronic acid alginate (US patent 2008/0050417).

TheraCyte Inc. (Laguna Hills, CA) also attempted to macroencapsulate islets in a minimally invasive device based on technology developed by Baxter Healthcare (Round lake, IL)^[101]. It is a robust, mesh-supported, and retrievable planar device consisting of a 3-layer membrane. An outer layer of woven polyester mesh supports a 5 μ m pore size polytetrafluoroethylene

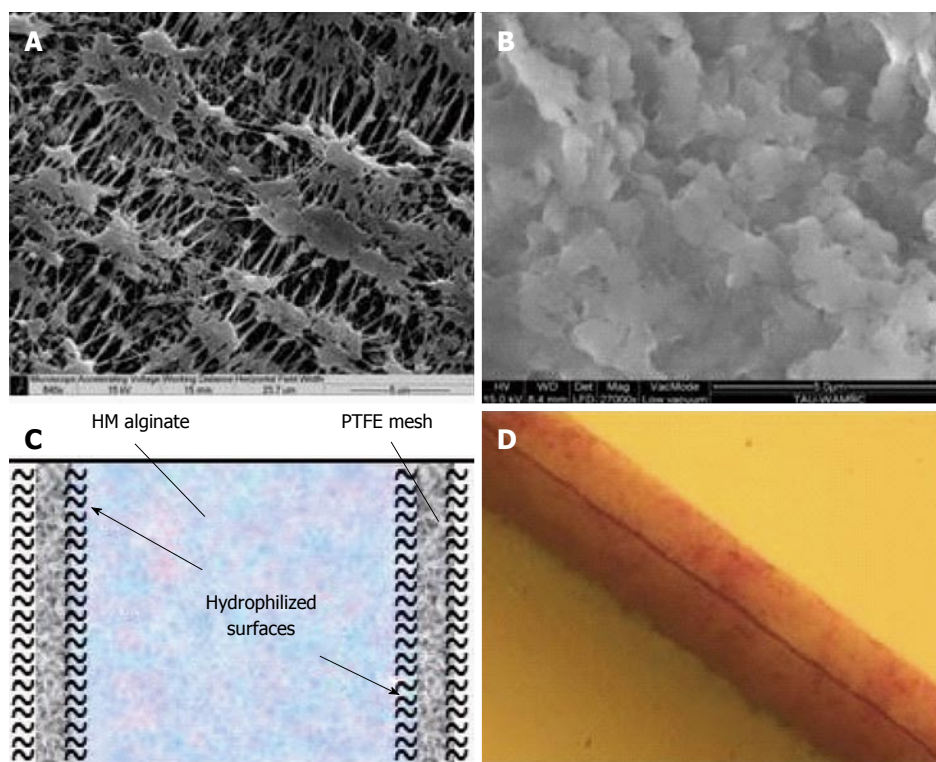


Figure 3 The β -Air immune barrier, a double hydrophilized polytetrafluoroethylene membrane impregnated with 6% high mannuronic alginate. A: Environmental scanning electron microscope (ESEM) surface image of a virgin membrane; B: ESEM surface image of impregnated membrane; C: Drawing of hypothetical cross section in one polytetrafluoroethylene (PTFE) membrane; D: Cross section of double PTFE membrane impregnated with colored alginate (total width = 60 μm).

(PTFE) leaf and an inner PTFE leaf with nominal pore size of 0.45 μm ^[102]. The 3-layer approach is designed to allow for development of dense vascularization on the outer part of the membrane in order to reduce the diffusion distance of nutrients and waste products from the vascular bed and the encapsulated cells. The most inner leaf of this structure is supposed to create an immune barrier between the graft and the host immune system although the nominal pore size seems to be inadequate for this purpose. Rat islets implanted within this device were functional for 4 wk in immunocompromized mouse recipients^[103], for > 6 mo in allogeneic rat recipients^[104] and for 30 d in a mouse model resembling autoimmune diabetes^[105]. Also, reversal of diabetes for a 16-wk period was reported when neonatal porcine islets were transplanted subcutaneously in nonobese diabetic mice^[106]. Successful reversal of diabetes by this device is currently limited to rodent recipients. Data on successful transplantation of donor islets into larger animal models are limited. Nonetheless, the device was transplanted in non-human primates, including a 3-mo trial with xenogeneic porcine islets^[106], and up to 12-mo trial with allogeneic NHP islets^[107]. However, cell doses in these studies were minimal (substantially below curing doses). ViaCyte, Inc. (San Diego, CA) is using a modified TheraCyte membrane (Encaptra) as an immune barrier in order to protect stem cells-derived β -cells from the host immune system. Preclinical data

on the efficacy of Encaptra as an immune barrier are yet to be published, but the company launched a phase I / II clinical trial in September 2014 (NCT 02239354). Practically, neovascularized devices are not easily retrievable because of bleeding and hematoma^[108].

A quite different macroencapsulation method was developed at the Rogosin institute (Xenia, OH)^[90,91]. Donor islets are encapsulated in double layer agarose macrobeads; a 5% external agarose film functions as the immune barrier. Using this method, porcine islets were shown to lower blood glucose in diabetic rats and reduce their insulin requirements for > 6 mo^[91,109]. Similar results were obtained when porcine islets encapsulated in these macrobeads were implanted into diabetic dogs; however, no complete remission of diabetic state was evident even with high islet dose^[110,111]. This macroencapsulation technology is currently awaiting regulatory approval for initiating Phase I studies.

Beta O2 Technologies (Rosh Ha'ayin, Israel) developed the β -Air device which includes a composite membrane serving as an immune barrier (Figure 3). This barrier includes 2 (25 μm each) hydrophilized PTFE membranes with pore size of 0.45 μm , similar to the inner leaf of the TheraCyte membrane. High viscous high mannuronic (HM) acid alginate (G = 0.46) at 6% (w/v) is impregnated into the membrane pores using mild vacuum^[112]. The β -Air composite membrane

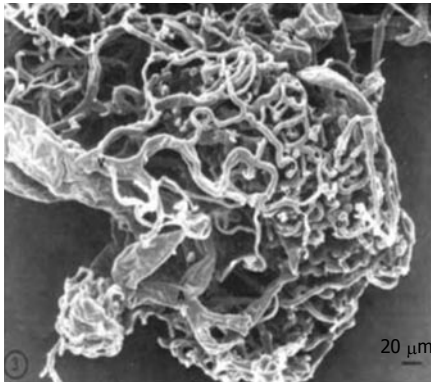


Figure 4 Vasculature of a large islet (300- μm diameter) as seen in scanning electron microscope. Republished with permission of the American Diabetes Association, from Ref. [119] permission conveyed through Copyright Clearance Center, Inc.

is strong but quasi-flexible. It does not allow host cells to permeate into the device (*e.g.*, CD3 cells; Barkai *et al.*, unpublished data), and is also impermeable to viruses, C1q and IgG molecules, while allowing free diffusion of glucose and insulin both inwards and outwards^[112].

OXYGEN SUPPLY

The vasculature of the pancreas consists of a complex network differentially adopted for the distinctive needs of the endocrine and exocrine parts of the organ. Pancreatic islets possess an autonomous mechanism of blood flow regulation, independent of that of the exocrine pancreas. The endocrine tissue, which in humans includes approximately 1 million IOL, is scattered in the exocrine pancreas and constitutes only 1%-2% of its biomass, while utilizing 10%-20% of the total blood flow into the organ^[113-115]. The proportion of arteriole endings and of vascular density in IOL and exocrine tissue is similar^[116,117]. IOL are supplied with arterial blood *via* one or more arterioles which, after penetrating the capsule, form dense, glomerular-like network of capillaries. They are wider than their exocrine counterparts and have much more fenestrae^[118]. The sinusoidal capillaries are drained *via* several efferent venules. Figure 4 (courtesy of Dr. Bonner-Weir^[119]) demonstrates the complexity of single islet vascularization. Vascular density is such that all endocrine cells are no more than one cell away from arterial blood^[120]. This architecture is dramatically changed following transplantation. Capillary densities of rodent islet grafts implanted under the kidney capsule average 500-700 capillaries/ mm^2 ^[121-124], which is approximately half the density of native pancreatic islets (1300 capillaries/ mm^2)^[123] and vascular density of murine islets transplanted into the liver is not more than 20% of the original density^[117]. The vascular density of the human subcutis is lower by an additional order of magnitude averaging only 60-100 capillaries/

mm^2 ^[125-127]. The density of local vasculature should be reflected in the perfusion characteristics of these organs. Basic pancreatic blood perfusion is measured at 200-300 mL/100 g per minute^[128-130]. So far, perfusion values for islet blood flow were not reported but they are expected to be higher than the average pancreatic values. Notably, subcutaneous blood flow is lower by 2 orders of magnitude^[131-133]. Thus, when addressing the question of islet transplantation into the subcutis, these differential values should be considered.

Oxygen supply to cells in tissues/organs is driven by a concentration gradient. Oxygen is solubilized from oxygenated hemoglobin on plasma membrane of red blood cells into the plasma, further diffuses into the interstitial space and then through the cell plasma membrane into the mitochondria. As it diffuses, a pressure gradient is formed. The oxygen transfer rate (flux) from the plasma to the mitochondria is dictated by the oxygen gradient, the distance it has to cross, and the diffusion coefficients in the various tissues being crossed. When oxygen consumption rate (OCR) of the mitochondria increases, local oxygen concentration decreases. Similarly, as distance between blood plasma and target mitochondria increases, the flux of oxygen decreases.

In the normal blood circulation, oxygen partial pressure (PO_2) in the large arteries starts at > 100 Torr. It then decreases to approximately 65 Torr in the smallest arterioles and further decreases to 40 Torr in the venous system. In pancreatic IOL, the average PO_2 measured *in situ* in anesthetized animals is 35-40 Torr^[134,135]. This level is slightly higher in healthy, wake animals and comparable to the PO_2 values measured in the hepatic portal vein used for clinical islet transplantation^[136]. However, following isolation and transplantation of IOL, this level changes dramatically. As IOLs are cut from their blood supply, oxygen is supplied from the periphery solely by diffusion and quickly becomes a rate-limiting nutrient. Transplantation is followed by neo-vascularization and IOLs transplanted into the subcapsular space of the kidney or into the hepatic sinusoids undergo a similar neovascularization process. Finally, they almost reach level of vascular density of normal pancreatic islets^[137]. However, the anatomy of this vascular bed is completely different than that of the native complex; blood is supplied from the periphery inside instead of the original core-shell direction. Consequently, under the kidney capsule, PO_2 of transplanted IOL is only 10 Torr^[134] and values in diabetic animals are even lower (5-6 Torr^[138]). This is also the level recorded for islets transplanted into the liver or spleen^[134,138]. Pimonidazole is an oxygen tension indicator signaling at ambient pressure of ≤ 10 Torr. In the native pancreas, approximately one third of the islets are pimonidazole positive. This proportion is doubled in islets isolated from a donor and infused into the liver of diabetic recipients^[139].

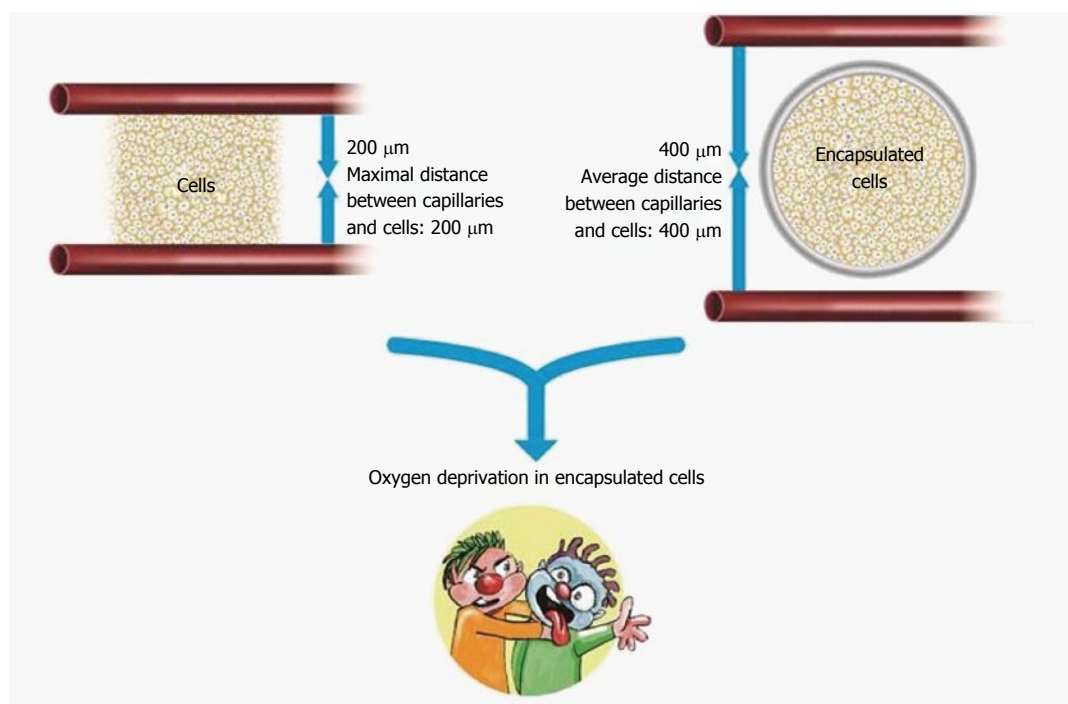


Figure 5 Cartoon representation limitations of oxygen supply to encapsulated islets of Langerhans.

While transplantation of islets into vascularized spaces presents perfusion limitation, encapsulation just aggravates this situation (Figure 5). As no revascularization process is allowed, the distance of these islet cells from the nearest capillary is extended substantially. A mathematical model developed by Johnson *et al.*^[140] predicts that whereas islets transplanted under the kidney capsule or into the portal venous system are exposed to ambient PO_2 of 40–50 Torr, encapsulation (in standard 500 μm width microspheres or planar macrocapsules) reduces the PO_2 to 25 Torr. Under these conditions, cells in a 50 μm cores of these islets are exposed to $PO_2 < 10$ Torr. Most encapsulation methods use an enveloping hydrogel with a width of 500–800 μm . If positioned at the geometric center of the capsule, the innermost islets cells are up to 400 μm away from the host vascular bed. To provide sufficient oxygen to mitochondria inside a cell, the maximal distance between capillary and the cell must not exceed 200 μm ^[141]. Cancer cells have relatively high OCR but OCR of cancer cell lines^[142] is only one third of that of islet cells. Even though, cancer cells placed > 100 μm away from capillaries become necrotic^[143]. Evidently, following encapsulation, the distance between the islets and the vascular bed becomes a major impediment for their normal physiological performance and even for their ability to survive.

Several mathematical models were developed in order to simulate oxygen transfer to encapsulated islets. In a detailed analysis, Dulong and Legallaise^[144] presented pessimistic data on the feasibility of producing a BAP device using microencapsulated islets or islets encapsulated in hollow fibers. Based on

oxygen transfer parameters, efficient performance of a human-type BAP requires a minimum of 570000 IEQ. These should be encapsulated in narrow, 250 μm diameter, hollow fiber measuring 270 cm. Under the same conditions, planar encapsulation is preferred. A sheet of 240 cm^2 surface area and 300- μm width containing 420000 IEQ suffices the needs but, increasing the width to only 500 μm , which is desirable to protect the islets from the host immune system, makes this design impractical. About 1 million islets have to be encapsulated in a sheet of 600 cm^2 surface area. Another model by Johnson *et al.*^[140] predicts that even at surface density of 500 IEQ/ cm^2 , the core of a standard encapsulated IEQ becomes hypoxic. These findings were confirmed in an independent mathematical model^[145]. Islets cultured under normoxic conditions in 1 mm high standard culture medium at density of 1600 IEQ/ cm^2 present hypoxic core when their size exceed a diameter of 100 μm .

A BAP device should continuously sense ambient glucose concentrations and respond to a glucose concentration change by releasing adequate amounts of insulin. This process is also PO_2 -dependent^[146,147]. Fractional secretion of islets decreases at PO_2 below 60 Torr and reaches 50% efficiency at 27 Torr. At PO_2 of 10 Torr, fractional secretion is only 10% of the normoxic level (Figure 6).

In their native environment, islets enjoy surface PO_2 of 40–60 Torr and the efficiency of insulin secretion is predicted to be high ($> 75\%$ of the normoxic level; Figure 6). In contrast, islets transplanted under the kidney capsule or into the hepatic sinusoids, as practiced in clinical transplantations, are exposed to PO_2 of ≤ 10 Torr^[134]. Diabetes and encapsulation

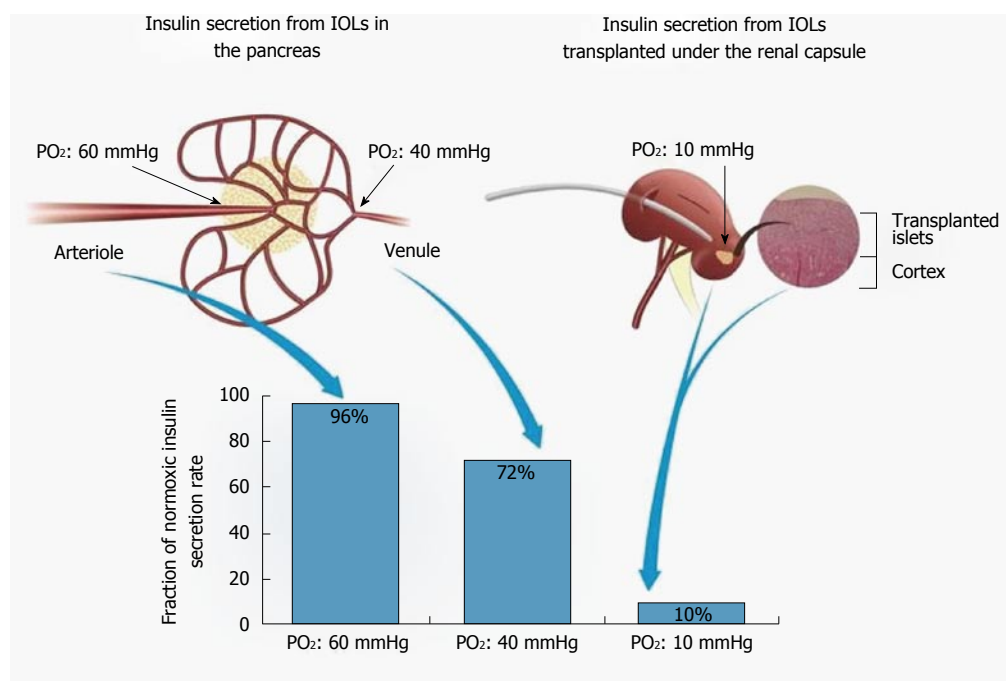


Figure 6 Efficiency of insulin secretion as a function of PO₂. PO₂ levels in native IOL (left) and when IOLs are transplanted under subcapsular space in the kidney (right). HE stained section of rat kidney demonstrating integration of isogeneic transplanted IOLs into the kidney tissue (far right). The association between PO₂ in each location and the efficiency of insulin secretion is shown (bottom). IOL: Islets of Langerhans.

just worsen this situation. Under surface PO₂ of ≤ 10 Torr, insulin secretion is expected to be reduced by an order of magnitude compared with physiological conditions. Also, short distance from capillaries and high perfusion rate which are characteristic of native islets are obstructed following encapsulation. As such, protection against the host immune system imparted by a standard permselective membrane is traded for low efficiency of insulin secretion.

A simple solution to this apparent oxygen deficiency is active delivery of oxygen by generating it *in situ* or using stored reservoirs. Some solutions were experimentally tested including a direct supply of oxygen to cultured cells using decomposition of solid calcium peroxide^[148], electrochemical generator^[149] (USP 8368592), or local photosynthesis^[150,151]. Unfortunately, none of these systems generated enough oxygen to maintain clinical doses of islet graft viable and functional for long periods of time. Recently, we published a series of manuscripts describing active oxygen supply to encapsulated islets from internal storage. The islets were packed in a planar slab at a very high surface density, 1400-3600 IEQ/cm² (5%-13% v/v). The device, β -Air, was implanted under the skin or into the pre-peritoneal space of diabetic recipients and gaseous oxygen was injected daily into a gas chamber that is an integral part of the device^[24,112,152-154].

THE β -AIR DEVICE

Hypoxia adversely affects the functionality of donor islets transplanted into a recipient and has emerged as the bottleneck in the development of efficient BAP

devices. The role played by hyperoxia is less explored. In culture, IOL exposed to atmospheric air survive and function properly for extended periods of time. Higher PO₂ levels, on the other hand, were reported to be toxic^[155-157], but the levels used in these experiments were extremely high (680-1300 Torr, 5-9 times the atmospheric pressure). We hypothesized that some degree of hyperoxia could be beneficial to implanted islets as high PO₂ at the surface of the encapsulated graft is necessary to fuel islet cells across the entire width of the capsule and all the way to the islet core. Also, hyperoxia may allow the use of denser islet grafts which may contribute to decreased device volume.

β -Air is a BAP device implanted under the skin or into the pre-peritoneal cavity, both of which are easily accessed by minimal surgical intervention. The rat variant of this device is composed of an integral macrochamber, access ports and connecting tubing (Figure 7). The device also holds an islet module containing 2400 IEQ [approximately 8000 IEQ/kg body weight (BW)] separated from an integral gas chamber by a rubber silicone membrane (Figure 8). Gas blend is infused into the gas chamber every 2 h (first prototype) or once a day using the access ports and a manual injector.

Using this device we exposed the islet module to increasing levels of PO₂ and tested the effect of hyperoxia on their functional performance under culture conditions and following implantation of the BAP into diabetic animals. At a dose of 2400 IEQ/device and surface density of 1000 IEQ/cm², none of 10 devices implanted in the subcutis without direct oxygen supply were functional for more than 3 d. On the other hand,

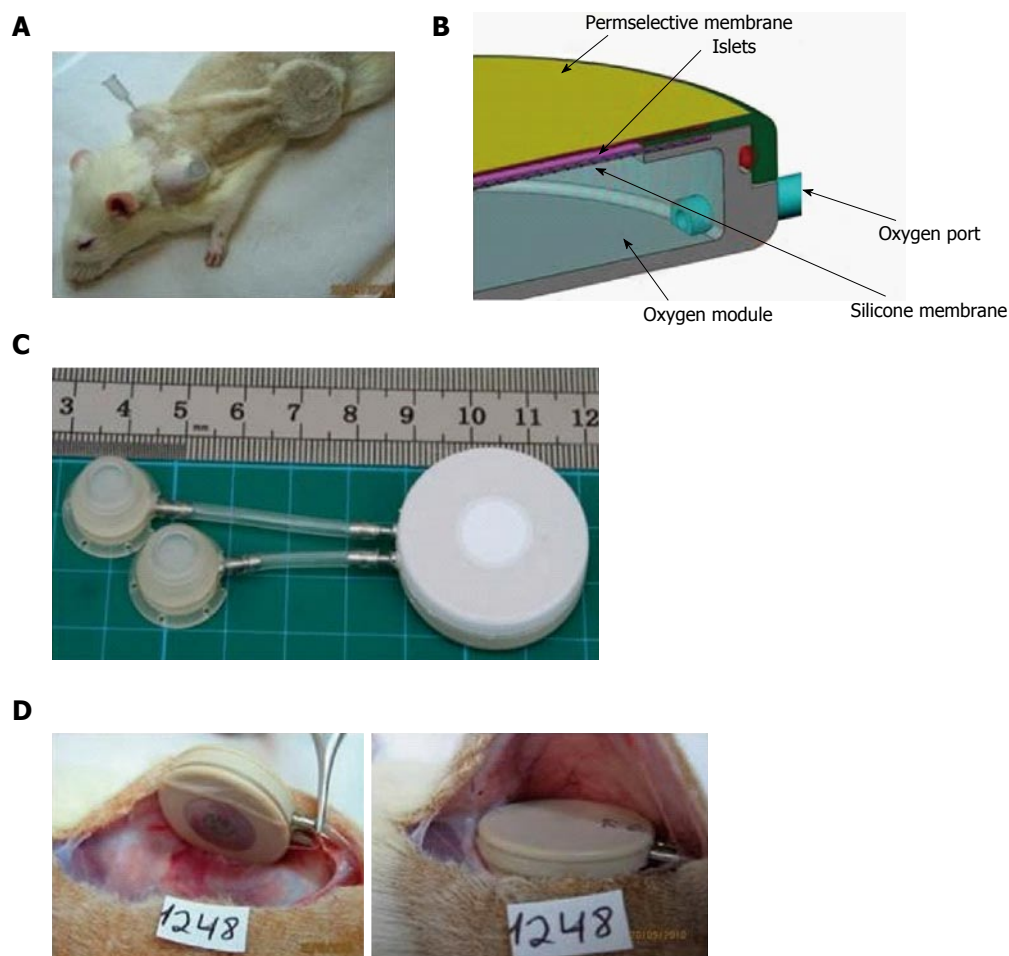


Figure 7 The rat variant of the β -Air device. A: Shaved animal demonstrating relative positions of the device, connecting tubes, and access ports. A syringe needle used for gas refueling is inserted into one of these ports; B: Schematic illustration of the device. Size of the gas chamber and the islets module is shown; C: The macrochamber and connected access ports (each square is 1 cm \times 1 cm); D: Implantation of the device under the skin of diabetic recipient (the inactive surface faces the skin and the active surface faces the fascia).

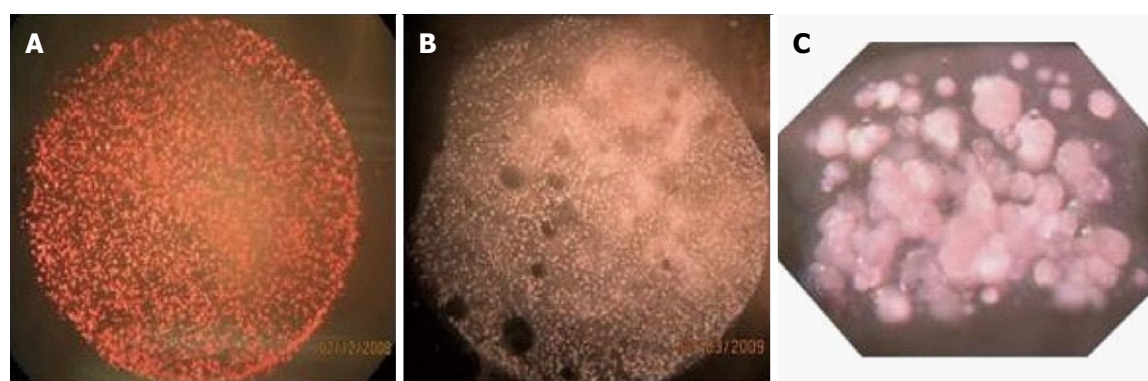


Figure 8 Islet modules of the β -Air device at surface density of 1000 IEQ/cm². A: Before implantation; B: At explantation (after 90 d); C: Cross section of an islet module before integration into the β -Air device.

refueling of 15 min every 2 h with atmospheric air was sufficient to maintain normoglycemia in diabetic recipients through the end of the experiments (up to 240 d)^[24]. Surprisingly, all the devices equipped with the same islet dose but at increased surface density (2400 IEQ/cm²) failed to cure diabetic animals for > 1 wk when refueled alike the former group. Similar

negative results were obtained when β -Air devices were refueled once a day with a gas blend at PO₂ of 230 Torr (30% O₂; Barkai *et al.*, unpublished data). As the null hypothesis was that this failure stemmed from under and not hyper oxygenation of islets, PO₂ in the gas chamber was raised further to 304, 456, and 570 Torr. Most of the diabetic animals implanted with

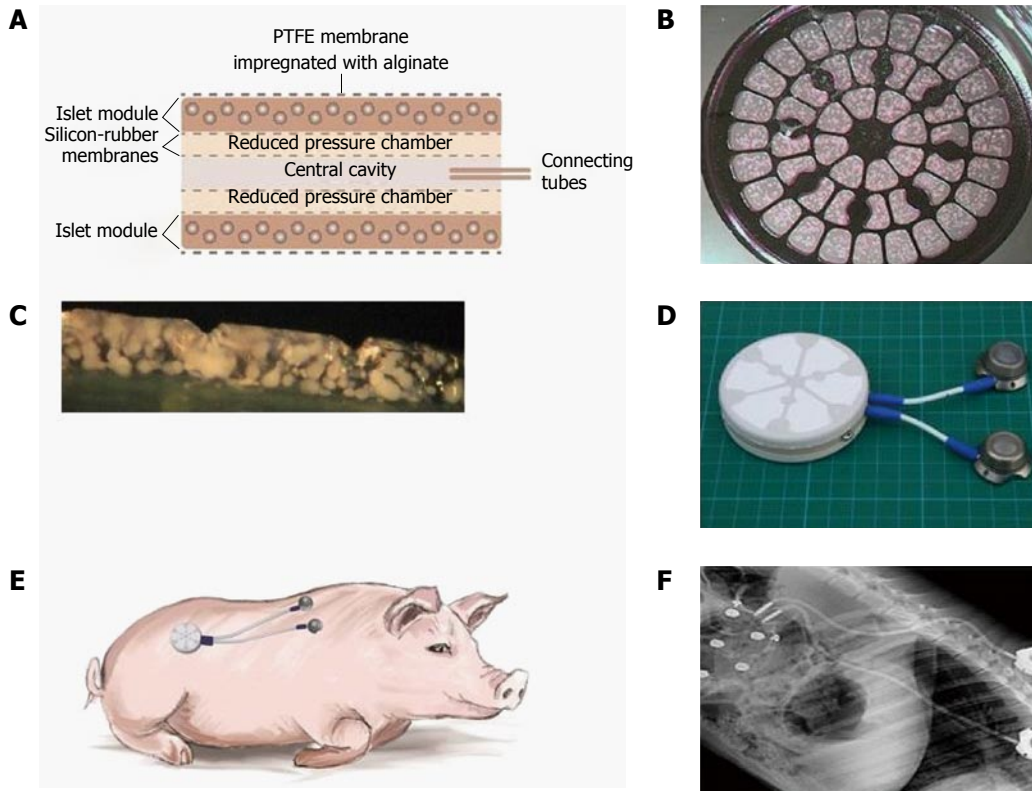


Figure 9 The design, makeup, and implantation site of the porcine-type β -Air device. A: Schematic cross section of a porcine-type β -Air device. The four dashed lines separating the central cavity from the “reduced pressure chambers” and the “reduced pressure chambers” from the islet modules are silicone rubber membranes; B: A surface image of an islet module; C: Cross section of an islet module; D: The macrochamber and connected access ports (each square is 1 cm \times 1 cm); E: Illustration of the device (including the subcutaneous access ports) implanted into a mini-swine recipient; F: X-ray image of an implanted device.

β -Air devices and refueled as such were cured from the disease for the entire study period (Evron, Barkai *et al*, unpublished data). Notably, no signs of oxygen toxicity to the islets were observed in devices refueled with oxygen at 304 and 456 Torr at surface densities of 2400 or even at 3600 IEQ/cm². Raising PO₂ level to 570 Torr led to inconclusive observations, with part of the animals refueled at this level failing to achieve normoglycemia for more than a month. Therefore, we concluded that any PO₂ < 550 Torr at the islet module-gas chamber interface is safe and maintains normoglycemia in implanted animals for long periods of time. These results also explain the toxic effects of oxygen observed at higher PO₂ (> 680 Torr) reported by others^[155,157,158].

The data collected with the rat-type β -Air device were used to design a larger, porcine-type device (Figure 9), which can maintain up to 180000 IEQ and is, theoretically, capable of supporting glycemic demands of diabetic animals of 25-30 kg at a dose of 6000-7500 IEQ/kg. The porcine-type device (Figure 9A and B) is a disc-shaped structure composed of 2 opposing islet modules attached to a gas chamber. The islet modules are composed of a planar, 600- μ m thick, alginate hydrogel encapsulating donor islets at surface density of 3600 IEQ/cm² (approximately 11% v/v). They are separated from the gas chamber by a porous gas-permeable membrane. The gas chamber is a 3-compartment structure. A central cavity is separated

from 2 “reduced pressure chambers” by a pair of porous membranes. It is connected by polyurethane tubes to subcutaneous access ports (Figure 9D). These ports allow direct injection of oxygen-enriched gas mixture (95% oxygen at 1.4 ATM; 1011 Torr) into the central cavity. Oxygen is diffusing from the central cavity into the “reduce pressure chambers” and from these chambers into the islet module where it is being dissolved in the aqueous environment of the hydrogel. The role of the 2 silicone membrane pairs separating the central cavity from the side chambers and the side chambers from the islet module is to reduce the PO₂ at the chamber-islet module boundary to < 550 Torr. A mathematical model developed for this purpose (Lorber, Barkai *et al*, unpublished data) predicted that this level is never crossed during a standard refueling cycle and that refueling every 24 h ensures minimal PO₂ at a critical value of 60 Torr, even at a depth of 450 μ m from this boundary (Figure 10). Porcine-type β -Air devices, equipped with xenogeneic rat islets, were implanted into 4 diabetic *Sinclair* mini swine with fasting blood glucose levels of > 350 mg/dL (Figure 11A). The device maintained close to normal blood glucose levels in the diabetic animals and was functional for 1 mo. The islet dose was 6700 \pm 600 IEQ/kg at the onset of the experiment and 5500 \pm 500 at time of explantation. When implantation time was extended to 90 d, BW increased by more than

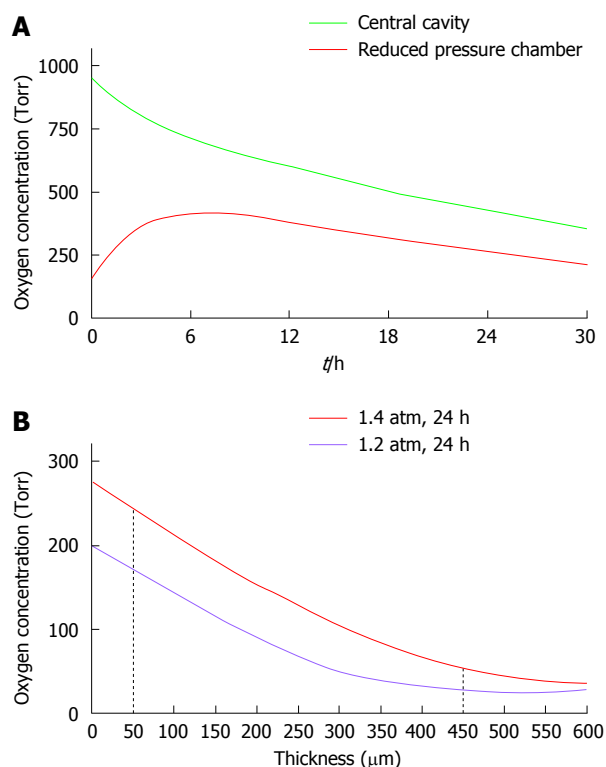


Figure 10 Predictions of the mathematical model for PO₂ levels. The parameters of the model were set as follow: Islet dose in each of the 2 islet modules, 60000 IEQ; surface density of 3600 IEQ/cm², and OCR of 3.6 pmoles/IEQ/min. A: PO₂ profile at the central cavity (green line) and at the "reduced pressure chamber" (red line) of a porcine-type β-Air device. The central cavity was refueled with 95% oxygen/5% CO₂ at 1.4 atm (1011 Torr); B: PO₂ across a section of an islet module of a β-Air device refueled with 95% oxygen at either 1.2 (purple line) or 1.4 (red line) atm. The red dashed lines represent distances of 50 and 450 μm from the chamber-islet module boundary.

60%, islet dose decreased to < 4000 IEQ/kg and, eventually, glycemic control was lost by day 75^[112]. These results clearly demonstrate that under proper oxygenation regime, xenogeneic islets dosed at 6000 IEQ/kg (half of the standard clinical dose) are curative^[112].

Our mathematical model predicted that upon refueling with oxygen at pressure of >1000 Torr, the PO₂ obtained at the "reduced pressure chamber" measured at the end of 24-h cycles (just before the next refueling), remains at > 100 Torr but never > 550 Torr. Actual measurements were made in 3 devices implanted in diabetic pigs for 90 d and are illustrated in Figure 11B. At the central cavity, oxygen tension was between 400 and 450 Torr and in both "reduced pressure chambers" it was approximately 300 Torr. These values are consistent with our mathematical model and also proved that the stored oxygen in this device is sufficient to maintain the demands of a graft comprising > 80000 IEQ for > 24 h.

A porcine-type β-Air device equipped with human donor islets was tested in first-in-human clinical trial^[154]. Images from the surgical procedure used for implantation are shown in Figure 12. Although the dose

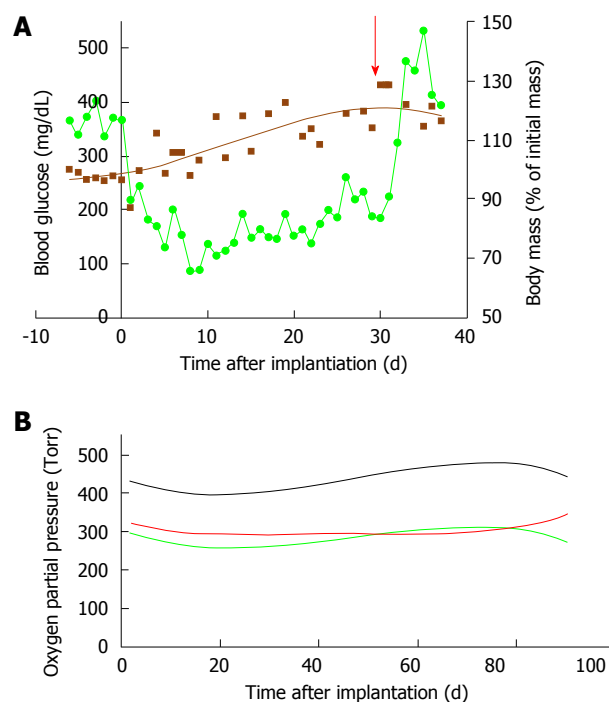


Figure 11 Implanting of the porcine-type β-Air device in diabetic Sinclair mini swine (n = 4). A: Blood glucose (green line) and body mass (brown line) of diabetic mini-swine implanted with β-Air devices are shown over time. The red arrow represents the day of explantation; B: PO₂ in the central chamber (black line) and in the 2 "reduced pressure chambers" (green, body side; red, skin side).

of donor islets used was < 20% of the standard clinical dose (approximately 2100 IEQ/kg), efficacy was clearly demonstrated. Ten months after implantation, the daily insulin requirement was reduced by approximately 15%, HbA1c decreased from 7.4% to 6.4%, and explanted islets stained for insulin and glucagon. The same device is now tested in a registered open labeled, pilot investigation clinical trial (NCT02064309).

In summary, the negative outcome of hypoxia on cultured or transplanted islets is a well-documented phenomenon. Shortage in oxygen supply must be resolved before long-term functional performance of macro-encapsulated islets graft is obtained. The studies described herein also set an upper level for long-term islet hyperoxia. Evidently, islets tolerate and are functional when directly exposed to PO₂ < 300 Torr, about 2 times the PO₂ in atmospheric air. Using these PO₂ levels, we were able to maintain isogeneic, allogeneic, and xenogeneic islet grafts in animal models and human diabetic recipients for extended periods of time.

AUXILIARY TECHNOLOGIES

Most of the BAP devices use physical encapsulation as a way to introduce donor islets into a recipient body. This approach is promising; yet, many unresolved obstacles still exist before a long-term functional BAP could be established. Auxiliary complementary

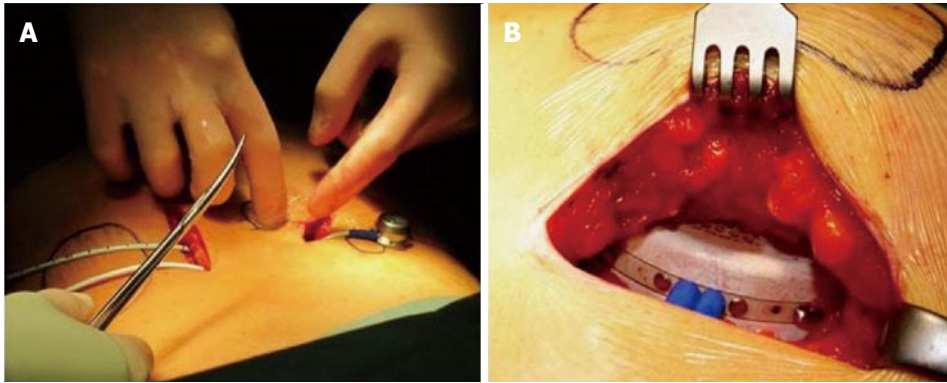


Figure 12 Implantation of the β -Air device into a patient. A: Relative positions of the device and the access ports; B: Insertion of the device into the subcutis.

technologies, especially introduced during the period immediately after transplantation, are needed to create a “friendly environment” and prevent loss of transplanted islets. In the previous chapter we provided evidence that hyperoxic oxygen supply is beneficial to graft function. However, parameters such as chronic inflammation and biocompatibility, uncontrolled loss of viable cells, distance from the vascular bed to support readily exchange of glucose, insulin, and nutrients and supportive microenvironment are still considerable hurdles to get over in order to optimize graft function.

Controlling inflammation

Implantation of a medical device is a 3-tier irritation process including: the surgical procedure; the chemistry and size of the implanted device; and the type and amount of contained cells. A tissue repair process is inevitable with any surgical procedure. It is aggravated by inserting an artificial device into the open wound and further intensified if the device includes cells. Inflammation during tissue repair process is a protective attempt of the immune system to remove the injurious stimuli and to initiate a healing process. It is a short-term process including vascular changes such as increased blood flow, vasodilation, infiltration of blood cells, and augmented permeability of plasma proteins. Inflammatory cytokines, prostaglandins, NO and ROS molecules that are locally produced by resident and imported immune cells are the major effectors of this response.

Primary malfunction of transplanted islets accounts for the bulk of graft losses (for example, see^[45,159,160]). The aforementioned encapsulation of islets in hydrogels, practiced for many years by many laboratories, is only a partial solution to this problem. Overgrowth of activated macrophages on just a fraction of implanted islet capsules negatively affects glucose responsiveness of the entire graft^[161]. Therefore, strategies to reduce inflammation are expected to improve long-term survival and proper operation of islet grafts. A pivotal approach in this direction involves using the protective mechanisms of immunomodulatory cells—Sertoli and mesenchymal stem cells (MSCs). MSCs are

described as an “injury drugstore” having antibacterial, immunomodulatory and trophic activities^[162]. They produce a curtain of activities behind which tissue regeneration is operable. These range of activities led Arnold Caplan to suggest changing the “MSC” acronym to “medicinal signaling cells”^[163]. Co-transplantation of islets and MSCs seeded on naked scaffold enhanced islet function^[164,165], and similar advantage were demonstrated following co-encapsulation of islets and MSCs^[166,167]. In our hands, rat islets co-encapsulated with marginal mass of pancreatic MSCs and cultured for 2 wk demonstrated enhanced insulin secretion capacity and better survival rate (Barkai *et al.*, unpublished data). Sertoli cells have similar effect on survival and functioning of islet graft in rodents^[168,169] and co-aggregates of core Sertoli cells and mantle β -cells promoted close-to-normal glycemic control in allogeneic recipients for > 100 d^[170]. Sertoli cells were also able to enhance survival of islets graft in xenogeneic recipients^[171–173]. Finally, co-encapsulated porcine islets and Sertoli cells were implanted into human subjects in a controversial Mexican clinical trial^[8,174,175]. Some of the transplanted patients experienced reduction in their requirements for insulin therapy for up to 3 years.

Acute phase proteins, a group of circulating plasma proteins, rapidly respond to inflammation. Hepatic alpha-1 antitrypsin (AAT), a member of this class, is abundant in the plasma and its level increases many-folds in response to inflammation. AAT protects tissues from proteases released from inflammatory cells. It also exhibits protease-independent anti-inflammatory activities against these cells and against the soluble effectors they release^[176,177]. Unlike immunosuppressive drugs, AAT helps the immune system to distinguish between desired responses against authentic threats and unwanted responses fueled by positive feedback loops^[178], thereby transforming devastating inflammation into beneficial immune tolerance. AAT was shown to prolong survival of transplanted islets in rodents^[179–181] and in non-human primates^[182]. It also induces immune tolerance in animals receiving transplantation of multiple allografts^[183]. We showed that, in diabetic animals implanted with β -Air devices, a

week treatment with systemic AAT resulted in improved survival of islet cells (Barkai *et al.*, unpublished data). Collectively, the findings suggest that proper control of inflammation may improve transplantation outcome of islets grafts.

Controlling apoptosis

Cysteine-aspartic proteases (caspases) play a pivotal role in apoptosis. Cell-permeable apoptosis inhibitors pentapeptides (V5 and DHMEQ) were shown to improve transplantation outcomes when used throughout the islet isolation process^[184,185]. Similar improvements in yield and quality of rat and porcine islets were obtained when the tetra-peptide z-DEVD-FMK (caspase 3 inhibitor) was included in the enzymatic blend used to digest the pancreas (Barkai *et al.*, unpublished data). With all the promise, there is only one anti-apoptotic drug, an orally delivered pan-caspase inhibitor (Emricasan, Conatus Pharmaceuticals Inc., San Diego, CA) that is currently evaluated as islet transplantation adjuvant therapy in a phase I / II clinical study (NCT01653899).

A subgroup of G-protein coupled receptors (GPCR) is the B-family GPCR consisting 15 members^[186], which bind relatively short peptides (20-50 amino acids long). A subset of this family of effectors includes incretin hormones (GLP-1, GIP), growth hormone releasing hormone (GHRH), and corticotropin-releasing hormone (CRH), all of which augment insulin secretion. GLP-1 was shown to inhibit apoptosis of pancreatic β -cells^[187-189], to reduce inflammation^[190], and is clinically used to treat type 2 diabetes. Less known are GHRH and CRH. Both ligands as well as their cognate receptors are expressed in pancreatic β -cells of rat and human^[191-194]. Upon binding, these ligands increases cell proliferation and decreases β -cells apoptotic rate. Both peptides also change the intracellular balance between the active and inactive glucocorticoid molecules in favor of the inactive form, thereby increasing insulin sensitivity^[191]. We tested one of these effectors in diabetic rats using the β -Air BAP. Devices loaded with islets pretreated with a GHRH agonist significantly enhanced graft function by improving glucose tolerance and β -cell insulin reserve^[153].

Controlling angiogenesis

BAP macro-devices are usually inserted under the skin. This site is characterized by poor vascularization to begin with, and adding the enveloping capsule creates a large diffusion distance between the capillary bed and the graft. Inducing dense angiogenesis at close proximity to the graft capsule may create a more supportive environment. Such induction attempts included temporal placement of pro-angiogenic membrane or mesh^[195,196], slow release of pro-angiogenic factors^[197-200], and using both these strategies concurrently^[201]. Enhanced angiogenesis that promoted long-term islet function occurred, but was validated only in rodent models. Also, from a

regulatory perspective, the use of pure pro-angiogenic factors that may promote growth of malignant cells may be problematic.

Many cells shed small (0.1-1 μ m) fragments of their plasma membranes into the circulation. Platelet micro-particles (PMP) derived from megakaryocytes are the most abundant circulating micro-particle subtype. PMP contain broad spectrum of bioactive molecules including a concentrated set of cytokines and signaling proteins. PMP are postulated to play a key role in angiogenesis^[202-204] and to treat hypoxia (WO patent 2006059329). Notably, PMP are regulated as a blood product. When freely mixed with the encapsulating hydrogel of β -Air devices and implanted for 3 wk in rats, PMP promoted denser and more mature angiogenesis of the capsule formed around the devices (Figure 13).

Controlling the Intra-capsular microenvironment

Research has focused on the inflammatory and immune responses against the capsule polymers, whereas the research on the compatibility of the intra-capsular milieu with the contained islets remains insufficient. Islets are very sensitive clumps of cells requiring nutritional factors, hormones, extracellular matrix (ECM), and a relative pliable microenvironment. Islets undergo a cellular transition immediately after encapsulation, during which islet cells are very sensitive to changes in the rigidity of the microenvironment and may die by a mechanotransduction process^[205]. The exact threshold at which islet cells are sensitive to mechanotransduction is unknown. Therefore, cell lines were used to explore whether increase in alginate-concentration in microcapsules could induce mechanotransduction-mediated cell-death. The study showed that the concentration as well as the type of alginate were critical in activating mechanotransduction^[206]. Alginates that are high in guluronic acid form stiffer gels and are associated with massive cell death as of a concentration of 2% while alginates containing more mannuronic acid exhibited optimal survival up to alginate concentrations of 3.4%^[206]. The contribution of micro-environmental rigidity to the enormous inter-lab variability in survival of encapsulated islets remains to be established and warrants further investigation and standardization.

Engineering the intra-capsular milieu with ECM molecules may decrease the effects of mechanotransduction. It has been suggested that integrins are the sensors of the cells for mechanical stress. A synthetic peptide RGD, mimicking the original tri-peptide part on the ECM molecule fibronectin is now being sold by Novamatrix (Sandvika, Norway). It binds and prevents clustering of integrins which form an essential step in mechanotransduction^[207,208]. Some groups have added RGD or IKVAV (another integrin binding epitope) to the intracapsular environment and demonstrated improved viability and functionality under culture

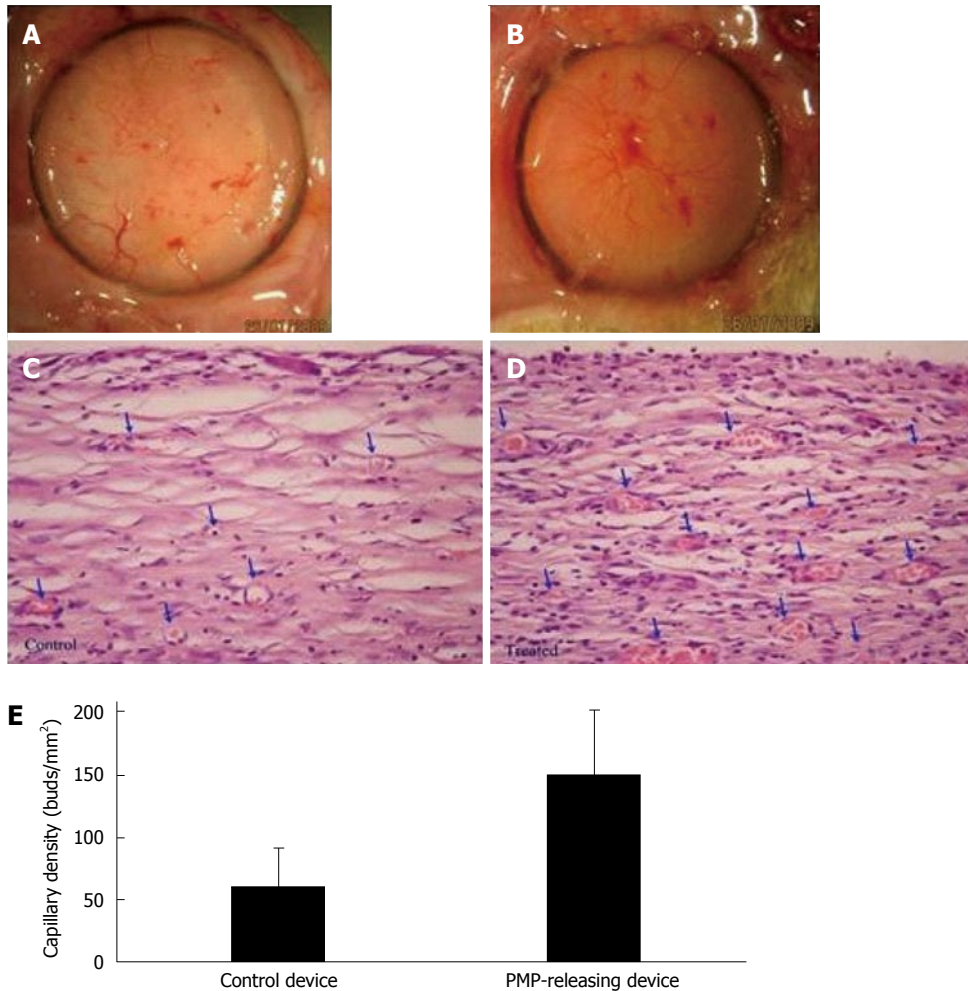


Figure 13 Platelet micro-particles-induced angiogenesis in capsules formed around β -Air devices ($n = 4$). A, B: Surface views of capsule formed around a control device (A) and PMP-releasing device (B); C, D: Histological sections of a capsule formed in close proximity with a control (C) and a PMP releasing device (D). Arrows indicate blood capillaries in the capsule. Original magnification, $\times 40$; E: Quantitative analysis of primary mature capillaries (SMA-stained) in the capsule. PMP: Platelet micro-particles; SMS: Smooth muscle actin.

conditions (for examples, see^[209,210]) and in animal models^[211]. However, ECM molecules may be necessary for additional processes contributing to prevention of anoikis and prolonging survival of islet cells as they are anchoring sites for many essential growth factors. To date, only little is known on the role played by the lack of specific ECM components on islet longevity^[45].

The quality of the intra-capsular milieu is far more than a step towards survival of more functional cells. It also contributes to prevention of pro-inflammatory immune responses against the grafts. Human encapsulated islets regularly undergo 4 processes of cell death: Necrosis, apoptosis, autophagy and necroptosis (de Vos *et al.*, unpublished data). In islets, all these cell-death processes ended with the release of significant amounts of danger-associated molecular patterns (DAMPs), which even in small amount activate immune cells. Microcapsules retain part of the DAMPs, however significant amounts are still released. Adding NEC-1, an inhibitor of necroptosis reduced DAMP release and activation of immune cells and rescued larger part of the islet cells^[212]. Combined, these data highlight that

the adequacy of the intracapsular microenvironment should be taken into consideration.

CONCLUSION

Encapsulation in permselective membrane is experimentally used in diabetes for progressing from drug- and standard cell-based therapy to immunosuppressive-free cell-based therapy. Cell encapsulation is a mandatory but not a sufficient requirement for an efficient curing technology. Adequate oxygen supply to the grafted cells constitutes the second tier of mandatory requirements. Fulfilling these requirements should enhance the practicability of clinical islet transplantation; however, successful implementation of a cell-based cure also depends on auxiliary technologies, some of which are portrayed in this review.

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Key psychosocial challenges in vascularized composite allotransplantation

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Abstract

Psychosocial factors are important elements in the assessment and follow-up care for vascularized composite allotransplantation (VCA) and require multidisciplinary evaluation protocols. This review will highlight differences between VCA with solid organ transplantation (SOT), provide information on the psychosocial selection of VCA candidates, ethical issues, psychological outcomes, and on the need for multicenter research. VCA is primarily a life-enhancing procedure to improve recipients' quality of life and psychological well-being and it represents a potential option to provide reproduction in case of penile or uterine transplantation. The risk benefit ratio is distinctly different than SOT with candidates desiring life enhancing outcomes including improved body image, return to occupations, restored touch, and for uterine transplant, pregnancy. The Chauvet Workgroup has been convened with membership from a number of transplant centers to address these issues and to call for multicenter research. A multicenter research network would share similar evaluation approaches so that meaningful research on psychosocial variables could inform the transplant community and patients about factors that increase risk of non-adherence and other adverse psychosocial and medical outcomes.

Key words: Vascularized composite allotransplantation; Psychological evaluation; Motivation; Psychosocial outcomes; Quality of life

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Core tip: A psychosocial evaluation for vascularized composite allotransplantation (VCA) is unique and should be informed by many characteristics that are described in this review article including the importance of multidisciplinary care and the need for careful selection of candidates for VCA. Important areas to

consider in the evaluation include: History of ability to comply with medical care, body image, adaptation to previous trauma and preparedness for transplantation, reasonable expectations, and presence of adaptive coping skills of the candidate. Multicenter research will support better understanding of psychosocial variables that predict outcome. Optimally, developing a common evaluation strategy to enhance comparison of candidates with good outcomes to those with less optimal outcomes will help in future selection of candidates.

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THE HISTORY OF VASCULARIZED COMPOSITE TISSUE ALLOTRANSPLANTATION

The rapidly expanding vascularized composite allotransplantation (VCA) field combines the technical challenges of surgery and microsurgery with the multidisciplinary care that characterizes solid organ transplantation (SOT)^[1,2]. The technical demands of VCA and complex psychosocial issues pertaining to the recipients significantly accounts for the discrepancy between these two related fields^[3]. Although VCA and SOT share a common history, VCA has not yet been performed on a scale approaching that of SOT^[1,4]. Currently, the following four main domains for VCA exist: hand, face, uterus, penis transplantation although other areas are emerging.

In the history of medicine there are several well documented cases that demonstrate the developing concept of reconstructive transplantation medicine^[2,5,6]. One such account is "The Legend of the Black Leg (Leggenda Aurea)", about twins Cosmos and Damian, who transplanted the leg of a man with that of an Ethiopian in 348 AD^[7]. In the 16th century, in Italy, Gaspare Tagliacozzi transplanted a nose from a slave to his master^[8]. Reports of tissue transplants occasionally were reported^[6]. Bunker^[9] performed a transplant involving a sheepskin. Carrel^[10] attached an artery from the arm of a father to the leg of his infant son who suffered from intestinal bleeding^[11]. Guthrie^[12] transplanted dog heads onto the neck of other-dogs. Although surgical techniques were created, the immunological challenges made transplant unfeasible^[13], until the discoveries of Medawar and colleagues^[14], who described rejection which allowed advances leading to modern transplant immunology^[5,15]. In 1957 Peacock *et al*^[16,17], coined the term composite tissue allograft

and in 1964, Robert Gilbert^[18], performed the first hand transplantation (HTx) in Ecuador. A single hand was transplanted to a bilateral hand amputee, but the graft was amputated three weeks later as a result of acute rejection. This early unsuccessful experience contributed to a 30-year period of stagnation in the field. Significant developments in immunosuppressive drug therapy facilitated the growth of SOT^[2,5]. The next two HTx were performed in 1998 by pioneers Dubernard *et al*^[19-21] in Lyon and in 1999 Warren Breidenbach^[22] in Louisville, thus starting the modern era of reconstructive HTx^[6]. Since 1998 73 HTx, 23 unilateral and 25 bilateral transplant, for a total of 48 patients have been reported^[23].

The encouraging outcomes in human hand transplants led to the development of human face transplant (FTx) programs^[6]. In 2003, surgeons in Nanjing, China transplanted a skin flap including an extensive part of the scalp and both ears^[24]. In 2005, by transplanting a triangular graft from the nose to the chin including the lips, Bernhard Devauchelle and Jean-Michel Dubernard from Lyon performed a partial face transplant on a woman disfigured by a dog bite^[13,25]. In April 2006, a 30-year man suffering from trauma from a bear, received the second face transplant^[26].

Face transplantation has garnered wide interest with the public and in the media due to the importance to identity that the face represents. Therefore, psychosocial issues in FTx are as important as in HTx or more so and the multidisciplinary evaluation and treatment has to ensure that these are addressed adequately. Since the first FTx in 2005, almost 32 face transplants have been performed worldwide with promising outcomes including reasonable functional improvements and reports of patients satisfaction^[23,27].

Recently, penile (PTx) and uterine transplantation (UTx) are the focus of VCA research. In 1992, a conceptual framework for human PTx was developed by Eberli *et al*^[28] in 2008 who transplanted bioengineered penises onto rabbits. In 2006, a Chinese man received the first donor penis, but the transplant had to be removed by surgeons at the request of both the patient and his partner. This first case emphasizes the psychological impact that transplants can have, especially with an organ as significant to sexual function and identity as the penis. The first successful PTx was performed on a 21-year-old man in December 2014 by André van der Merwe and Frank Graewe at the University of Stellenbosch in South Africa^[29]. Subsequently, the recipient has been reported to have recovered function in the organ (including urination, erection, orgasm, and ejaculation), and has, remarkably, since successfully conceived a child^[30].

The earliest UTx was performed in 1931 on a transgender woman in Denmark who died from rejection three months after transplantation^[31]. The development of *in vitro* fertilization in the late 70s resulted in decreased interest in this area^[32]. Two UTx

attempts by teams with no preceding research records in this field followed. In Saudi Arabia in 2000 an UTx was performed from an older hysterectomy patient into a 26-year-old. The graft failed due to vascular occlusion^[33,34]. In 2011, the second transplant involved a uterine graft from a deceased female multiorgan donor^[35]. This case resulted in two pregnancies but with early miscarriage^[36]. The first mother-to-daughter uterine transplant was performed in 2012 in Sweden^[37]. Following extensive preliminary research that UTx is a treatment for absolute uterine factor infertility (AUI) and that also this AUI treatment, which combines *in vitro* fertilization and UTx, this is now a viable option for selected infertile patients^[38]. The UTx project encompasses a total of 9 recipients and the first live birth after UTx was reported^[39]. Because of the risks of an invasive organ transplant procedure and to avoid the need for lifetime immunosuppression, this is considered a temporary transplant with the expectation of hysterectomy after couple of successful pregnancies^[38].

As already determined from SOT, transplant outcomes depend on the selection of an optimal combination of immunological, surgical, and psychosocial factors. The history of VCA underscores the importance of interdisciplinary assessment before surgery. A patient's psychosocial suitability for VCA is as important as the surgeon's technical ability and the effectiveness of postoperative immunosuppression^[3]. Several cases of noncompliance with immunosuppression and physical therapy reveal how allograft survival needs to be supported by psychosocial stability and an ability to comply with complex medical care^[3]. This is especially critical when the graft is involved in tasks related to a part of the body that senses, supports instrumental tasks of daily living, and is visible to others^[2,3]. Additionally, what all kinds of VCA have in common is the fact that there are still ethical concerns regarding the entire procedures, especially because the VCA is a life-enhancing not life-saving procedure, with psychosocial issues like quality of life (QOL), body image, psychological well-being, etc. weighing significantly in the risk benefit ratio of candidates considering VCA^[3,40].

At present the number of successful VCAs is increasing and several transplant centers worldwide have developed specific VCA programs^[40]. Although research provides some understanding of functional and sensory outcomes, psychosocial outcomes have been minimally reported^[3]. We will discuss in this paper aspects of VCA transplantation that have been reported in the literature and extrapolate from literature in SOT to anticipate key areas of interest to enhance psychosocial outcomes in VCA and discuss the key psychosocial challenges we face in VCA today.

PSYCHOSOCIAL IMPLICATIONS OF VCA

As already discussed, certain characteristics of VCA are uniquely different from SOT, particularly because

VCA is primarily a procedure to improve the recipients' QOL and psychological well-being or represents a potential option to provide reproduction in case of PTx and UTx. Since candidates considering VCA present no life-threatening illness, their motivation related to improved functional outcomes, occupational attainment, improved body image, restored touch, and in uterine transplantation, pregnancy^[3]. Therefore, scientific consensus exists that the assessment of the candidates' desire for VCA is a psychologically complex and warrants a customized psychosocial evaluation protocol that fully addresses the issues noted above^[3].

Again, comparing the psychosocial characteristics of VCA with SOT, the visible nature of the allograft strikingly changes the experience of transplantation for VCA recipients^[40,41] (other than UTx). Visible grafts could adversely effect the recipients' sense of themselves as an integrated whole, leading to rejection of the grafts as undesirable^[42]. Several cases demonstrated the importance of the successful psychological integration of the allograft for post-transplant outcomes, *e.g.*, amputation of the first successfully transplanted penis because of the recipient's and his partner's coping inability. Notably, patients must accept a new graft while adapting their loss of a part of their body that was unique to them^[43]. This requires alterations in their sense of who they are, how the graft fits in with their body, and ultimately acceptance of the allograft as part of themselves^[44].

When considering factors that could impair candidates' adherence with medications and physical therapy^[45-47], relevant information will be obtained by examining their psychiatric history, coping abilities, and social support^[48]. In Coping styles, support from family and friends, financial, and logistical factors emerge as important predictors of successful outcomes^[48]. Therefore, the evaluation protocol should additionally provide an assessment of family relationships and anticipate stress that might come from media attention which has occurred in a number of VCA cases^[49]. Patients will experience an initial decrease in function and caregivers will need to prepare for increased recipients needs for instrumental tasks of daily living potentially while also carrying a heavier burden of caring for children and maintaining employment^[3].

Ethical considerations

Aside from considerations of technical demands regarding modern transplant programs and costs, the field of VCA involves a number of ethical issues^[50]. The principle of patient autonomy is necessary for these procedures balanced by nonmaleficence to support limited risk to patients. It would appear that beneficence and justice are equivocal in this population^[51].

No instruments exist to fully measure the impact of hand(s) loss, facial distortion, the loss of penis, and reproduction inability^[3]. This makes the assessment process in VCA especially challenging^[51]. Prospective research and qualitative studies should focus on the

unique qualities of this experience including the highly individual nature of the VCA including, spiritual and cultural factors that also may be important^[52]. Ethical issues are myriad and collaborating with biomedical ethics experts would do justice to the complex issues that may arise for this patient population^[3].

Three important ethical considerations are patient selection, patient advocacy, and informed consent^[53]. When assessing for decision-making capacity and the candidates' overall ethical suitability to receive a VCA, the ethical guidance process should be based on this rubric of questions^[54,55]. Similar to living donation, the Lyon team viewed the first HTx decision as being one in which the candidate had to weigh the pros and cons from themselves^[56]. Informed consent for VCA recipients is a detailed process focusing on risks in surgery and anaesthesia and post-surgical complications (e.g., immunosuppressive effects, psychiatric disorders, etc.)^[53,54,56]. Consent related to the donor, is also an area of interest with some countries having an "opt-out" system with implications for how families may experience the donor related experience^[56].

Ethical considerations were noted in the "Montreal Criteria for the Ethical Feasibility of Uterine Transplantation"^[57] that describe a set of criteria for the ethical practice of UTx in humans and we refer interested readers to the original paper on this. Key points include that the candidate has failed other therapy and is not eligible for other options such as adoption. An assessment of the candidates' ability to manage the tasks of motherhood is noted. The donor must have decided that their reproductive years are concluded and be able to consent to donate and be free of coercion. Finally, the institution must have all the needed staff and facilities to provide the care and ensure informed consent for donor and recipients as well as protection of anonymity in the process.

In addition, another important and challenging question is a philosophical one related to how allograft represents personal identity including implications for how one communicates with others^[56]. In case of PTx we have to consider the function of physical intimacy. The intimate nature of the grafts may have implications for others with whom the donors have been intimate and for future partners of the recipient^[6,50,56,58].

In summary, the ethical issues in VCA are quite complex and are unique to this population and effect the recipients very sense of being^[50], which may impact post-transplant motivation^[59,60]. Utilizing biomedical ethics consultation on a case basis may be especially helpful for this population^[51].

Risk-benefit considerations

As noted in the international literature, VCA is life enhancing rather than life saving such as in the case in SOT^[1,56]. VCA candidates may overestimate the benefits of the procedure while minimizing the recovery period and not fully acknowledging the

surgical risk, demanding post-transplant medication regimen, and rehabilitation requirements^[3,61]. The risk-benefit ratio is quite different than SOT in which the risks are offset by the lifesaving nature of the procedure^[3,40,51]. VCA candidates have to face potential episodes of acute rejection^[62] and immunosuppression-related complications which are typical but can be reversed with proper medical treatment^[63,64]. Chronic allograft rejection that is predicted by the frequency and timing of rejection episodes has become a primary cause of long-term allograft failure^[62]. Particularly, the risks of nonspecific immunosuppression^[50,65] and the lengthy rehabilitation are the most important critical aspects that may lead to demoralization and non-adherence in rehabilitation^[52,66]. Rejection episodes and delayed function, difficulty with the rehabilitation, and long-term side effects of immunosuppressive treatment (e.g., malignancy, metabolic infections/disorders, diabetes, renal failure, etc.)^[50,65] may cause mood changes, anxiety as well as depressive reactions that substantially impact patients' adherence and require supportive treatment.

Although immunoregulatory protocols continue to be developed with decreased toxicity^[67] immunosuppressive medications are still required^[3], necessitating careful patient selection given the problematic nature of the risks of these therapies^[68] including infection, metabolic derangements^[46,47,69,70], toxicity^[70-73], and cancer^[69-74]. This potential improved function must be balanced against this significant risks^[63,67]. Patients have different risk thresholds which contribute to their decision making about how much risk they are willing to accept for improved function^[55,66,75-77], especially taking the psychosocial aspects of VCA into account (e.g., QOL factors, sense of identity, understanding of the treatment and its limitations, etc.)^[50]. In summary, the risk vs benefit decisions has to be judged on wider criteria that must include all relevant psychosocial aspects of VCA^[78].

Despite the encouraging results regarding the aesthetic and functional outcomes that have been achieved in patients who have undergone HTx in the last 15 years, risks persist^[50,66,75,76]. The International Registry on Hand and Composite Tissue Transplantation (IRHCTT)^[23,64] represents the world's largest database and research initiative to collect information from each case of VCA or composite tissue allotransplantation (CTA), thus it provides a comprehensive overview about what is happening in this new field of transplantation medicine. Currently, the IRHCTT includes cases of upper extremity and face allotransplantation performed all over the world^[23] with rejection rates of 85% of the hand and face patients in the first year and three recipients have died^[23,64]. Seven hand grafts were lost due to rejection in China^[23,63] and a similar number have been lost to rejection and other complications in European and American experience^[23,63,64,79,80]. Fortunately rejection was often detected and treated

without loss of graft^[23,63,64].

This literature highlights the need for careful patient selection to ensure that proper adherence to medication regimens occurs^[3,68]. Unilateral amputees appear to be more risk adverse due to the less compelling need for the graft while bilateral hand patients may be willing to accept the risk of rejection which is offset by the potential for significantly enhanced independence^[3,77].

Similar to the risk-benefit profile of HTx candidates, those who consider FTx also have to face specific risks and make their decision on the expected benefits^[81]. Beside the documented benefits of FTx, such as the improved functionality (e.g., ability to breathe, speak, swallow, smile, etc.), the restoration of a near-normal facial appearance, and the reduction of pain and discomfort (FTx is one large procedure, whereas conventional face reconstruction involves many surgeries), there are certain risks that tend to be peculiar to FTx. For example, the donor's appearance is not transferred to the recipient and the recipient is not typically recognizable immediately following surgery, so that the patient potentially may feel upset about having a new (changed) face^[81-84]. The IRHCTT^[64] data document episodes of acute rejection in 60% during the first year after FTx (on average two episodes per year). One FTx team declared a case of "chronic" rejection whereas other teams described chronic rejection to the IRHCTT. When looking at the patients' survival: One patient (simultaneous face and bilateral hand transplantation) died for cerebral anoxia on day 65; one patient died for lung failure 11 mo after transplantation; one patient died for pharyngolaryngeal neoplasia 3 years after transplantation. Only one graft has been removed for unknown causes. In addition, the following complications/side effects have been reported: opportunistic infections (e.g., herpes virus, bacterial infection, etc.), metabolic complications (e.g., hypertension, increased creatinine values, etc.), malignancies (e.g., basal cell carcinoma, pharyngolaryngeal neoplasia), and other side effects (e.g., neurofibromatosis of the transplanted face, trauma of grafted face, etc.)^[27].

Candidates who consider PTx or UTx share the same burdens and risks that are characteristic of VCA. The candidates have to face the risks of the surgical procedure, of ischemic injury, of graft loss, and psychosocial complications (e.g., inability to accept the allograft, interpersonal conflicts, non-adherence, etc.)^[85]. In the case of UTx, additionally, the risks of living donors (in most cases the mother of the female recipient became the donor who provided the uterus) need to be considered since they have to bear the particular burden of hysterectomy. Notably, the examination of mental conditions and QOL after hysterectomy is important, because a donor may have decreased QOL due to complications (e.g., affected sexuality). Donors after hysterectomy may have unstable mental conditions including anxiety and

depression, and may have additional burden from severe stress due to postoperative pain^[85]. Because the uterus is a symbol of femininity, childbearing, sexuality, vitality, youth, attractiveness^[86-88], the hysterectomy can lead to postoperative regression^[89-92], distortion of body image^[87,93], and loss of feminine self-image^[94].

PSYCHOSOCIAL RESEARCH IN HAND TRANSPLANTATION

While it is universally accepted that a psychosocial evaluation is needed in SOT^[95,96], the literature is still evolving and no single evaluation strategy has emerged^[3]. Although no standard approach has been published^[20,22,41,49,51,97-113], several domains have emerged as important and predictive of increased risk^[3,114-121]. Recent efforts in research are occurring to attempt to address this deficiency in the literature^[40].

Generic instruments have been developed to identify areas relevant to transplant populations (e.g., psychiatric disorders, adherence, transplant health literacy, etc.)^[3,122-124], but are not designed for areas specific for HTx such as satisfaction with prostheses, body image, physical limitations, and phantom limb pain^[40]. Creating a screening instrument customized for these patients is a goal for the field^[40,125].

A review of psychosocial evaluation strategies has been previously reported^[40] which includes semi-structured psychiatrist or psychologic evaluations and/or psychometric and projective testing^[20,22,41,49,51,97-113]. Case studies focusing on patients QOL, satisfaction with outcomes, and body image improvements have been a large part of the research reported^[40,101]. Overall, the majority of recipients reported having psychologically integrated the hand, and reported improved confidence in appearance and in social situations^[102,105]. The recipients assimilated the transplanted hand(s) into their body-/self-image and were able to develop a sense of "ownership". Another important outcome was the observed improvements in QOL and ADLs^[3].

Unmet expectations and either new or recurring psychiatric conditions have been reported^[126]: Including suicide attempts following hand transplant^[105]; request for amputation because the recipient could not integrate the grafted hand into his sense of self^[111]. The inability to psychologically incorporate the transplanted hand(s) may result in non-adherence with medications^[40,45-47], which in turn will lead to rejection and may necessitate amputation^[45]. Additionally, recipients may be frustrated with the lengthy process of recovery including loss of ability to do tasks while rehabilitating leading to decreases physical QOL at least initially^[3,63].

Optimally, candidates will have a strong motivation for transplant and have demonstrated good compliance with medical care in the past, have strong family support, utilize acceptance, flexibility and problem

solving in adapting to the loss of function from the injury/deficit and for future rehabilitation following transplant^[3,127-129]. Having appropriate expectations regarding immunosuppressive risks, surgical complications, and realistic understanding of functional gains after transplant is the best scenario for a psychologically prepared candidate^[55,61].

The optimal assessment includes: Health literacy regarding transplantation, assessment of pain related to amputation and phantom limb pain, family support, adaptation to prosthesis, financial and family stressors, assessed through multiple interactions with a variety of assessors including psychiatrists, psychologists, social workers, hand therapists, and all team members^[3,48,130]. Future research efforts directed at sharing similar evaluation strategies across centers in research protocols to determine best practices and predictive factors for optimal outcomes are needed^[3]. Another important component of interdisciplinary screening should be the identification of at-risk candidates. Intervention strategies to assist these candidates might then lead them to be eligible for this treatment and might especially be beneficial in supporting their ability to succeed with medication adherence and overall QOL post transplantation^[3,49,131].

PSYCHOSOCIAL RESEARCH IN FACE TRANSPLANTATION

FTx results in a visible change that affects social interactions and self-esteem in a profound way^[81,132], because the face is closely linked with a person's identity^[83] and can be conceptualized as an allotransplant with various functions (including communication, expression of emotion, perfection, *etc.*)^[133]. For this reason, FTx is never performed for cosmetic reasons alone^[134]. In the case of facial disfigurement, several difficulties, such as depression, anxiety, low self-esteem and QOL, poor marital and social relationships, and changes in body image have frequently been reported^[135]. What all types of VCA have in common, including FTx, is the fact that increased emphasis is placed on informed consent for a life-enhancing surgical procedure. Speech therapy and reintegrating into social settings are important^[134] as are tracheotomy care and strategies for maintaining nutrition^[81,136]. Plans for managing graft failure with a skin graft or flap are also described in the literature^[134].

When selecting candidates for FTx, the idea that the ideal candidate should not manifest some degree of anxiety and depression may be unrealistic, because patients with facial disfigurement suffer from painful dentition, chronic pain disorders related to damaged orofacial structures, and may have residual symptoms of PTSD. The candidate's adaptation to disfigurement using adaptive strategies rather than avoidance has been described^[81]. Similar to other types of VCA, there are specific psychosocial domains that need to

be considered in FTx evaluation protocols, including perception of appearance, mood disorders, presence of chronic pain, social ostracism, QOL, confidence, and social connectedness and integration^[81]. In addition to the semi-structured psychological interviews that are used to assess potential candidates for FTx, specific rating instruments (predominantly self-report measurements) have been developed for the purpose of prioritizing candidates for FTx: (1) the Perception of Teasing-FACES^[137]; (2) Facial Anxiety Scale-State^[138]; and (3) the Cleveland Clinic FACES score^[134,136], analogous to the MELD score. Usually, the pre-transplant psychosocial evaluation protocol used to identify the suitability of candidates for FTx, served as basis for the comparison in the post-transplant period^[83]. To improve the candidates' pre-transplant assessed suitability and to give them adequate support during the course of FTx, psychiatric and psychological consulting/treatment were performed^[84].

Concern about depersonalization towards the transplanted face and identity confusion with the donors face have not been reported^[27], and psychological outcomes for recipients of FTx have been generally favorable^[139,140]. The review of international literature about the assessment of psychological outcomes after FTx shows lower rates of depression and verbal abuse and significantly improved body image and social integration^[81,82,134,141-145]. Some studies report an initial decrease of psychological functioning and QOL immediately after FTx^[81,83,134]. In such cases the recipients have often adjusted to their deficits before transplantation and the extensive rehabilitation may lead to a temporary decrease of these psychosocial factors. In addition, psychological findings point to less psychological distress and depression, less verbal abuse, improved affective responsiveness, and social integration^[84]. Patients acceptance of the transplant and report of improved QOL is encouraging^[27], with additional psychosocial improvements after FTx (*e.g.*, return to work, *etc.*)^[82,84,141,143,144,146-148]. Two adaptive coping styles were common to almost all recipients, namely use of active coping and emotional support, and recipients reported normal to high self-esteem^[83]. Particularly, the rigorous preoperative psychosocial evaluation and follow-up of well selected candidates has led to an overwhelmingly positive psychological outcome^[27,149]. One exception is the non-adherent patient who used traditional medicinal approaches leading to multiple episodes of rejection and ultimately death^[27,142]. This highlights the need for careful patient selection, transplant health literacy, and careful ongoing monitoring for non-adherence following transplant^[27].

PSYCHOSOCIAL RESEARCH IN PENILE AND UTERINE TRANSPLANTATION

At present, the existing literature on psychosocial

evaluation and outcomes in PTx and UTx is limited and these still experimental surgical procedures have been performed in small numbers of patients. However in the field of PTx and UTx there exists the scientific consensus that psychosocial factors are important and the psychosocial evaluation is crucial for all candidates considering transplantation. By considering the already developed psychosocial evaluation and follow-up protocols for other VCA populations, *e.g.*, of hand(s) as well as face, almost the identical psychosocial aspects are of great importance. Nevertheless there are specific psychosocial aspects that are characteristic for PTx and UTx. Particularly, the function of physical intimacy of the allograft is one great difference and the motivation for PTx or UTx can emerge from the desire to restore bodily integrity, body image concerns, and even the hope to get pregnant/to beget a child, *etc.*^[150,151]. In case of UTx, moreover, the graft will not be for lifelong use and will be removed after the patient has had a limited number of children^[38,39], which may result in the recipient having limited time to partly adapt to the post-transplant regimen^[150].

Currently, the Swedish uterus transplant experience presents the most established VCA program for female candidates considering UTx^[38], and this was derived from a previously created face transplant protocol^[152]. The colleagues from the Sahlgrenska University of Gothenburg have developed a standardized evaluation protocol that uses a comprehensive pre-transplantation selection process that determines the suitability of the candidates and donors (*e.g.*, including psychological questionnaires regarding QOL and mood as well as semi-structured interviews with partners) and identifies potential vulnerabilities that need additional supportive treatment. Both the candidates and donors are assessed for psychiatric disorders, chemical dependency, social support, interpersonal conflicts, unrealistic expectations, and other factors related to lifestyle^[150].

Nine UTx have been performed, with two grafts removed in the first few months^[39,150]. The other seven women adapted well and following the initiation of menses, expressed relief in organ function and happiness about having a return to possible reproductivity. According to the follow-up outcomes 6 mo after UTx, the couples reported readjustment to baseline QOL and satisfactory sexual experience (no difference in sexual function or satisfaction). Despite the couples feeling well prepared and well informed about complications, couples with graft failure and subsequent removal had worse physical and psychological outcomes. Recipient-donor relationships returned to their pre-transplant state, which occurred more quickly with mothers/daughter pairs. However, the recipients who received a graft from someone other than their mother felt guilt related to an increased sense of responsibility to the donor^[150]. Finally, the Swedish UTx program highlights the importance of a multifaceted evaluation strategy and

that the evaluation should include identifying adaptive coping strategies and a strong alliance characterized by assertive and fluid communication with the transplant team^[38].

Penile defect is rare and only two cases of PTx are documented in the international literature^[151,153]. Although, the currently existing data of psychosocial aspects in PTx is limited, we can hypothesize that the psychosocial evaluation and follow-up are equally crucial as for any other life-enhancing types of VCA. The first case of PTx occurred in a 44-year-old male with previous trauma of the penis. Following transplant, the penis had to be removed because of psychological problems between the patients and his spouse at day 14 postoperatively^[151]. The psychological consequences of PTx showed that it is not easy to use and permanently see the allograft that was derived from a dead person. Nevertheless, in December 2014 a successful PTx was performed on a 21-year-old man following an unsuccessful circumcision procedure at age 18. Currently, the results of the psychological evaluation and follow-up were not reported, but the recipient previously had threatened to commit suicide if not considered for PTx^[153]. According to latest media reports, the recipient has in the meantime successfully conceived a child^[30].

ROLE OF MULTICENTER RESEARCH

Because there is still a lack of quantifiable data in the field of VCA^[40] and the inhomogeneous psychosocial protocols that have been developed from the transplant centers worldwide^[3,40], we feel strongly that our understanding of psychosocial predictors of outcomes will only be identified when sufficient numbers of patients are studied in multicenter research protocols^[3,154]. Because VCA is still uncommon, candidates who agree to undergo the surgery may be atypical in ways that are difficult to appreciate. Hence, it is recommended that transplant centers consider selecting several assessment and follow-up protocols to be administered collaboratively and consistently to all VCA recipients to strengthen and deepen our knowledge about psychosocial issues in VCA^[83,132], including prospective measurements across the continuum of time points from pre to post transplant^[3]. Therefore, it will be important that all transplant teams adhere to well-defined psychosocial guidelines and provide necessary multidisciplinary expertise^[6]. In addition, quality improvement strategies and qualitative research as well as demonstrable improvements in efficacy and financial cost offsets should take place^[3,67]. Once this occurs, VCA will become increasingly attractive to patients, insurance providers, and the medical community^[6].

CONCLUSION

In modern multidisciplinary transplantation medicine

the four areas of VCA (to date hands and faces have been transplanted in larger numbers, but also penile and uterine transplantations have occurred) represent an evolving field^[155] where psychosocial factors are important in successful outcomes^[3,40,48,49]. This review contrasted VCA with SOT and provided information to guide psychosocial selection and risk-benefit assessment of VCA candidates^[1,4]. VCA is primarily a life-enhancing procedure to improve the recipients' QOL and psychological well-being. The candidates' motivation for VCA is multifaceted and fundamentally different from SOT^[3,48].

Although it is clear that successful outcome requires a multi-staged multi-disciplinary psychosocial process to select candidates best equipped for VCA^[3], standardized evaluations have not been determined^[40,48]. Collaborative research on psychosocial predictors of outcome is needed^[3]. Additionally interventions to enhance the coping strategies of candidates and support their innate resilience are needed for them to best adapt to post transplant life^[3,49,156-158]. Thoughtful consideration of ethical challenges related to informed consent and the balance of autonomy and nonmaleficence is needed and future collaboration with experts in biomedical ethics is welcomed. We support and are involved in the development of multidisciplinary/-multicenter VCA research to identify psychosocial factors that can impact outcomes following VCA and will lead to further improvements for this patient population^[3,40,49].

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Renal transplantation with expanded criteria donors: Which is the optimal immunosuppression?

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allograft survival rates and, generally, worse outcomes than standard criteria donor kidneys, recipients of ECD kidneys generally have improved survival compared with wait-listed dialysis patients, thus encouraging the pursuit of this type of kidney transplantation. The relative benefits of transplantation using kidneys from ECDs are dependent on patient characteristics and the waiting time on dialysis. Because of the increased risk of poor graft function, calcineurin inhibitor (CNI)-induced nephrotoxicity, increased incidence of infections, cardiovascular risk, and malignancies, elderly recipients of an ECD kidney transplant are a special population that requires a tailored immunosuppressive regimen. Recipients of ECD kidneys often are excluded from transplant trials and, therefore, the optimal induction and maintenance immunosuppressive regimen for them is not known. Approaches are largely center specific and based upon expert opinion. Some data suggest that antithymocyte globulin might be the preferred induction agent for elderly recipients of ECD kidneys. Maintenance regimens that spare CNIs have been advocated, especially for older recipients of ECD kidneys. CNI-free regimens are not universally accepted due to occasionally high rejection rates. However, reduced CNI exposure and CNI-free regimens based on mammalian target of rapamycin inhibitors have shown acceptable outcomes in appropriately selected ECD transplant recipients.

Key words: Expanded-criteria donors; Outcomes; Kidney transplantation; Immunosuppression; Survival

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Abstract

The growing gap between demand and supply for kidney transplants has led to renewed interest in the use of expanded criteria donor (ECD) kidneys in an effort to increase the donor pool. Although most studies of ECD kidney transplantation confirm lower

Core tip: Kidney donor shortage is chronic, persistent and increasing in most countries worldwide. Therefore, there has been renewed interest in the use of expanded criteria donors (ECD) to increase donor pool. Compared to standard criteria donor kidneys, ECD kidneys are associated with up to a two-fold increased

risk of delayed graft function, acute rejection, and graft loss. The optimal induction and maintenance immunosuppressive regimen for ECD transplant recipients is not known due to shortage of randomized trials. Induction with antithymocyte globulin and maintenance with calcineurin inhibitors-sparing regimens have been advocated, especially for older recipients of ECD kidneys. This review provides insights into topics such as selection of appropriate candidates for kidney transplantation from ECDs, optimal management of ECD transplant recipients and discusses literature data on the immunosuppressive regimens that have been used in this patient population.

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INTRODUCTION

Kidney transplantation has been proven unquestionably the treatment of choice for most patients with end stage renal disease (ESRD) compared with other alternatives for renal replacement therapy. Survival, cardiovascular stability and quality of life have been found superior in allograft recipients compared with similar patients on the wait list^[1]. This benefit has been observed among recipients older than 60 years of age as well^[2].

There is a large gap between the number of patients waiting for a transplant and the number receiving a transplant. This gap has widened over the last two decades leading to renewed interest in the use of expanded criteria donor (ECD) kidneys in an effort to increase the donor pool. ECD kidneys are used to expand the number of deceased-donor kidney transplants, particularly for elderly recipients.

The Organ Procurement and Transplantation Network (OPTN) instituted a formalized definition of marginal kidneys in 2002 with the advent of ECD^[3]. ECD kidneys are those either from a brain-dead donor ≥ 60 years of age, or a donor 50 to 59 years of age with at least two of the following features: History of hypertension, terminal serum creatinine > 1.5 mg/dL (133 mmol/L), or cerebrovascular cause of death^[4]. These criteria for the definition of ECD were based on the presence of variables that increased the risk for graft failure by 70% (relative hazard ratio 1.70) compared with a standard criteria donor (SCD) kidney^[5]. Kidney transplants coming from donation after cardiac death (DCD) are not included in this definition. SCD was defined as a donor who does not meet criteria for DCD or ECD^[5].

United Network for Organ Sharing (UNOS) allocation policy has required that patients who

enter the waiting list for transplantation consent for consideration of ECD kidneys. Patients who agree to be placed on the list waiting for an ECD kidney are also eligible to receive SCD kidneys. Based upon patient age, there may be a survival advantage or disadvantage to waiting longer for a living donor or SCD kidney compared with a shorter wait for an ECD kidney^[6]. Several studies have shown that, for younger patients, it is generally worth waiting for a higher-quality kidney. For older patients, a prolonged wait for a SCD kidney is not in their interest^[7,8]. In the absence of a living donor, accepting an ECD kidney rather than waiting for a SCD kidney has significantly improved survival in the older ESRD patient. Furthermore, ECD kidneys were associated with higher mortality and higher risk of transplant loss among recipients between 18 to 70 years of age, whereas no significantly increased mortality or increased risk of transplant loss were noted among recipients older than 70 years of age^[7]. However, if older patients are fortunate to live in a geographical area where waiting times are relatively short, then it may be in their interest to wait somewhat longer for the higher-quality organ^[9].

The Eurotransplant Senior Programme (ESP) began in January 1999 with the aim of achieving a more efficient use of kidneys from elderly donors and offering transplantation in elderly patients. It allocates kidneys within a narrow geographic area (Austria, Belgium, Germany, Luxembourg, The Netherlands and Slovenia) from donors aged ≥ 65 years to recipients ≥ 65 years regardless of human leukocyte antigen (HLA) system. This allocation scheme was based on the concept of donor to recipient age matching policy, an alternative to the usual HLA-driven allocation procedure^[10]. To reduce ischemic damage, kidneys should be transplanted within the Eurotransplant region with the shortest possible cold ischemia time (CIT). Local or regional allocation minimized CIT compared to standard centralized Eurotransplant allocation system. Furthermore, to reduce immunological risk, only non-immunized [*i.e.*, panel-reactive antibody (PRA) $< 5\%$] first transplant recipients were included. The ESP allocation scheme furthermore included the option of transplanting both kidneys to a single recipient in cases in which the donor creatinine clearance was < 70 mL/min. Since initiation of the ESP, availability of elderly donors doubled and waiting time for ESP patients decreased. Local allocation led to shorter CIT and less delayed graft function (DGF) but 5%-10% higher rejection rates were reported. A 5-year analysis of ESP revealed that graft and patient survival were not negatively affected by the ESP allocation when compared with the standard allocation^[11].

ECD KIDNEY TRANSPLANTATION

OUTCOMES

Inherent to the definition of an ECD kidney is a 70%

Table 1 Expanded criteria donor kidney transplantation: Epidemiological data

Pro	Contra
Annual mortality rate in dialysis patients exceeds 20% ^[2]	70% increased risk for graft failure vs SCD kidneys ^[12]
Rapidly growing transplant waiting lists and, subsequently, increasingly longer waiting times ^[1-3]	17% primary graft non-function vs SCD kidneys ^[12]
Survival advantage of ECD kidney transplant recipients over dialysis patients remaining on transplant waiting list ^[2,4,6,15]	38% of ECD kidneys were discarded vs 9% for all other kidneys ^[12]
	Increased treatment cost and resource use ^[3,4]
	Mortality in perioperative period greater in ECD kidney recipients ^[4,13]
	Higher DGF rates, more acute rejection episodes and decreased long-term graft function in ECD vs SCD kidneys ^[12-14]

ECD: Expanded criteria donor; SCD: Standard criteria donor; DGF: Delayed graft function.

increased risk for graft failure compared with a SCD kidney in both older and younger recipients, but to a greater extent in recipients older than 50 years^[3,4,12]. Of note, 75% of ECD recipients are more than 55 years old^[3,4]. Nonetheless, diminished allograft survival does not suggest lack of therapeutic benefits. Although most studies of ECD kidney transplantation confirm lower allograft survival rates, recipients of ECD kidneys generally have improved survival compared with matched dialysis-treated patients^[4,6]. In addition to poorer allograft outcome, grafts from ECD kidneys are associated with increased treatment cost and resource use, primarily resulting from longer length of hospital stay, increased requirement for dialysis after transplantation and a greater number of readmissions^[3,4].

Many large retrospective database analysis compared outcomes of ECD with SCD kidney transplants. Overall, mortality in the perioperative period was greater in ECD kidney recipients^[4,13]. Kidneys transplanted from ECDs have higher DGF rates, more acute rejection episodes and decreased long-term graft function. Several factors, including prolonged CIT, increased immunogenicity, impaired ability to repair tissue and impaired function with decreased nephron mass may explain these findings^[14]. Furthermore, among organs procured from ECDs, 38% were discarded vs 9% for all other kidneys^[12]. An ECD kidney transplant recipient has a projected average added-life-years of 5.1 years compared with 10 years for a kidney recipient from a SCD^[6]. Despite these inferior results, these transplants have definitely survival advantage over dialysis patients remaining on transplant waiting list^[4,15]. Therefore, according to a longitudinal study of mortality in a large cohort of ESRD patients, the long-term mortality rate was 48% to 82% lower among

transplant recipients (annual death rate, 3.8 per 100 patient-years) than patients on the waiting list, with relatively larger benefits among patients who were 20 to 39 years old, white patients, and younger patients with diabetes^[2]. The average increase in life expectancy for recipients of "marginal" kidneys (defined as kidneys procured from old donors with comorbidities such as hypertension or diabetes or with prolonged CIT) compared with the waiting list dialysis cohort that did not undergo transplantation was 5 years^[15]. The main pros and cons for ECD kidney transplantation according to epidemiological data are summarized in Table 1.

Long-term relative mortality risk was 17% lower for ECD recipients (RR = 0.83; 95%CI: 0.77-0.90; $P < 0.001$) according to a large retrospective cohort study using data from a US national registry of mortality and graft outcomes among kidney transplant candidates and recipients and comparing mortality after ECD kidney transplantation vs that in a combined standard-therapy group of non-ECD and those still receiving dialysis^[4]. The survival benefit was apparent only at 3.5 years after transplantation due to high early mortality rate in ECD recipients. Subgroups with significant ECD survival benefit included patients older than 40 years, patients of low immunological risk, those with diabetes or hypertension, as well as recipients in organ procurement organizations with long median waiting times (> 3.7 years)^[4]. In areas with shorter waiting times, only recipients with diabetes demonstrated an ECD survival benefit^[4]. Another study using data from the United States Scientific Registry of Transplant Recipients (SRTR) showed that in wait-listed patients > 70 years of age the risk of death was significantly lower with deceased-donor transplantation vs remaining on the waitlist and this benefit extended to those who received an ECD kidney^[16]. Schold and Meier-Kriesche^[7] found that patients 65 years and older had a slightly longer life expectancy if they accepted an ECD kidney within 2 years of starting dialysis therapy (5.6 years) rather than waiting 4 years to receive either a SCD (5.3 years) or a living donor (5.5 years) kidney. A systematic review of kidney transplantation showed that patients younger than 40 years of age or scheduled for kidney retransplantation should not be listed for an ECD kidney due to poor outcomes^[6]. Primary transplant recipients 40 years or older might be listed for an ECD kidney transplant if they have diabetes or are listing in a program with more than 4 years of median waiting time for a SCD kidney^[6]. In conclusion, the relative benefits of transplantation using kidneys from ECDs are dependent on patient characteristics and the waiting time on dialysis. Therefore, wait-listed dialysis patients who are older and diabetic and/or hypertensive have poorer survival rates, but typically achieve the greatest relative gains in overall survival and quality of life after transplantation compared with those remaining on dialysis^[4,6,15]. The most well established indications for ECD kidney transplantation or, in other words,

Table 2 Subgroups with significant survival benefit after expanded criteria donor kidney transplantation according to epidemiological data^[4,6,7,16]

Patients older than 40 yr
Long median waiting time (> 4 yr)
Patients with diabetes or hypertension
Patients of low immunological risk
Dialysis patients with vascular access problems
Dialysis patients whose life expectancy in dialysis is lower than the estimated waiting time for kidney transplantation

subgroups with significant survival benefit after ECD kidney transplantation, according to epidemiological data, are shown in Table 2.

A few single-center observational studies suggested that the patient and graft survival achieved by using ECD kidneys was similar to that obtained with SCDs^[6]. However, it is noteworthy that no United States Registry report or European multicenter analysis that included large numbers of patients supported this conclusion. The vast majority of single-center studies and all available multicenter or registry reports showed significantly worse 1- to 15-year patient and graft survival rates after kidney transplantation using ECD kidneys compared with SCD kidneys^[6].

Our group demonstrated equivalent graft survival rates in a mean follow-up time of 36.4 mo between recipients from ECD and SCD or living donors > 60 years in the period 2005-2011^[17]. Estimated GFR at first year was found statistically different between the ECD and SCD groups (eGFR: 49.9 mL/min per 1.73 m² vs 64.6 mL/min per 1.73 m², $P < 0.001$), but still satisfactory at first year, and at end of follow-up period. Furthermore, comparison of the patients, who received transplants from ECD, even older than 70 years, with those from living donors > 60 years revealed equivalent renal function in short and long term. In conclusion, several studies suggest that in the absence of a living donor, older patients with ESRD should consider accepting an ECD kidney, especially if they have diabetes or face a long wait for a non-ECD kidney^[4,7,16,17].

Although graft function, allograft survival, and perhaps, patient survival may be adversely affected by the older donor, the results are still acceptable, including patient and graft outcomes^[18]. Furthermore, graft survival from older donors may be mostly related to recipient age. Whereas there is an increase in graft loss and an increased incidence of acute rejection among young recipients who receive kidneys from older donors, the age of the donor has little impact on graft function among older recipients. Therefore, graft survival steadily improves with increasing recipient age, the frequency of acute rejection decreases with every decade of increasing recipient age, and, most importantly, the graft and patient survival are superior when older, deceased donors are transplan-

ted into older recipients^[19]. In an analysis of the SRTR database, among recipients > 70 years of age, transplantation of an ECD kidney was not associated with significantly increased mortality, compared with a non-ECD kidney^[8]. On the contrary, transplantation of an ECD kidney was associated with increased mortality for recipients < 70 years^[8]. However, a single-center, retrospective review of all deceased-donor kidney transplantation demonstrated increased morbidity and mortality in elderly recipients of ECD kidneys^[9]. Patients ≥ 60 years that received ECD kidneys had significantly worse patient survival and graft survival, higher rates of acute rejection, and more complications in the perioperative period than similarly aged recipients receiving SCD kidneys. Further, upon comparing younger (age 40-59 years) ECD recipients with those ≥ 60 years of age, patient and graft survival rates and perioperative complications were significantly higher in the older age group^[9].

THE IMMUNOLOGICAL RISK OF ECD KIDNEY TRANSPLANT RECIPIENTS

Kidneys from older donors are generally more immunogenic than kidneys from young donors. Experimental studies have shown an intense inflammatory response and increased T-cell immune reactivity in recipients of deceased or older donor kidney allografts^[20-22]. Subsequently, increased incidence of acute interstitial rejection episodes has been observed among ECD kidney transplant recipients in the early post-transplantation period. The ESP demonstrated acute rejection rate on the order of 30%^[11]. It is well established that acute rejection episodes result in functional deterioration. Contrary to interstitial rejection in kidneys from younger donors, kidneys from older donors seem to have an impaired ability to restore tissue^[14]. A study by Diet *et al*^[23] questioned the increased immunogenicity of ECD transplants. In contrast with previous studies, the incidence of biopsy-proven acute rejection was not higher in recipients of transplants from ECD or donors aged ≥ 50 years than in recipients of transplants from optimal donors or donors aged < 50 years after adjustment for the immunological risk. These findings underline the fact that the risk of rejection depends on the immunological risk, recipient's age and immunosuppressive regimen rather than the donor status^[23].

At the same time, ECD kidney transplant recipients are mostly of advanced age. It is well established that the immune response is significantly affected by the ageing process. Although there is heterogeneity among individual patients, in general terms, both innate and adaptive immunity decrease with increased age, resulting in a decreased likelihood of immunologic rejection and increased risk of infection^[24]. For patients 18 years of age, the rejection rate was 28% compared to only 14% for those aged 70 years^[25]. This finding

Table 3 Expanded criteria donor kidney transplantation: Maximizing benefit

Modifying allocation rules for ECD kidneys in an effort to match the appropriate kidney to the appropriate recipient
Minimizing risk factors for DGF: Lowering CIT, pulsatile perfusion preservation
Preimplantation renal biopsy for ECD kidney recipients
Simultaneous dual ECD kidney transplantation
Restricting the use of ECD kidneys to patients of low immunological risk
Applying individualized immunosuppressive regimens

ECD: Expanded criteria donor; DGF: Delayed graft function; CIT: Cold ischemia time.

is consistent with the previous experimental data showing that ageing is associated with a reduced cellular immunity and CD4⁺ T-cell response and a reduced ability to reject the skin allograft^[26]. However, immune senescence is likely to be affected by the accumulation of memory T cells observed in aged recipients who often have an alloimmune response to transplantation^[27]. This paradox may be explained by recent data showing that aged mice are able to reject a skin allograft at a similar rate to that observed for young transplant recipients, independently of donor age, but display an interleukin (IL)-17-mediated response mediated by memory CD4⁺ cells rather than a classical interferon (IFN)-response^[28]. Thus, ageing seems to cause more qualitative rather than quantitative changes in the alloimmune response.

Independent of the real rejection rates in the elderly transplant recipients the risk of transplant loss from rejection is increased in older recipients compared with younger patients. Importantly, these differences in rejection and infection were independent of baseline immunosuppression. It is possible that elderly patients received less overall immunosuppression than younger recipients because of their decreased rate of rejection, yet the older patients still had an increased risk of infectious death, which emphasizes the vulnerability of the older transplant candidate^[29]. Despite the potential decrease in acute rejection rate, there is an increased risk of chronic allograft nephropathy among older recipients, which is enhanced if the allograft is from an older donor, as it is the case in ECD kidney transplant recipients^[30].

OPTIMAL IMMUNOSUPPRESSION IN ECD KIDNEY TRANSPLANT RECIPIENTS

General principles

The goal of any immunosuppression protocol should be to achieve an adequate immunosuppression level that offers a minimal risk of infection without increasing the risk of rejection. This is particularly important among older patients because patient death is the most common cause of graft loss and

infection is a leading cause of death. As already mentioned, the majority of ECD transplant recipients are of advanced age. Although the relative incidence of acute rejection among older adults is unclear, increased immunosuppression to suppress rejection may increase vulnerability to infection^[31]. In addition, the pharmacokinetics and effects of drugs are altered in older adults^[29]. Therefore, initial calcineurin inhibitor (CNI) doses should be reduced because, at any given dose, higher than normal blood levels result from a decline in cytochrome P450 activity. Moreover, rapid corticosteroid tapering is recommended since corticosteroids have many untoward effects in older adults. On the other hand, ECD transplants are complicated by increased rates of DGF and acute rejection, especially in the early post-transplantation period, and adequate level of immunosuppression is desired under these circumstances. Therefore, optimal management is a challenge in ECD kidney transplant recipients.

In any case, older patients and recipients of ECD kidneys often are excluded from transplant trials and, therefore, the optimal induction and maintenance regimen for them is not known. Approaches are largely center specific and based upon expert opinion.

Management for an ECD kidney is based on potential nephron-protecting strategies, including CIT minimization, pulsatile perfusion preservation, immunosuppression focused on nephrotoxicity minimization, and adequate infection prophylaxis^[29,30]. Routine donor preimplantation renal biopsy may be useful to evaluate the integrity of renal anatomy in ECD kidneys and select the viable grafts. Furthermore, the successful use of ECD kidneys can be enhanced by restricting the use of these kidneys to unsensitized patients receiving a first graft, and minimizing, if feasible, other risk factors for acute tubular necrosis, such as hemodynamic stability and total ischemic time^[32]. In addition, limited evidence also suggests that transplanting two ECD kidneys, rather than one, in one recipient might help improve outcomes^[33]. Lastly, we should always underline the importance of appropriately matching organs with recipients, particularly for ECD organs. Modifying allocation rules for ECD kidneys should be considered in an effort to match the appropriate kidney to the appropriate recipient^[5-7]. In general, the life expectancy of the recipient should approach the expected survival of the allograft. The main strategies to maximize benefit in ECD kidney transplantation are summarized in Table 3.

Although CNIs are excellent drugs, nephrotoxicity is a major concern, especially in older recipients of ECD kidneys. These kidneys may be more vulnerable to the adverse effects of immunosuppressive medications such as CNIs. Therefore, various strategies of CNI withdrawal, minimization as well as avoidance or CNI addition after induction have been utilized by a number of investigators. Of note, in kidneys with

Table 4 Modifying and individualizing the immunosuppressive regimen in expanded criteria donor kidney transplantation: Main strategies

Induction with ATG
Reduce overall immunosuppression burden, especially in elderly recipients of ECD kidney transplants
Reduced CNI exposure regimens (target CNI blood levels 25%-50% lower)
Delayed CNI introduction regimens
CNI-free regimens based on MMF and steroids with ATG induction
CNI-free Belatacept-based regimens
Reduced CNI exposure and CNI-free mTOR-inhibitors-based regimens

ATG: Antithymocyte globulin; ECD: Expanded criteria donor; CNI: Calcineurin inhibitor; mTOR: Mammalian target of rapamycin; MMF: Mycophenolate mofetil.

assumed reduced nephron mass such as ECD kidneys, the immunological risk should be kept as low as possible by accurate pretransplant risk assessment and risk-adjusted immunosuppression during the post-transplant period to avoid further damage^[6].

Although the optimal immunosuppressive regimen for ECD kidney transplant recipient has not been determined as yet, several maneuvers and modifications have been proposed in an effort to improve outcomes in this high-risk patient population. These are briefly presented in Table 4 and further discussed later in this review.

Induction immunosuppression

There are limited data concerning the benefits and adverse effects associated with different induction regimens in ECD kidney transplant recipients. A retrospective analysis of United Network of Organ Sharing (UNOS) data from 2003 to 2008 among high-risk older (> 60 years) recipients who received high-risk kidneys showed that, in the entire cohort, older recipients who received rabbit antithymocyte globulin (rATG) had the lowest cumulative rate of acute rejection within the first year after transplantation compared with those who received interleukin-2 (IL-2) receptor antagonists or alemtuzumab^[34]. Despite the high rejection rates, IL-2 receptor antagonists were associated with transplant loss in only high-risk recipients who received high-risk donor organs. These data suggest that ATG might be the preferred induction agent for high-risk elderly recipients of a high-risk donor organ, such as an ECD kidney. No significant difference in death-censored graft survival was noted on multivariate analysis in patients who received anti-IL-2 receptor antibody or rATG. However, there was an increased risk of death among recipients of anti-IL-2 receptor antibody compared with rATG. Patients induced with alemtuzumab had an increased risk of death-censored graft loss and death compared with rATG. In the abovementioned study, a high-risk recipient was defined as one having a peak panel reactive antibody > 20% or a prior kidney transplantation or of black race. High-risk

donor kidneys included ECD kidneys, kidneys following cardiac death or kidneys having a CIT > 24 h^[34].

It is in the current practice of our group to use in ECD transplant recipients induction with rATG to ameliorate preservation injury and moreover minimize the state of DGF due to acute tubular necrosis^[17].

Maintenance immunosuppression

The optimal combination of medications for maintenance immunosuppression among ECD kidney transplant recipients is unknown. Regimens that spare CNIs have been advocated, especially for older recipients of ECD kidneys^[29]. However, such regimens, as well as those associated with the withdrawal of CNIs, have been associated with an increased incidence of acute rejection^[35]. Guidelines suggest that tacrolimus and mycophenolate should be used as first-line maintenance immunosuppressive agents following transplantation, but there are no separate recommendations for older recipients^[36]. In the abovementioned retrospective analysis of UNOS data from 2003 to 2008, tacrolimus use was associated with a decreased risk of rejection for high-risk elderly patients who had a high-risk donor, but there was no decrease in risk of rejection with low-risk donor-recipient combinations^[34]. Although there was no association between tacrolimus use and death-censored transplant loss, tacrolimus was associated with a decreased risk of death (RR range, 0.77-0.85 depending on risk group). Interestingly, mycophenolic acid use was associated with a significant decrease in transplant failure and death in both high- and low-risk patient groups. For example, in a recipient with low immunologic risk who received a high-risk donor transplant, such as from an ECD, mycophenolic acid use was associated with a 28% decrease in transplant failure (RR = 0.72; 95%CI: 0.59-0.89) and a 16% lower likelihood of death (RR = 0.84; 95%CI: 0.72-0.98)^[30]. Steroid use had no significant effect on either patient or transplant survival. Although there are no randomized comparisons, the recent data from Gill *et al.*^[34] suggest that tacrolimus and mycophenolic acid might be the preferred immunosuppressive agents in patients older than 60 years with respect to patient and transplant survival.

Several suggestions have been made on the optimal combination of immunosuppressants to preserve renal function following kidney transplantation from ECD kidneys. However, randomized trials, necessary to better define the optimal induction and maintenance regimen for ECD kidney transplant recipients, are largely lacking.

Reduced steroid exposure regimens

The goal of immunosuppression in elderly should consist of a reduction of the risk of CNI nephrotoxicity along with a limited use of steroids because of the increased risk of infections, fractures, myopathy, and other steroid-related side effects. Aull *et al.*^[37] showed that an early corticosteroid withdrawal regimen of

rATG induction, tacrolimus, and mycophenolate mofetil is associated with excellent patient and kidney graft survival in a population consisted of 55% deceased donor kidney transplants, 46% of whom were ECD. However, the success of steroid-sparing strategies has not been proved in ECD kidney transplantation to date because all trials available were mainly developed with SCD kidney transplantation^[6]. Segoloni *et al.*^[38] described a series of 88 patients receiving kidneys from marginal donors whose immunosuppressive protocol consisted of monoclonal anti-IL-2 receptor antibodies, mycophenolate mofetil (MMF), and steroids. When serum creatinine levels were less than 2.6 mg/mL, tacrolimus was started and MMF was subsequently withdrawn when the tacrolimus through level increased above 15 ng/mL. Steroid was tapered to 5 mg at day 45 and then progressively reduced. The acute rejection rate was 13.6%. At 3 years and 4 years after transplant, 80% and 100% of patients, respectively, were off steroids with a 4-year patient and graft survival of 98% and 79%, respectively. Incidence of infections and malignancy were also acceptable.

Reduced CNI exposure and CNI-free regimens

Recipients of ECD kidneys are at increased risk for graft dysfunction/loss, and may benefit from immunosuppression that avoids CNI nephrotoxicity. CNI-induced vasoconstriction and subsequent hypoxia could be more detrimental in elderly organs. On a molecular level calcineurin inhibitors accelerate pathways already activated during physiological ageing^[29-31].

CNIs are the mainstay of immunosuppression in renal transplantation. Their use has decreased acute rejection rates and improved short-term patient and graft survivals. However, they are associated with chronic graft dysfunction as well as increased risks of cardiovascular disorders and of malignancies^[36]. ECD kidneys may be particularly susceptible to CNI-mediated vasoconstriction that may prolong ischemic injury in the early post-transplant phase. In the long term, chronic CNI nephrotoxicity is a major concern^[23,25]. Furthermore, CNIs may be associated with worse short- and long-term graft function, particularly in ECD kidneys, with frequent preimplantation structural damage.

Reduced CNI exposure regimens have been examined in a number of clinical studies with the aim of minimizing nephrotoxicity. Two possible strategies have been proposed for CNI toxicity minimization: To delay CNI introduction until a certain level of renal graft function is achieved, and more radical, complete CNI-free strategies^[6]. Another maneuver in the context of reduced CNI exposure regimens could be to target towards lower CNI levels in ECD as compared with SCD kidney transplant recipients. This strategy has not been evaluated so far and, therefore, no

recommendation can be made. However, it is in the practice of our group to target about 25%-50% lower CNI levels long term in this patient population with satisfactory preliminary results regarding patient and graft survival as well as renal function in short- and long-term^[17].

Delayed CNI introduction has been analyzed in several nonrandomized studies, including induction therapy with anti-IL 2 receptor antibodies or ATG^[38-43]. Reported acute rejection rates were low at 6% to 23%, DGF rates were 31% to 54%, and patient and graft survival were within the reported ranges for SCD kidney transplantation. In a long-term study including 101 ECD kidney recipients, Stratta *et al.*^[44] used ATG or alemtuzumab with MMF and steroids, and, only when serum creatinine level was less than 4 mg/dL, a moderate tacrolimus dose was introduced. With 4-year patient and graft actuarial survival rates of 93% and 74%, this trial constitutes potentially the best long-term experience to date on delayed CNI introduction.

Regarding CNI-free initial immunosuppression, several European studies analyzed experiences based on MMF and steroids with ATG induction, showing acute rejection rates of 24% to 26%, a DGF rate of 30%, and 5-year actuarial graft survival rates of 65% to 70%^[45-48]. For example, Arbogast *et al.*^[45] investigated a therapeutic regimen consisting of a CNI-free, MMF-based immunosuppressive induction/maintenance protocol in conjunction with a short course (4-10 d) of rATG in 89 patients of mean age 63.8 years who received an organ from an elderly cadaver donor (mean age 66.8 years). Cumulative 5-year patient and graft survival was excellent with 88% and 70%, respectively, but only a historical control group under CNI therapy was available for comparison. The same group subsequently investigated a regimen of strictly monitored MMF [target mycophenolic acid (MPA) trough levels between 2-6 mg/mL] and steroids combined with a polyclonal-monoclonal induction regimen consisting of a low dose, single shot of rATG and the IL-2-receptor-antibody basiliximab^[46]. Thirty elderly recipients (67.8 ± 3.8 years) of renal transplants from deceased donors (69.4 ± 13.3 years) were recruited consecutively for this 5-year prospective, open, single center, pilot trial. One-year patient and renal allograft survivals were 87% and 83%, respectively; death-censored 1-year graft survival was 97%. Mostly steroid-sensitive rejection episodes were observed in 46% of patients, with only 3 patients requiring antibody therapy^[46]. However, CNI-free regimens have been occasionally complicated by unacceptably high acute rejection rates. Therefore, in a study of basiliximab induction and MMF and steroid maintenance therapy, a large subgroup of patients experienced acute rejection rate of 45% and was subsequently converted to CNI therapy^[49].

Belatacept, a selective costimulation blocker, may preserve renal function and improve long-term

outcomes vs CNIs. BENEFIT-EXT (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial-EXTended criteria donors) is a 3-year, Phase III study that assessed a more (MI) or less intensive (LI) regimen of belatacept vs cyclosporine in adult ECD kidney transplant recipients^[50]. The co-primary endpoints at 12 mo were composite patient/graft survival and a composite renal impairment endpoint. Patient/graft survival with belatacept was similar to cyclosporine (86% MI, 89% LI, 85% cyclosporine) at 12 mo. Fewer belatacept patients reached the composite renal impairment endpoint vs cyclosporine. The mean measured glomerular filtration rate was 4–7 mL/min higher on belatacept vs cyclosporine, and the overall cardiovascular/metabolic profile was better on belatacept vs cyclosporine. The incidence of acute rejection was similar across groups. Overall rates of infection and malignancy were similar between groups; however, more cases of posttransplant lymphoproliferative disorder (PTLD) occurred in the central nervous system on belatacept^[50]. More recently the 3-year results of this trial have become available and the abovementioned promising findings of this CNI-free regimen have been confirmed^[51].

Reduced CNI exposure, mTOR-inhibitors-based regimens

Mammalian target of rapamycin (mTOR) inhibitors (sirolimus, everolimus) appear to permit a CNI-sparing regimen among stable kidney recipients. However, the promising initial results in SCD kidney transplantation using CNI-free sirolimus and MMF-based immunosuppression after basiliximab induction have not been confirmed in larger scale randomized controlled trials, which showed increased acute rejection rates and complications, worse graft function but equivalent graft survival^[52].

Some small nonrandomized studies assessed the potential of combined sirolimus and MMF in patients after ECD kidney transplantation^[53–61]. Therefore, CNI-free sirolimus-based therapy compared with MMF-based treatment in kidney transplantation with advanced-age donors was associated with an acceptable outcome, but increased proteinuria in sirolimus-treated patients was noted in the intention-to-treat analysis^[58]. CNI-free immunosuppression regimen, consisting of ATG induction, sirolimus, MMF and steroids, have been applied in recipients of dual kidney transplantation from elderly donors^[54]. Excellent results have been demonstrated with a lower DGF rate and a better renal function as compared with earlier dual kidney transplant recipients treated with CNI-based regimen. However, in another study, the investigators were not able to find an advantage in acute rejection and graft function with their CNI-free approach for dual kidney transplantation using ECDs compared with the results of a conventional cyclosporine A and MMF strategy^[59]. A study analyzed

the results obtained with the use of a CNI-free immunosuppressive protocol (ATG induction, plus sirolimus, MMF, and low doses of steroids) in terms of graft and patient survival as well as posttransplant clinical complications over 2 years in recipients of ECD kidneys^[55]. Under this immunosuppressive protocol, 78.04% of the patients completed the follow-up. A protocol biopsy was performed in 17 patients (53.1%) within 2 years posttransplant of which 82.31% were diagnosed as chronic allograft nephropathy grade I. The incidence of clinical complications was low and not significantly different from that reported with other immunosuppressive schemes. Death-censored graft survival was 95.12%. Another study introduced the idea of a CNI-free regimen in 13 recipients of ECD kidneys treated with induction therapy and maintained on sirolimus, MMF and prednisone and demonstrated excellent 2-year patient and graft survival and good renal allograft function although longer follow-up in larger randomized controlled trials are necessary to establish these findings^[60]. Similarly, low-dose sirolimus-based triple immunosuppression with ATG induction offered 100% patient and graft survival in 27 ECD kidney transplant recipients with the achievement of stable renal function over a mean follow-up of 20.2 mo^[61]. However, mild progression of histological damage and increased risk of bacterial infection detected in this study are a major concern.

In a large report on the potential for CNI-free immunosuppression, the United States registry has shown that the adjusted hazard ratio for overall graft loss for patients on sirolimus and MMF therapy at discharge doubles that observed with tacrolimus and MMF^[62]. Only 33% of the kidney transplantation procedures included in this report used kidneys from donors older than 50 years, and no specific analyses are available for ECDs. One may conclude that the potential for CNI-free sirolimus and MMF-based therapy in ECD kidney transplant recipients has not been adequately established to date. Consequently, extrapolation of the best results obtained with anti-IL-2 receptors, MMF, steroids, and moderate exposure to tacrolimus might constitute an advisable strategy^[52].

It is well established that first attempts to minimize CNI nephrotoxicity by reducing the dose or withdrawing CNI from the immunosuppressive regimen have been limited by high acute rejection rates^[63]. More recently, an early abrupt conversion from cyclosporine to everolimus has shown a significant increase in renal function with an acceptable acute rejection rate at 6 mo after transplantation^[64]. Furthermore, a clinical trial in patients with no immunological risk, who received conventional immunosuppression for 6 mo, showed that patients converted from cyclosporine to everolimus displayed lower acute rejection rates and improved renal function vs those who remained on treatment with MMF or cyclosporine^[65]. In a retrospective registry-based study from Portugal,

everolimus appears to be an effective, safe alternative to CNI for maintenance therapy in selected kidney transplant recipients^[66]. The potentially protective role of everolimus on renal allograft dysfunction offers an attractive option in recipients of ECD kidneys.

Trials of everolimus combined with reduced-exposure CNI have yielded good renal function whilst maintaining efficacy. The combination of everolimus with reduced-exposure CNI may offer advantages both for young as well as for older transplant recipients who receive an ECD graft. Everolimus, by allowing reduction in CNI exposure, has the potential to improve outcomes and minimize CNI-associated toxicities. Given the vulnerability of older patients (and older grafts) to CNI-induced nephrotoxicity, minimization of CNI dose is highly desirable in "old-for-old" patients^[67]. There is good rationale for using reduced-exposure CNI regimen from the outset in ECD transplant recipients and, in case of low immunological risk, CNI withdrawal is a feasible option. CNI-free regimens are particularly desirable in recipients with advanced baseline histopathological lesions and/or GFR < 50 mL/min^[67].

We have always to take into account when interpreting study results that initial studies are generally characterized by suboptimal use of everolimus and sirolimus (high trough levels, high loading dose). On the contrary, today CNI-free schemes appear to perform much better than those applied 10 years ago.

As already mentioned, it is in the practice of our group to target about 25%-50% lower CNI levels long term in an attempt to diminish the nephrotoxicity effect in ECD transplant recipients. Furthermore, it is in our practice as well, when considered safe, to switch to a CNI-sparing regimen using an mTOR inhibitor^[17].

CONCLUDING REMARKS

The data presented so far regarding reduced CNI exposure or even CNI-free regimens may justify the use of such immunosuppressive regimens, at least in ECD transplant recipients of low immunological risk. However, a recent study from Switzerland showed that in ECD kidneys recipients of low immunological risk, defined as the absence of pretransplant donor-specific HLA antibodies, 1-, 3- and 5-year graft survival was significantly better when recipients were treated with Tacrolimus than when they were treated without Tacrolimus and comparable to SCD kidneys during the first six years. Furthermore, ECD kidneys recipients treated with Tacrolimus had a higher median estimated creatinine clearance than those treated without Tacrolimus. Graft function from one to three years was better preserved in ECD recipients treated with Tacrolimus compared with those treated without Tacrolimus. According to this study, in recipients with low immunological risk Tacrolimus-based immunosuppression seems to improve graft survival and to preserve graft function in kidney transplants with

reduced baseline nephron mass, such as ECD kidneys, which are highly vulnerable to additional hits^[68].

It is unclear whether the choice of maintenance immunosuppression modulates the negative effect of advanced donor age on outcome after renal transplantation. A study from Austria evaluated patient and graft survival based on donor age and immunosuppressive therapy in 1829 patients who received their first transplant between 1990 and 2003^[69]. This study concluded that in median follow-up time of 7 years, use of CNIs 90 d after kidney transplantation is associated with improved patient survival even after adjustment for confounders, but its beneficial association with actual and functional graft survival is lost or at least reduced if kidneys from donors older than 50 years are used^[69].

Apart from being more susceptible to CNI-induced nephrotoxicity, kidneys from ECDs may elicit a strong inflammatory response, predisposing recipients to an increased risk of cancer after transplantation. This association between different donor types and the risk of cancer was assessed in a study using the Australian and New Zealand Dialysis and Transplant Registry^[70]. Compared to recipients of living donor kidneys, recipients of ECD kidneys were at an increased risk of cancer, particularly for genitourinary cancer and post-transplant lymphoproliferative disease, over a median follow-up period of 4.4 years. Therefore, this study demonstrated that recipients of ECDs have an overall increased risk of cancer by at least 1.5 times compared to recipients of SCD and living-donor kidneys independent of age, sex, and time on dialysis^[70]. With increasing utility of ECD kidneys worldwide, it is conceivable that the use of these organs is contributing to the escalating burden of cancer in transplanted patients. However, the impact of cancer on the overall and cause-specific survivals in the context of receiving ECD compared to SCS kidneys and the trade-off between death on the waiting list and the increased risk of cancer after receiving ECD kidneys remains to be determined. Strategies to ensure adequate cancer surveillance in these recipients should be considered, particularly in those with other risk factors for cancer development, such as older recipients, Epstein-Barr Virus naive recipients, or the use of T cell depleting antibody as induction or as treatment for acute rejection.

ECD kidneys and elderly recipients usually are excluded from randomized clinical trials assessing the efficacy and safety of new immunosuppressive drugs and combinations. Consequently, results for pharmacological regimens in the lower risk transplant recipients may not be valid in this higher risk population. Specific well-designed controlled trials of immunosuppressive strategies are urgently needed in ECD kidney transplantation. Therefore, recommendations regarding optimal immunosuppressive regimen in this patient population should be made with caution. However, reducing the overall immunosuppression burden appears

to be a prudent approach in this high-risk kidney transplant recipients. Reduced CNI exposure regimens or even CNI-free regimens, in selected cases, may improve survival of ECD kidney transplants. In the context of such regimens, m-TOR inhibitor everolimus appears to offer advantages in ECD kidney recipients both in terms of improving outcomes and preserving renal function as well as in terms of minimizing CNI-associated adverse events, such as cardiovascular morbidity/mortality and malignancies, particularly prevalent in this patient population. Finally, we should always bear in mind that, apart from applying individualized immunosuppressive regimen, appropriate selection of ECD kidney transplant recipients and close peri- and post-operative follow-up are of prime importance in order to maximize the benefits associated with the increasingly widespread use of ECD kidney allografts.

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Continuous internal counterpulsation as a bridge to recovery in acute and chronic heart failure

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Abstract

Cardiac recovery from cardiogenic shock (CS) and end-stage chronic heart failure (HF) remains an

often insurmountable therapeutic challenge. The counterpulsation technique exerts numerous beneficial effects on systemic hemodynamics and left ventricular mechanoenergetics, rendering it attractive for promoting myocardial recovery in both acute and chronic HF. Although a recent clinical trial has questioned the clinical effectiveness of short-term hemodynamic support with intra-aortic balloon pump (IABP, the main representative of the counterpulsation technique) in CS complicating myocardial infarction, the issue remains open to further investigation. Moreover, preliminary data suggest that long-term IABP support in patients with end-stage HF is safe and may mediate recovery of left- or/and right-sided cardiac function, facilitating long-term weaning from mechanical support or enabling the application of other permanent, life-saving solutions. The potential of long-term counterpulsation could possibly be enhanced by implementation of novel, fully implantable counterpulsation devices.

Key words: Counterpulsation; Recovery; Intra-aortic balloon pump; Heart failure; Cardiac remodeling; Reverse remodeling

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Core tip: The counterpulsation technique induces beneficial effects on systemic hemodynamics and left ventricular mechanoenergetics. In this manner, it may facilitate myocardial recovery in acute and chronic heart failure (HF). The intra-aortic balloon pump (IABP) remains the main representative of the counterpulsation technique. Although recent data have questioned the effectiveness of short-term hemodynamic support with IABP in cardiogenic shock complicating myocardial infarction, the issue remains open to further investigation. Preliminary data suggest that long-term IABP support in patients with end-stage HF is safe and may mediate recovery of left- or/and right-sided cardiac function. Novel, fully implantable counterpulsation devices, which enable long-term counterpulsation, are described in this manuscript.

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INTRODUCTION

Heart failure (HF) is a true pandemic, responsible for 5% of hospitalizations globally^[1]. HF, in its most severe forms, can manifest as two lethal clinical entities: (1) acute HF with cardiogenic shock (CS), with post-myocardial infarction (MI) CS mortality rates approaching 50%^[2]; and (2) end-stage chronic HF, with 1-year mortality of approximately 80% (worse than most types of cancer)^[3]. Despite significant advances in development of drug and device-based therapies, cardiac recovery from these two destructive forms of HF remains an often insurmountable therapeutic challenge. As we will see, the meaning of "recovery" and the remedial goal differ between acute and chronic HF.

RECOVERY IN ACUTE HF

Any cause of acute, severe left ventricular (LV) or right ventricular (RV) dysfunction may lead to CS. The most important cause of CS is severe LV dysfunction following a large acute MI^[4]. Despite the fact that the vast majority of these patients suffer from acute ST elevation MI, CS also occurs in approximately 2.5% of non-ST elevation MIs^[5]. Moreover, mechanical complications, such as ventricular septal rupture, acute severe mitral regurgitation and contained free wall rupture may lead to CS and must be suspected in patients with CS complicating non-anterior MI^[6]. Other less frequent causes include acute myopericarditis, isolated RV failure, Takotsubo cardiomyopathy, hypertrophic cardiomyopathy, acute valvular regurgitation (typically caused by endocarditis or chordal rupture), cardiac tamponade, excess beta or calcium channel blockade, dilated cardiomyopathy, peri-operative low output syndrome, and CS associated with cardiac catheterization complications^[7].

The meaning of "recovery" in the setting of acute HF and, thus, the treatment goal, is hemodynamic support during acute cardiac decompensation, including measures that allow the injured myocardium to recuperate and overcome the need for acute support^[8]. The therapeutic means to achieve this goal varies significantly depending on the cause of CS.

RECOVERY IN CHRONIC HF

Cardiac remodeling is a deleterious component of HF progression associated with poor prognosis^[9,10]. It

comprises molecular, cellular and interstitial changes, manifested clinically as changes in size, shape and function of the heart following cardiac overload or injury^[11]. Adverse changes at the organ level include alteration of LV geometry (less elliptical and more spherical LV shape)^[12,13], wall thinning^[14], LV dilatation (increase in LV end diastolic and end-systolic volumes) and decline in LV ejection fraction (EF)^[15]. Cellular and molecular changes include myocyte hypertrophy, loss of myocytes due to apoptosis^[16] or necrosis^[17], fibroblast proliferation^[18] and fibrosis^[19].

The therapeutic goal in chronic HF is to improve symptoms and life expectancy. That can be achieved by prevention of the adverse components of LV remodeling and reversal of already completed LV remodeling. Today we know that any level of reverse LV remodeling is associated with an analogous increase of survival in the patients suffering from HF^[20].

The term "bridge to transplantation" (BTT) for patients with chronic HF by use of mechanical assistance with an LVAD was introduced by the cardiac surgeons who were surprised to find a normal or almost normal recipient heart at the time of transplantation. Subsequently, "recovery" in chronic HF refers to sustained reversal of the aforementioned alterations, a process known as reverse remodeling with near normalization of LV function in patients on an LVAD as a BTT followed by a "safe" LVAD explantation. So, the definition of LV recovery presupposes that the patient can tolerate a large cardiac operation for LVAD explantation and remain clinically and hemodynamically stable thereafter.

This presupposition does not apply to patients assisted by a device easily explantable, like the percutaneous intra-aortic balloon pump (IABP). An example is one of our patient with chronic HF due to IDC complicated by CS requiring mechanical assistance by IABP. After 3 mo of continuous IABP support, he was successfully weaned from mechanical assistance and 5 years later he remains asymptomatic. He did not have to be subjected to a major cardiac surgical procedure to remove his bridging device, which may be the reason he did so well.

The patient mentioned above is now a 25-year-old man. He had had a history of progressively worsening HF when he presented at age 21 with CS, an LVEF of 17%, a BNP of 2800 pg/dL and a myocardial biopsy showed dilated cardiomyopathy. The patient was placed on intravenous infusion of inotropes and furosemide but further deteriorated. The patient was placed on IABP mechanical assistance and, although he was offered biventricular mechanical assistance (BiVAD), he preferred protracted IABP assistance. Initially he did not tolerate any anti-remodeling drug treatment. At the end of the 3 mo period on IABP his clinical and hemodynamic improvement permitted weaning from the IABP with a LVEF = 25% and a BNP = 207 pg/dL and 5 years later he remains asymptomatic with a LVEF = 30%, and VO_{2peak} =

Table 1 Criteria of sufficiency of recovery with easily-explantable counterpulsation devices and continuous flow left ventricular assist devices

Counterpulsation devices	
EF	↑ 5%
BNP	< 500 pg/mL
Continuous flow LVADs	
LVEDD	< 60 mm
LV end-systolic diameter	< 50 mm
EF	> 45%
LV end-diastolic pressure/PCWP	< 12 mmHg
Cardiac Index (resting)	> 2.8 L/min per square

EF: Ejection fraction; LV: Left ventricular; LVAD: Left ventricular assist device; PCWP: Pulmonary capillary wedge pressure; LVEDD: Left ventricular end-diastolic dimension.

29 mL/kg per minute. Thus, recovery no longer must presume a patient's ability to withstand an arduous LVAD explantation procedure.

In our experience, in patients who undergo mechanical assistance by a device that is easy and safe to explant (like the IABP), myocardial recovery can be considered adequate for termination of mechanical assistance when all of the following criteria are met (Table 1): (1) absolute increase in LVEF \geq 5% (measured by echo at the end of a 24-h reduced (1/4) pump function test) compared to baseline; and (2) BNP \leq 500 pg/mL (measured at the end of a 24-h reduced pump function test).

However, for the continuous flow LVADs which require a large and high risk operation for explantation, the recovery can only be considered adequate if the very demanding established criteria are met (Table 1): LVEDD < 60 mm, LV end-systolic diameter < 50 mm, and EF > 45%; LV end-diastolic pressure or PCWP < 12 mmHg, resting cardiac index > 2.8 L/min per square; and maximal oxygen consumption with exercise (mVO_2) > 16 mL/kg per square^[21].

COUNTERPULSATION

Counterpulsation was first conceived by Kantrowitz^[22] in the early 1950s, who managed to augment coronary blood flow by delaying arterial pulse in canine experimental models. In 1962, Mouloupoulos *et al.*^[23] developed the IABP, which was applied in human subjects 6 years later for the management of post-MI CS^[24]. Nowadays, IABP remains the single most widely-used short-term circulatory assist device in acute cardiac decompensation^[25]. However, the application of long-term IABP counterpulsation in the setting of chronic HF remains limited; the potential of long-term counterpulsation could possibly be enhanced by implementation of novel, fully implantable counterpulsation devices. These include the para-aortic counterpulsation device (PACD)^[26], representing the initial version of the pressure unloading LVAD (PULVAD) described below, the Kantrowitz CardioVAD (KCV)^[27], the

Table 2 Effects of counterpulsation on systemic hemodynamics and left ventricular mechanoenergetics

Decrease
Systolic aortic pressure
End-diastolic aortic pressure
LV systolic wall stress (afterload)
Myocardial oxygen/LV energy consumption
End-diastolic ventricular volume (preload)
Mean pulmonary capillary wedge pressure
Increase
Diastolic aortic pressure (augmentation)
LV mechanical performance (ejection fraction, stroke volume, cardiac output)
LV contractility and active relaxation (in the reperfused failing heart)
Coronary blood flow (post-ischemia, when coronary autoregulation is impaired and flow is pressure-dependent) ^[33]
Cerebral, renal, mesenteric and pulmonary blood flow
Mean arterial pressure (in patients with shock)

LV: Left ventricular.

Symphony counterpulsation device^[28,29] and C-pulse^[30].

How does counterpulsation promote recovery? Insights from experimental studies

Several experimental studies have demonstrated that counterpulsation exerts numerous beneficial effects on systemic hemodynamics and LV mechanoenergetics (Table 2), rendering it attractive for induction of recovery in both acute and chronic HF^[31-35]. In brief, counterpulsation unloads the LV (decreases LV afterload), decreases LV energy consumption and concurrently improves LV mechanical performance (EF, stroke volume, cardiac output). In addition, counterpulsation improves LV contractility and active relaxation of the reperfused failing heart, possibly through augmentation of coronary blood flow^[34]. However, it should be highlighted that the magnitude of the aforementioned beneficial effects varies widely, depending on several factors, such as the volume of counter-pulsated blood, the position of the device in the aorta, aortic compliance, heart rate/rhythm and systemic pressures and resistances^[36,37].

Counterpulsation in acute HF

IABP remains the most widely-used circulatory assist device in patients with CS complicating acute MI^[38]. Until 2012 IABP support was considered to be a class I treatment in the setting of post-MI CS^[39,40]. However, the clinical effectiveness of short-term IABP support in patients with CS post-MI has recently been called into question, mainly on the basis of the results of the IABP-SHOCK II trial, the largest randomized IABP trial to-date, which demonstrated no benefit of IABP support on either 30-d or 1-year all-cause mortality^[41,42]. Criticism of IABP SHOCK II study design and methodology have arisen^[43,44], mainly focusing on: (1) the late timing of IABP insertion (once revascularization had been completed), which could undermine the effectiveness of IABP support^[45];

and (2) the lower than expected mortality rate, which raises concerns about the severity of CS in the enrolled patient population. The interpretation of the trial's results is also complicated by methodological difficulties inherent to the design and execution of randomized trials in gravely ill patients with CS (e.g., need for rescue LVAD implantation, need for rescue IABP insertion in patients randomized to the non-IABP group). Overall 17% of the patients randomized to conventional treatment received mechanical assistance by IABP or LVAD. Furthermore, in everyday clinical practice only 22% of patients with post-MI CS undergo IABP assistance^[46], most likely only those with the most severe CS. So, the strong message of that study is that not all patients with post-MI CS need mechanical assistance by the IABP. Nevertheless, the lack of hard evidence regarding clinical effectiveness of IABP support resulted in reconsideration of American and European guidelines, which have downgraded the routine use of IABP support in post-MI CS to class II a and III treatments, respectively^[47,48]. It should be noted, though, that the absence of evidence should not necessarily be interpreted as evidence of absence of clinical effectiveness; given that mortality in CS remains unacceptably high^[41,42], new, appropriately-powered and carefully-designed, clinical studies are needed to clarify the role of IABP support in promoting cardiac recovery in this setting.

Counterpulsation in chronic HF

Patients with advanced chronic HF face a grim prognosis, with 1-year mortality rates approaching 80%. These vulnerable patients have limited access to donor hearts for cardiac transplantation and chronic mechanical circulatory support is often used as a last resort. Intriguingly, clinical observation shows that chronic mechanical unloading can occasionally reverse the complex process of myocardial remodeling to the point that a subset of patients can be weaned from the device after restoration of basic cardiac function^[9]. Yet, myocardial recovery induced by conventional left ventricular assist devices (LVADs) is disappointingly rare^[49]. A prominent reason for the low rate of recovery is the physiologic mechanism through which conventional LVADs provide salutary hemodynamic effects. These LVADs bypass the LV and unload the failing LV independently of its systolic reserve. As a consequence, the LV is rendered ineffective to generate adequate pressure to surpass the mean arterial pressure generated by the LVAD itself. Thus, clinically available LVADs assist the LV at the cost of severely suppressing native LV function, rendering the LV incapable of sustaining its conditioning and therefore compromising recovery. In addition, pulsatility of flow seems to play an important role for cardiac reverse remodeling; recovery in patients with IDC may be as low as 3% for currently-used continuous flow LVADs, yet 25% with older-generation pulsatile alternatives^[50].

Chronic counterpulsation can overcome the aforementioned limitations of conventional LVADs and therefore appears attractive, at least from a theoretical standpoint, for promoting cardiac reverse remodeling and recovery, as it: (1) unloads the LV and decreases its energy consumption; (2) utilizes the LV systolic reserve; (3) enhances native LV functional performance (unlike clinically-used LVADs which suppress it); (4) retains pulsatility of flow and; and (5) preserves heart integrity.

The aforementioned reasons theoretically rationalize the expansion of the indications of counterpulsation implementation, beyond that of short-term hemodynamic stabilization. New potential indications could include use of long-term counterpulsation as a bridge to decision making (cardiac surgery, LV assist device implantation or transplantation), bridge to transplantation and bridge to myocardial recovery. However, long-term IABP support is not risk-free; major complications include acute limb ischemia, severe bleeding, embolic events, infection and sepsis^[51]. However, sheathless implantation technique in combination with thinner catheters application significantly minimized the rate of complications from 20.7% for 12 French catheters to 8.4% for 9.5 French catheters. Though more recent data are not available, it is reasonable to assume that the contemporary complication rate with the use of 6 and 7 French IABP catheters is significantly lower. In addition, several recent studies (described later in this review) have demonstrated that long-term IABP support appears to be associated with a favorable safety profile^[52-58]. The potential of long-term counterpulsation could possibly be enhanced by implementation of novel, fully implantable counterpulsation devices (described later) and mobilization of the patient.

IABP FOR CHRONIC LV HF

Converging data suggest safety and possibly efficacy of long-term circulatory support with IABP in patients with end-stage LV HF. In the study by Gjesdal *et al.*^[52], 32 patients were successfully bridged to transplantation *via* IABP, after a mean IABP support of 21 d (range: 3-66 d), with few IABP-related complications. Importantly, mortality and hemodynamic indices at 1 year post-transplantation were similar in patients bridged to transplantation *via* IABP and in a control group, comprising 135 electively transplanted patients not needing circulatory support in the pre-transplant period. In the study by Cochran *et al.*^[53], 4 patients with end-stage ischemic HF were successfully bridged to transplantation *via* IABP, after a mean duration of IABP support of 31 d (range: 12-70 d). Long-term IABP resulted in a 10 to 50-fold decrease in cost compared to the cost associated with the use of LV assist devices as a bridge to transplantation. In the study by Russo *et al.*^[54], 14/17 patients with end-stage HF were successfully bridged

Table 3 Potential roles of long-term intra-aortic balloon pump support in chronic heart failure

Improves patients' clinical status and their hemodynamic indices, rendering them suitable candidates for heart transplantation (BTT)
Improves RV functionality and peripheral organ function, increasing the candidacy rates of patients who are ineligible for additional mechanical interventions (BTC)
Enhances native LV functional performance and unloads LV while maintaining its integrity, promoting reverse remodeling and cardiac recovery (BTR)

BTT: Bridge to transplantation; LV: Left ventricular; RV: Right ventricular.

to transplantation and 3/3 patients were successfully bridged to recovery *via* IABP after a mean support of 17 d (range: 3-48 d). In the study by Estep *et al*^[55], 50 patients received IABP support for a median of 18 d (range: 4-152 d) as a bridge to transplantation. Prolonged IABP support was associated with remarkable improvements in mean pulmonary artery pressure (MPAP) as well as in creatinine and total bilirubin concentrations. Forty-two patients (84%) were successfully bridged to transplantation and 38 of them (90%) were alive 90 d after transplantation. Additionally, in the study by Terrovitis *et al*^[56], 7 patients with end-stage HF (INTERMACS 2) due to idiopathic dilated cardiomyopathy underwent long-term circulatory support with IABP. One patient was successfully bridged to cardiac surgery, 4 patients were successfully bridged to assist device implantation, while the remaining 2 patients were successfully bridged to recovery and remained asymptomatic (NYHA class I) for at least 6 and 20 mo post-IABP removal^[56]. Finally, Tanaka *et al*^[57] investigated 88 patients with decompensated advanced HF who were implanted with IABP either as BTT and mechanical support ($n = 82$) or as bridge to recovery ($n = 6$). More than 90% of the patients succeeded the therapeutic goal with minimal rates of morbidity and mortality, while 3 out of 6 BTR patients experienced cardiac recovery.

IABP FOR CHRONIC RV HF

RV dysfunction is both a cause and an effect of HF progression, often leading to treatment dead-ends. On the one hand, patients with RV dysfunction are considered to be bad candidates for LVAD implantation^[59], as LVAD support aggravates pre-existing RV dysfunction through an increase in RV preload^[60]. On the other hand, the use of biventricular assist devices (often viewed as the only treatment option for these patients) is complicated and associated with poor long-term survival^[61]. We recently investigated the effects of long-term IABP support in a cohort of 15 patients suffering from biventricular end-stage HF (all classified as NYHA class IV, INTERMACS profiles 1 or 2), who were ineligible for any alternative LV interventional procedure^[58]. Long-term IABP support (median 72 d,

range: 13-115 d) resulted in substantial RV reverse remodeling, decreasing RV end-diastolic diameter and mean right atrial pressure. In addition, long-term IABP support increased cardiac index, increased RV stroke work index, and improved peripheral organ function. Clinical outcomes were encouraging, as 3 patients experienced complete clinical recovery and remained alive in NYHA class I at least 6 mo after IABP removal. Six patients exhibited partial clinical recovery, as long-term IABP support induced reversal of contraindications rendering them eligible for LVAD implantation. Four patients (all in INTERMACS profile 1) continued to deteriorate clinically and eventually died, while 1 patient died from septic shock on the 155th day of support and 1 from systemic inflammatory response syndrome on the 90th day. Putative mechanisms underlying the counterpulsation-induced recovery of RV function include an increase in RV myocardial blood flow and restoration of an optimal interventricular septal geometry, by relieving the septal shift induced by overload of the left ventricle. Regardless of the precise mechanism, these findings suggest that long-term counterpulsation may have a role in promoting recovery of the failing RV and could be used as a therapeutic strategy to increase the candidacy rates of patients who don't qualify for additional mechanical interventions.

The potential roles of long-term IABP support in chronic LV and RF HF are summarized in Table 3. Converging data suggest safety and efficacy of long-term IABP support as a bridge to transplantation or bridge to LVAD implantation. In addition, limited clinical data suggest that long-term IABP support may promote myocardial recovery. However, additional studies are warranted in order to clarify whether IABP-induced myocardial recovery can be consistently achieved or represents an anecdotal experience. The potential for myocardial recovery would undoubtedly be enhanced by prospective identification of patients who are more likely to undergo cardiac recovery^[62].

KCV FOR CHRONIC HF

KCV is a pneumatically-driven counterpulsation circulatory assist device, surgically implanted in the descending thoracic aorta by thoracotomy under cardiopulmonary bypass^[27]. The KCV system consists of a 60-cc pumping chamber (sutured to the descending aorta), a percutaneous access device (PAD, implanted in a subcutaneous pocket), and an external controller. With regard to clinical application, the device was implanted in 5 patients with end-stage HF refractory to pharmacological medical treatment, but responsive to IABP support. The first patient died intra-operatively due to technical complications, whereas the following 4 patients demonstrated improvements in cardiac index, pulmonary capillary wedge pressure,

right atrial pressure, and NYHA class.

C-PULSE FOR CHRONIC HF

C-Pulse is an implantable extra-aortic balloon (EAB) counterpulsation device, consisting of an inflatable cuff positioned around the ascending aorta^[63]. The polyurethane cuff is implanted through thoracotomy and is wrapped around the patient's ascending aorta without any contact with the aortic blood^[64]. The cuff is synchronized to inflate inwardly during the diastolic notch, producing a stroke volume between 20 and 30 mL, depending on the cuff size and the aortic diameter.

Hayward *et al.*^[63] investigated the feasibility and safety of C-Pulse support in 5 patients with advanced HF (NYHA class III or IV). All patients improved by 1 NYHA class, however, infectious complications were observed in 3/5 patients (with 2 patients suffering mediastinal infection necessitating device explantation). Recently, Abraham *et al.*^[64] performed a multicenter study to investigate the feasibility, safety and preliminary efficacy of C-Pulse support in 20 patients with advanced HF (NYHA class III or ambulatory class IV). No 30-d mortality was observed and no neurological events or myocardial infarctions were recorded during the 1 year of follow-up. However, one patient suffered a device-related death (due to mediastinal infection) and 40% of patients experienced drive line exit site infections. In terms of efficacy, C-Pulse support produced significant improvements in NYHA functional class, quality of life and 6-min walk distance. Currently, a prospective, multicenter, randomized trial investigating the safety and efficacy of C-Pulse support in moderate to severe HF is underway (NCT01740596); 388 patients will be randomized to undergo C-Pulse implantation of optimal medical treatment (1 year follow up)^[36]. The primary efficacy point of the trial is freedom from worsening HF resulting in hospitalization, LVAD implantation, cardiac transplantation or death during 1 year of follow-up.

THE SYMPHONY DEVICE FOR CHRONIC HF

The Symphony device (Symphony) is an implantable counterpulsation device designed to be implanted *via* a minimally-invasive superficial technique, without the need to open the thoracic cavity. Symphony comprises a valveless, single chamber 40-mL polyurethane-lined pumping sac, which is designed to fit in a pacemaker-like pocket on the right side of the thorax, in the subclavian fossa^[29]. The pumping sac is connected to the systemic circulation by a short vascular graft, which is anastomosed to the subclavian artery. The driveline is tunneled out through the skin and attached to the driving console.

An anatomical cadaver-fit study suggested that a 40-mL Symphony might not be suitable for a large

number of patients, including women and small-sized men and that a smaller-sized device (32 mL) should be examined^[29]. An experimental study in 8 calves with toxin-induced cardiomyopathy demonstrated that the 32 mL-Symphony device was superior to the 40 mL-IABP in decreasing LV myocardial oxygen consumption and increasing the ratio of diastolic coronary artery flow to left LV external work, and inferior to the IABP in decreasing aortic end-diastolic pressure. Giridharan *et al.*^[65] compared the effects of Symphony and IABP on aortic, carotid and coronary flows in a bovine experimental model of monensin-induced heart dysfunction. Compared to IABP, Symphony eliminated retrograde systolic blood flow (observed during IABP support) and increased total blood flow (despite producing less diastolic flow augmentation compared to IABP).

The first clinical application of Symphony was performed in a 64-year-old man with ischemic HF (NYHA III b)^[66]. Within 72 h of implantation, Symphony support increased cardiac index, and decreased right atrial pressure, pulmonary capillary wedge pressure and serum creatinine. However, following the patient's ambulation and increased activity, low flow to the pump and loss of right radial pulse were observed with cephalad movement of the right arm. This was attributed to compression of the subclavian artery due to device movement and the Symphony was explanted on the 10th postoperative day.

PULVAD

The PACD^[67,68], consists of a round valveless pumping chamber driven by an IABP console. The PACD is implanted in the thoracic cavity and is connected to the ascending aorta *via* a Dacron vascular graft. The PACD is superior to IABP in unloading the failing heart and increasing cardiac output^[69]. The PACD was implanted in 3 patients suffering from CS refractory to conventional treatment, including IABP; one patient died 4 h after the device implantation due to anesthesia-induced peripheral vasoparalysis, while the other two died due to septic shock 8 and 54 d after implantation, respectively^[26].

The PULVAD is the improved version of the PACD (Figure 1). It is smaller than PACD and can be driven by any conventional IABP console. In pigs weighing 80-100 kg and calves weighing approximately 100 kg it proved to be 3 times more effective than an IABP in reducing the systolic and end diastolic aortic pressure^[70,71].

The PULVAD'S ease of implantation (not requiring extracorporeal circulation), low cost of manufacture, wide availability of driving consoles and the fact that it provides only pressure unloading of the LV (which should prevent myocyte atrophy^[72,73] and cardiac fibrosis^[74], and promote myocardial recovery) make the PULVAD an attractive long-term alternative to the more expensive and complex LV assist devices currently used

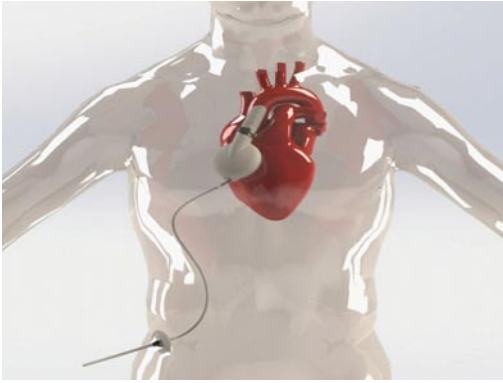


Figure 1 Pressure-unload left ventricle assist device.

in patients with end-stage decompensated HF.

DISCUSSION

Modern LVADs rely on continuous flow, and, while successful at prolonging life, LVAD-induced myocardial recovery is disappointingly rare. Clinically available LVADs bypass the LV and unload the failing LV independently of its systolic reserve. As a consequence, the dilated LV is rendered unable to generate at a basal pressure and LVEF is severely reduced because of the non-coupling of preload/afterload to LV systolic reserve. In other words, the continuous flow LVADs decrease LV preload but increase or maintain excessive afterload, driving LV function towards the bottom left of the Frank-Starling curve, reducing its functional performance. In general, we know that the lower the functional performance of the LV (*i.e.*, the lower the LVEF), the more vigorous is the process of adverse LV remodeling. In contrast to continuous flow LVADs the counterpulsation devices decrease LV afterload, thereby enhancing LV functional performance, and utilizing the LV systolic reserve to meet the peripheral metabolic demands. At the same time, the LV, based on the Frank-Starling curve, physiologically adjusts (decreases) its preload.

The IABP has been safely and effectively used for bridging chronic HF patients to transplantation^[52-56], to LVAD implantation and to recovery^[57,58]. Today, there are 4 counterpulsation devices (KardioVAD, C-Pulse, Symphony, and PULVAD) suitable for chronic mechanical assistance. These devices preserve heart integrity, unload the LV, decrease its energy consumption, enhance native LV functional performance and retain pulsatility of flow. In addition, the C-Pulse, Symphony and PULVAD counterpulsation devices do not require extracorporeal circulation for their implantation or explantation procedures. Knowing that recovery appears usually within the first 3-6 mo on mechanical assistance^[75], we propose that counterpulsation devices could be used temporarily (3-6 mo) as a bridge to recovery.

These devices appear suitable as a bridge to re-

covery not only for patients with acute HF but also for those with chronic HF, especially the ones with non-ischemic cardiomyopathy. We propose that when these patients become candidates for mechanical assistance the following practical rule can be followed: First assist them with IABP up to 2 wk and if the patients are hemodynamically stabilized (no need for IV inotropes/diuretics, no indication of peripheral organ malfunction, tolerance of HF medications, CVP \leq 10 mmHg, HR \leq 80 bpm, mean BP \geq 65 mmHg) then proceed to implantation of a counterpulsation device suitable for chronic mechanical assistance as a BTR. However, in the case of non-stabilization or further deterioration on IABP, proceed with implantation of a continuous flow LVAD or a BiVAD.

In conclusion, counterpulsation devices appear attractive for cardiac recovery not only for acute but also for chronic HF. Their simplicity of design and ease of implantation/explantation may allow much more widespread use compared to that of the currently-used continuous flow LVADs. To that end, further experimental and clinical studies are urgently needed to better define the role of counterpulsation devices in HF.

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Post-transplant dyslipidemia: Mechanisms, diagnosis and management

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Abstract

Post-transplant dyslipidemia is highly prevalent and presents unique management challenges to the clinician. The two major outcomes to consider

with post-transplant therapies for dyslipidemia are preserving or improving allograft function, and reducing cardiovascular risk. Although there are other cardiovascular risk factors such as graft dysfunction, hypertension, and diabetes, attention to dyslipidemia is warranted because interventions for dyslipidemia have an impact on reducing cardiac events in clinical trials specific to the transplant population. Dyslipidemia is not synonymous with hyperlipidemia. Numerous mechanisms exist for the occurrence of post-transplant dyslipidemia, including those mediated by immunosuppressive drug therapy. Statin therapy has received the most attention in all solid organ transplant recipient populations, although the effect of proper dietary advice and adjuvant pharmacological and non-pharmacological agents should not be dismissed. At all stages of treatment appropriate monitoring strategies for side effects should be implemented so that the benefits from these therapies can be achieved. Clinicians have a choice when there is a conflict between various transplant society and lipid society guidelines for therapy and targets.

Key words: Cholesterol; Dyslipidemia; Triglycerides; Statins; Immunosuppression

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Core tip: Post-transplant dyslipidemia is highly prevalent in all solid organ transplant recipient populations. Guidelines for therapy are derived mostly from general population experiences, although the mechanisms for dyslipidemia due to immunosuppression are distinct and known. Statin therapy has understandably received the most attention in transplant populations but the potential efficacy of other therapeutic strategies should not be ignored.

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INTRODUCTION

The great success of solid organ transplantation (SOT) over the past 50 years is demonstrated by the fact that both excellent short-term allograft survival and adequate long-term allograft function without the development of overwhelming comorbidity are routinely expected. Immunosuppressive medication regimens have advanced to the point that acute rejection has declined significantly, and even chronic forms of rejection are being delayed and their effects mitigated. As a result, increased clinician attention is being focused on the general well-being of transplant recipients, apart from allograft health *per se*, towards which cardiovascular (CV) health is an important component. In turn, each of the traditional CV disease (CVD) risk factors has received a share of the thrust on management strategies in transplant populations^[1], including dyslipidemia^[2]. However, most interventions are typically mapped to transplant recipients on the basis of evidence garnered from the general population. While the mechanisms for post-transplant dyslipidemia have largely been worked out, it is still not sufficiently known whether there is value to measuring isolated cholesterol subfractions, designing interventions for specific subfractions, or altering immunosuppressive medication regimens towards the goal of improving lipid profiles and CV health.

This review article provides a comprehensive overview of dyslipidemia in SOT recipients, based on the currently available literature. The prevalence and types of post-transplant dyslipidemia are first described, followed by the factors associated with lipid abnormalities, mechanisms of dyslipidemia after transplantation, the consequences of dyslipidemia, and finally its clinical diagnosis, monitoring, and treatment.

PREVALENCE AND TYPES OF DYSLIPIDEMIA

At one time, the prevalence of hyperlipidemia, which is the most common form of dyslipidemia, was estimated to be as high as 80% in kidney transplant recipients (KTR)^[3]. Reports of the high prevalence of hyperlipidemia go back as far as 1973^[4]. In the azathioprine-corticosteroid era of post-transplant immunosuppression, the prevalence rate was estimated at 50%-78%^[5-7]. Hypertriglyceridemia was just as common as hypercholesterolemia. However, with the introduction of cyclosporine, hypercholesterolemia has become the predominant abnormality^[8], particularly low density lipoprotein (LDL) cholesterol elevation^[9]. An early prevalence estimate of hyperlipidemia of over 50% has been reported in heart transplant recipients

(HTR)^[10]. Lung transplantation has been associated with a prevalence of hypercholesterolemia and hypertriglyceridemia of 32% and 41% respectively^[11]. Estimates of dyslipidemia in liver transplant recipients (LTR) include 43%^[12] and 31%-51%^[13]. The point prevalence of hyperlipidemia is unlikely to vary over time post-transplant. In KTR, hyperlipidemia is persistent if untreated. It is also possible that the prevalence is higher with time, due to inadequate surveillance in long-term patients. Cumulative factors such as advancing age, immunosuppression, weight gain, and the development of diabetes may all contribute to developing hyperlipidemia over time. Hyperlipidemia has also been documented in children after kidney transplantation^[14].

Dyslipidemia is not synonymous with hyperlipidemia, so it is conceivable that dyslipidemia may still be present despite normal lipid levels. Increased levels of very low-density lipoprotein (VLDL) cholesterol and decreased high-density lipoprotein (HDL) cholesterol levels despite normal "total" cholesterol levels are well-described^[15]. A low HDL has been noted in lung transplant recipients^[16] but not necessarily in HTR^[17]. In particular, a low level of the HDL2 sub-fraction has been reported after kidney transplantation^[18]. There is also a higher amount of oxidized LDL cholesterol^[19,20]. The lipid profile of LTR has also recently been elaborated. Compared to controls with no chronic medical disease, LTR had higher apolipoprotein B, small dense LDL cholesterol, and VLDL cholesterol concentrations^[21]. VLDL cholesterol concentration was also related to cyclosporine levels^[21]. Despite the initial excitement surrounding HDL sub-fractions and oxidized LDL cholesterol^[18-20], measurement of these lipid forms has yet to reach clinical practice almost thirty years after their description. The prevalence of small dense LDL cholesterol has been estimated at 26%-33% in KTR^[22]. Elevations in serum apolipoprotein B and lipoprotein (a)^[23], as well as decreased apolipoprotein A-I^[24], and decreased ratios of apolipoproteins C-II to C-III^[25,26] also generated significant interest, but at the present time none of these are routinely measured in a clinical setting. More recently, "non-HDL cholesterol", which is simply the total cholesterol minus HDL cholesterol level, has received attention in transplant patients^[27]. However, the importance of this particular measure has not yet been placed in full context.

FACTORS ASSOCIATED WITH LIPID ABNORMALITIES

Given the variety of lipid abnormalities seen, it is useful to divide factors contributing to dyslipidemia into those that contribute primarily to hypercholesterolemia and those that contribute primarily to hypertriglyceridemia, notwithstanding their qualitative impact that cannot be routinely assessed in the clinic. These risk factors are summarized in Table 1 (partially adapted from^[8]).

Table 1 Factors associated with lipid abnormalities after transplantation

Hypercholesterolemia	Hypertriglyceridemia
Genetic predisposition	Genetic predisposition
Age	Excessive dietary intake of carbohydrates, cholesterol, and saturated fat
	Obesity
Excessive dietary intake of cholesterol and saturated fats	Proteinuria
Obesity	Renal insufficiency
Proteinuria	Corticosteroids
Anti-hypertensive agents, <i>e.g.</i> , diuretics, beta-blockers	
Corticosteroids	Mammalian target-of-rapamycin inhibitors (sirolimus)
Calcineurin-inhibitors (cyclosporine, possibly tacrolimus)	
Mammalian target-of-rapamycin inhibitors (sirolimus, everolimus)	

Hypercholesterolemia is considered more prevalent based on the available literature, although the literature is dominated by North American and Western European publications. Genetic predisposition may be based on the prevalence of various polymorphisms of the lipoprotein system. For example, the GA genotype of the apo A-1 promoter region has been associated with a greater rise in LDL cholesterol after heart transplantation^[28]. Conversely, some genes such as the TP-binding cassette subfamily B member 1 (*ABCB1*) lose their association with LDL cholesterol after heart transplantation^[29]. Advanced age is another non-modifiable risk factor. However, modifiable risk factors such as a diet high in saturated fat may be just as important as a contributor to hypercholesterolemia. Obesity, proteinuria either as a result of native or transplant kidney disease, or the use of thiazide diuretics or beta-blockers for hypertension and heart disease may also contribute. Corticosteroids, cyclosporine, and sirolimus may all cause elevations in cholesterol levels^[8]. Although tacrolimus is generally believed to cause less elevation in LDL cholesterol than cyclosporine, this may not always be the case, particularly in LTR in whom lipid levels may correlate with tacrolimus levels^[30]. The association with sirolimus is particularly strong. LDL cholesterol levels were higher in the sirolimus arm of the Symphony study^[31].

In the case of post-transplant hypertriglyceridemia, as with hypercholesterolemia, genetic predisposition plays an important role. The apolipoprotein E 2/2 and 2/3 genotypes are associated with elevated triglycerides after kidney transplantation^[32]. The apo A-1 promoter region^[28] also correlates with elevated triglycerides. The development of hypertriglyceridemia in response to sirolimus has been subject to genetic analysis, with positive associations demonstrated with the *ABCB1* 1236 TT homozygote and the interleukin-10 1082AA homozygote in the case of KTR^[33]. Age, however, seems to be less important as

a risk factor for hypertriglyceridemia. A diet rich in simple sugars predisposes to hypertriglyceridemia, and although obesity and proteinuria are also associated with hypertriglyceridemia, poor renal function *per se* appears to be an additional risk factor^[8]. Sirolimus is more strongly associated with hypertriglyceridemia than hypercholesterolemia, with even a lower drug exposure leading to this abnormality^[31], although the contribution of other immunosuppressive drugs is less clear. More common is the association of hypertriglyceridemia with other metabolic syndrome components^[1].

MECHANISMS OF POST-TRANSPLANT DYSLIPIDEMIA

Immunosuppressive agents contribute significantly and specifically to lipid abnormalities after SOT.

Corticosteroids induce insulin resistance. The resultant hyperinsulinemia leads to increased hepatic uptake of free fatty acids (FFA)^[34]. FFA constitutes the main substrate for VLDL cholesterol synthesis. FFA synthetase and acetyl-CoA carboxylase are also increased by steroids^[35] and so hepatic synthesis of VLDL is increased. Insulin resistance also leads to a reduction in lipoprotein lipase, which leads to reduced triglyceride clearance^[36]. There is an increased conversion of VLDL to LDL cholesterol, leading to a rise in LDL cholesterol levels. Yet another contributory mechanism is down-regulation of LDL receptor expression^[37]. Finally, corticosteroids increase the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which is the rate-limiting step in the cholesterol biosynthetic pathway^[37].

Cyclosporine interferes with the binding of LDL cholesterol to the LDL receptor. As a result, there is a decline in LDL clearance, leading to a rise in LDL cholesterol levels. In this respect, there may be an additive effect of cyclosporine with corticosteroids. Cyclosporine also interferes with bile acid synthesis^[38] by interfering with the enzyme 26 hydroxylase^[15]. Decreased bile acid synthesis in turn leads to LDL receptor down-regulation, further reducing the clearance of cholesterol. Cyclosporine, by virtue of being highly lipophilic, is transported within the core of LDL cholesterol particles. In the process, it may change the molecular configuration of LDL^[39] and alter the normal feedback regulation of cholesterol synthesis^[8]. Glucose intolerance may even potentiate the effect of cyclosporine on lipid levels. The effects of tacrolimus on lipid metabolism are generally similar to those of cyclosporine, so it remains unclear why tacrolimus is associated with less hyperlipidemia.

Sirolimus provides a fascinating instance of a strong connection between pharmacotherapy and dyslipidemia on the one hand, yet ongoing debate about its cardiovascular effects both harmful and protective on the other. Sirolimus may inhibit lipoprotein lipase^[40]

and decrease lipolysis. There may also be hepatic over-production of lipoprotein in general^[41]. Other effects include a decrease in apolipoprotein B100 catabolism^[42]. Finally, sirolimus alters insulin signaling, increases the activity of tissue lipase, and increases the secretion of VLDL cholesterol^[40]. Sirolimus is almost never used as monotherapy for transplant-related immunosuppression and so likely acts in a synergistic manner with other immunosuppressive agents in promoting dyslipidemia. Sirolimus is also used as an anti-proliferative agent in endovascular stents, but the amount of exposure is unlikely to promote lipid abnormalities in that instance.

CONSEQUENCES OF DYSLIPIDEMIA POST-TRANSPLANTATION

SOT recipients, especially KTR, are at high risk for the development of post-transplant CVD. The link between dyslipidemia and CVD may not be as strong as, for instance, diabetes^[1], but there is no reason to believe that the association does not hold in transplant populations as it does in the general population. The underlying assumptions, however, are not so straightforward. Atherosclerosis is accelerated after transplantation^[8], and this can be linked at least retrospectively to cardiovascular events^[43]. The association of elevations in cholesterol to cardiovascular events may be stronger with cholesterol than with triglycerides, and likewise, more associated with ischemic heart disease than other forms of CVD such as cerebrovascular disease or peripheral vascular disease^[44]. It has been estimated that an increase in LDL cholesterol concentration by 2 mmol/L doubles the risk for major adverse cardiac events (MACE), comparable to an age increase by 23 years^[45]. A low level of HDL cholesterol has been associated with a threefold increase in post-transplant MACE^[46] and also an increase in all-cause mortality^[46]. Non-HDL cholesterol has been found to be as powerful a predictor of MACE as diabetes in KTR^[47].

Despite some correlative success between various lipid level abnormalities and MACE, consistent demonstration of the association remains quite difficult, since a large proportion of MACE is explained by unmeasured risk factors outside of the traditional Framingham risk factors, including dyslipidemia^[48]. Moreover, hyperlipidemia has not been found to be an independent risk factor for MACE in non-Caucasian populations in whom non-traditional risk factors may be more important^[49]. The Assessment of Lescol in Renal Transplantation (ALERT) study database^[50] has formed the basis for significant understanding of the link of dyslipidemia to human pathology, but all links remain associative. Data from another large database, the Patient Outcomes in Renal Transplantation study, however, indicate that dyslipidemia adds little predictive value to more transplant-specific and

graft-related variables in predicting acute myocardial infarction (MI), coronary artery revascularization or sudden death^[51]. Nonetheless, hypertriglyceridemia in particular has been associated with the progression of coronary artery calcification (CAC) in KTR^[52], although it must be understood that CAC is only a surrogate marker for CVD and is itself controversial in that respect at best. Information regarding dyslipidemia and CVD risk in SOT outside of kidney transplantation is limited. In HTR, hypercholesterolemia has been associated with non-fatal MACE in a retrospective analysis^[53]. Although LTR display a higher CVD risk and CVD is the leading cause of non-graft related deaths^[54], demonstration of dyslipidemia as a CVD risk factor lags behind other risk factors such as diabetes and hypertension^[54]. While other studies in liver transplantation have also either not addressed or failed to demonstrate a relationship of dyslipidemia to CVD^[55], a link with CVD has been found with metabolic syndrome and hypertriglyceridemia^[56].

Dyslipidemia, or at least one aspect of it (hypertriglyceridemia and low HDL cholesterol), is one among five components constituting the metabolic syndrome. Therefore, it is helpful to understand the contribution of dyslipidemia to post-transplant morbidity relative to its sister CVD risk factors such as hypertension, microalbuminuria, obesity and dysglycemia. As one example, in a cohort study of 1182 stable KTR with close to 7500 patient-years of follow-up, dyslipidemia did not attain statistical significance as a stand-alone CVD risk factor, but provided additive value to dysglycemia and microalbuminuria in predicting MACE ahead of hypertension and obesity^[1]. Interventions for dyslipidemia have an impact on reducing cardiac deaths and non-fatal MI in clinical trials specific to the transplant population^[2]. Therefore, attention to dyslipidemia is indeed warranted.

In contrast to other populations, SOT permits the assessment of the relationship of dyslipidemia to the performance of the allograft itself. It is possible, at least theoretically, that an allograft is predisposed differently to metabolic injury compared to a native organ due to its intersection with injury from the actions of the immune system. Hyperlipidemia is a paradigmatic contributor to chronic kidney allograft injury as a "non-immune" risk factor^[57]. Atherosclerosis is believed to be an integral part of the rejection process, by virtue of the accumulation of oxidized LDL cholesterol in the kidney interstitium leading to fibrosis^[58]. However, this may be a bidirectional relationship, with lipid abnormalities perpetuated by allograft dysfunction. Hypercholesterolemia has been associated with kidney allograft loss in the context of prior acute rejection^[59]. Hypercholesterolemia itself may predispose to acute rejection, by altering cyclosporine pharmacokinetics and increased binding with less tissue release^[60]. At a clinical level, overall there has been little progress in understanding beyond earlier studies that demonstrate associations between early post-kidney transplant lipid

levels and subsequent graft function or death-censored graft loss^[61,62]. A demonstrable effect of lipid levels on graft function may be blunted by more aggressive lipid lowering in transplant recipients for cardiovascular protection with the advent of other potent medical therapies, as well as due to data on safety and efficacy of lipid-lowering therapies from studies such as ALERT. Effective immunosuppressive therapy, and other graft-related variables such as donor organ quality may also be too overpowering to allow for demonstrating any effects of lipid profiles on graft function.

DIAGNOSIS AND MONITORING

The diagnosis of dyslipidemia in SOT recipients typically starts with a lipid profile obtained after 8 to 12 h of fasting. Although non-fasting lipid level measurement has been occasionally recommended for the general population, transplant recipients should be considered a high-risk group for CVD and should therefore be subject to fasting measurements. Normal "cut-offs" for hyperlipidemia are typically the same as those used for the general population^[15], in the absence of any evidence to the contrary. Measurements of lipid parameters beyond total, HDL and LDL cholesterol, or triglycerides are rarely performed outside of research studies. All recipients require at least one such fasting lipid profile, with the first profile obtained at some point during the first year. An initial evaluation as soon as three months post-transplant has been recommended^[8]. A Canadian commentary on the 2009 KDIGO Clinical Practice Guideline^[63] advises initial measurement 2-3 mo post-transplant, 2-3 mo after a change in treatment, and annually thereafter^[63]. Annual monitoring is corroborated by older European guidelines^[64]. More recently, the need for repeat lipid level measurement in many forms of chronic kidney disease has been questioned^[65], mostly on the basis of lack of evidence for utility and the absence of clinical trial data. A useful approach might be to gauge the transplant recipient's overall cardiac risk profile, and reserve lipid monitoring to those at a perceived higher CV risk, understanding that chronic graft dysfunction may itself be a high-risk equivalent.

TREATMENT

All transplant recipients require consultation with a dietician on a regular, if infrequent basis. A diet low in total fat, saturated fatty acids, and cholesterol can be prescribed as an initial measure, particularly in KTR who by definition have chronic kidney disease (CKD). Hypertriglyceridemia may be controlled with the help of a diet low in simple sugars and alcohol. The American Heart Association Step I diet can be considered as a starting point for those with an elevated LDL cholesterol level. Limiting dietary cholesterol intake to under 300 mg/d and caloric intake from fat to under 30% of the total caloric intake

may be helpful. A further Step II approach would be to limit these further to under 200 mg/d and 10% respectively. However, evidence of the efficacy of such diets in transplant recipients is lacking. Balance of the saturated to polyunsaturated fat intake should be sought. Losing excess body weight is important, and control of total caloric intake is likely to have the biggest impact^[3]. Improved glycemic control will also help to improve hyperlipidemia. Adherence to prescribed diets can be highly variable, and so culture-specific dietary interventions may be needed to improve adherence. Incorporation of soy protein into the diet^[15] has not been tested in SOT recipients. The success of dietary intervention alone at improving dyslipidemia has been estimated at under 20% in KTR^[66].

Non-conventional pharmacological therapies have received some attention, particularly in KTR. There may be attempts by SOT recipients to reduce their lipid levels through herbal supplements. Obviously, this can be quite dangerous in the context of immunosuppressive medication. For example, red yeast rice (*Monascus purpureus*) is a remedy designed to lower cholesterol levels. Red yeast rice contains varieties of mevinic acid, a naturally occurring statin, that has been associated with rhabdomyolysis^[67]. Since statin concentrations show batch variability and production is unregulated, herbal remedies should be discouraged. Fish oil is rich in omega-3 polyunsaturated fatty acids and can lower serum triglycerides^[68] by reducing its hepatic synthesis. Fish oil may even have a beneficial effect on graft function^[69], although further studies are clearly needed before this therapy can be endorsed. Finally, the use of antioxidants particularly antioxidant vitamins has also been considered based on the rationale that oxidized LDL cholesterol is particularly atherogenic. However, antioxidants are not considered efficacious at preventing CVD in the general population^[70]. The administration of homocysteine-lowering therapies is also not recommended^[68].

HMG-CoA reductase inhibitors, or statins, are widely used in KTR, LTR and HTR. They are potent reducers of LDL cholesterol levels, and are generally considered safe as long as patients are appropriately monitored. Some statins may have modest beneficial effects in lowering serum triglycerides and raising HDL cholesterol levels^[15]. There are also claims that statins have pleiotropic effects, involving a favorable modulation of endothelial function that translates into improved CV health^[71]. Since CKD may be a high-risk equivalent for CVD, this paradigm seems appealing. Perhaps the most commonly used statin is atorvastatin, despite the fact that the single prospective randomized trial of statins vs placebo in KTR, the ALERT study, utilized a different but older statin, namely fluvastatin^[2]. This large trial was successful in demonstrating benefit for secondary CVD endpoints, but not the primary composite

endpoint. Since a greater reduction in LDL cholesterol is believed to translate into greater cardiovascular advantage, atorvastatin or another more potent statin such as rosuvastatin may be preferred by clinicians. Atorvastatin and rosuvastatin are not as dependent on time of day for administration as the other statins^[15]. Maximum doses used are generally less than those for the general population, although the rationale for this practice in SOT recipients is based more on the known interaction of calcineurin-inhibitors through the CYP3A4 isoenzyme system^[72] than clinical evidence. Transplant recipients are also prescribed multiple other medications that can interact through this busy enzyme system, and so regular monitoring for the major statin-induced side effects, namely myositis or rhabdomyolysis, as well as hepatitis, is warranted. Simvastatin has recently been singled out as an offender with regards to rhabdomyolysis^[15]. However, statins remain appealing agents to use, being once-daily drugs and especially since they have also been shown to improve patient survival^[73]. Detailed guidelines on the use of specific statins in KTR are available^[15]. The recommended target for LDL cholesterol is a level under 2.0 mmol/L^[63] although this may be based more on extrapolation from the general population. A non-HDL cholesterol target of under 3.36 mmol/L in adults and 4.14 mmol/L in adolescents is a recommendation that serves as a surrogate for forms of cholesterol besides LDL cholesterol^[63]. It might be easier to initiate statin therapy early after the transplant, when other medications are being adjusted and patients are more receptive to new suggestions for optimizing their overall health. As more time elapses post-transplant, longer-term risks such as CVD may become less appreciated and the introduction of new medications may be perceived as an unnecessary risk or potential threat to allograft health.

Statins are also used in other SOT recipients besides KTR. Statins are generally considered safe in LTR with no severe complications^[74], although pravastatin in particular has been recommended^[75]. Statins also reduce accelerated graft atherosclerosis and mortality in HTR, especially pravastatin and simvastatin^[76], although atorvastatin has also been studied^[77]. The benefit of statins has also been extended to pediatric and adolescent HTR^[78]. Although the literature with other solid organs is not as expansive as that for KTR, there is no reason to believe that safety and efficacy concerns are substantially different among them.

If a maximal dose of statin proves to be insufficient at bringing the LDL cholesterol level to target, then consideration can be given to adding a second agent. Ezetimibe inhibits cholesterol absorption at the level of the intestinal brush border. Ezetimibe is generally safe in KTR^[79] although consultation at this point with a lipid metabolism specialist could be considered, particularly when increased transaminase levels have previously been noted with statin therapy. There are no time-of-day restrictions with ezetimibe. Ezetimibe can be

considered for use in LTR^[75,80] and in HTR^[81] in whom it has also been tested as monotherapy^[82]. Ezetimibe also increases HDL cholesterol levels in some HTR^[83].

Fibrates reduce hepatic VLDL cholesterol synthesis and increase lipoprotein lipase activity, decreasing triglyceride levels and increasing HDL cholesterol levels to some extent. LDL cholesterol levels may also decline, but not to the same extent as triglycerides. Among fibrates, fenofibrate is generally preferred over gemfibrozil due to less myotoxicity when added to a statin, as a result of less drug interaction. A concern regarding fibrate use is the potential for decline in kidney function in the presence of existing renal insufficiency^[84]. The use of fibrates should be avoided in advanced CKD since fibrates are metabolized by the kidneys^[15]. Their efficacy at preventing cardiac events in other population groups such as type 2 diabetes has also been seriously questioned^[85] and they are rarely, if ever used in combination with statins. Fibrates are believed to be generally well tolerated in LTR^[86]. Severe hypertriglyceridemia however may require plasma exchange in order to manage the associated pancreatitis^[87].

Niacin and bile acid sequestrants have both been explored for use in SOT recipients. Niacin could be considered as an option for monotherapy to reduce LDL cholesterol levels in those intolerant to statins^[15]. Niacin has been studied favorably in combination with simvastatin in the general population at preventing coronary disease^[70], although this has also been questioned^[88]. If used, a gradual dose escalation is required, and liver enzyme monitoring is warranted. Bile acid sequestrants are not popular in transplant recipients due to their gastrointestinal side effects including nausea and bloating, which patients are often already prone to as a result of immunosuppressive drug therapy. They can also interfere with the absorption of immunosuppressive drugs and should be separately administered from them by at least two hours.

Table 2 provides one suggested summary approach to post-transplant hyperlipidemia that can be tailored to individual clinic circumstances. However, relevant national society guidelines should preferably be followed. Clinicians have a choice when there is a conflict between various transplant society and lipid society guidelines for therapy and targets. There are few, if any clinical trials where modification of immunosuppressive therapy has been pursued with the intention of addressing dyslipidemia or reducing CVD risk and similarly, large database reviews are not sufficiently informative in this respect.

CONCLUSION

Post-transplant dyslipidemia is highly prevalent and presents unique management challenges to the clinician. There are two major outcomes when considering post-transplant therapies: preserving or

Table 2 A suggested approach to managing post-transplant dyslipidemia

Initial post-transplant period	Manage acute graft-related concerns Optimize immunosuppressive medication to graft function
2-3 mo post-transplant If LDL cholesterol and/or triglyceride level above target ¹	Measure 8-12 h fasting lipid profile Dietician consult
2-3 mo post-dietary intervention If LDL cholesterol and/or triglyceride level still above target ¹	Measure 8-12 h fasting lipid profile Initiate statin therapy, <i>e.g.</i> , atorvastatin 10 mg/d or rosuvastatin 5 mg/d Assess for potential drug interactions Monitor creatine kinase and liver transaminase levels
2-3 mo post-statin initiation If LDL cholesterol and/or triglyceride level still above target ¹	Measure 8-12 h fasting lipid profile Repeat all of the above until targets are achieved. Increase statin dose as tolerated to a maximum acceptable dose with each measurement not at target. If targets are not achieved then consider adding a supplemental agent, <i>e.g.</i> , ezetimibe 10 mg/d
If LDL cholesterol and/or triglyceride level still above target ¹	Consider consultation with lipid specialist
LDL and triglyceride target levels achieved	Annual monitoring of lipid levels. Consider more frequent monitoring for side effects
At all times post-transplant	Gauge overall cardiovascular risk

¹See text for relevant targets but also consult relevant local transplant and lipid society guidelines. LDL: Low density lipoprotein.

improving allograft function and reducing cardiovascular risk. Attention to dyslipidemia is warranted because interventions for dyslipidemia have an impact on reducing cardiac events in clinical trials specific to the transplant population. Dyslipidemia is not synonymous with hyperlipidemia. Numerous mechanisms exist for the occurrence of post-transplant dyslipidemia, including those mediated by immunosuppressive drug therapy. Statin therapy has received the most attention in all SOT recipient populations, although the effect of proper dietary advice and adjuvant pharmacological or non-pharmacological agents should not be dismissed. At all stages of treatment appropriate monitoring for side effects should be implemented so that the benefits from these therapies can be achieved.

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Kidney transplantation in obese patients

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Abstract

The World Health Organization estimated that in 2014, over 600 million people met criteria for obesity. In 2011, over 30% of individuals undergoing kidney transplant had a body mass index (BMI) 35 kg/m² or greater. A number of recent studies have confirmed the relationship between overweight/obesity and important comorbidities in kidney transplant patients. As with non-transplant surgeries, the rate of wound and soft tissue complications are increased following transplant as is the incidence of delayed graft function. These two issues appear to contribute to longer length of stay compared to normal BMI. New onset diabetes after transplant and cardiac outcomes also appear to be increased in the obese population. The impact of obesity on patient survival after kidney transplantation remains controversial, but appears to mirror the impact of extremes of BMI in non-transplant populations. Early experience with (open and laparoscopic) Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy support excellent weight loss (in the range of 50%-60% excess weight lost at 1 year), but experts have recommended the need for further studies. Long term nutrient deficiencies remain a concern but in general, these procedures do not appear to adversely impact absorption of immunosuppressive medications. In this study, we review the literature to arrive at a better understanding of the risks related to renal transplantation among individuals with obesity.

Key words: Body mass index; Overweight; Obese; Kidney transplant; Transplant complications; Transplant outcomes; Patient survival; Graft survival

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Core tip: Extremes of body mass index (BMI) appear to impact survival in kidney transplant recipients, but this effect appears to parallel that seen in the general population. Skin and soft-tissue complications, particularly wound infections and lymphocele formation, are higher among obese patients. In addition, the

rate of delayed graft function is also higher, and contributes to longer length of stay following transplant in this population. New onset diabetes after transplant also appears to be influenced both by BMI at time of transplant as well as increasing BMI following transplant. Measures of central adiposity, such as waist-to-hip ratio, may enhance risk assessment. Bariatric surgery appears promising to aid in reducing excess weight both pre- and post-transplant, but further studies are needed. Obesity should not constitute an absolute contraindication to transplantation but individualized risk assessment is necessary.

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INTRODUCTION

The World Health Organization defines overweight and obesity as having a body mass index (BMI = weight in kg/m² height) of ≥ 25 m/kg² and ≥ 30 m/kg², respectively. Using these definitions, WHO has estimated that in 2014, more than 1.9 billion adults were overweight of whom, over 600 million met criteria for obesity^[1].

A number of recent studies have confirmed the relationship between overweight/obesity and a number of important comorbidities - including risk for diabetes, cardiovascular disease (CVD), many cancers, gallbladder disease, and osteoarthritis^[2-5]. Extremes of BMI are strong predictors of increased mortality^[6] and rising BMI increases both direct healthcare costs and indirect costs related to reduced productivity and premature mortality^[7].

In 2011, 23% of United States kidney transplant recipients met criteria for obesity (BMI 30-34.9), 9.4% for morbid obesity (BMI 35-39.9), and 2.1% for very-morbid obesity (BMI ≥ 40)^[8]. Given the rising prevalence of obesity among kidney transplant candidates, we sought to review the literature to arrive at a better understanding of the risks related to renal transplantation among individuals with obesity.

LITERATURE SEARCH

A literature search was conducted on PubMed using search terms "obesity" AND "renal transplantation", "obesity" AND "kidney transplantation". In addition, the bibliographies of selected articles were reviewed for additional references. Cohort studies comparing outcomes between BMI categories, case series, systematic reviews, meta-analyses, and studies using data from established registries (*i.e.*, SRTR, UNOS) were preferentially selected. Authors reviewed

the available literature and synthesized findings in collaboration to produce the following review of obesity-related complications following renal transplantation. Where feasible, complication rates were categorized as described below and reported rates across series summarized as mean, median and range.

RECIPIENT RISKS ASSOCIATED WITH OBESITY

Recipient risks can be categorized as skin and soft tissue complications (such as wound infections and wound dehiscence), anastomotic and perinephric complications (such as lymphocele, hematoma, vascular), complications related to intrinsic allograft function [such as delayed graft function (DGF), immunologic rejection, graft survival], and systemic complications [such as sepsis, hospital readmissions, new onset diabetes after transplantation (NODAT), and patient survival]. Data of interest were derived from cohort studies comparing outcomes between BMI groups, case series, case control studies, meta-analyses, analyses of large transplant registries, and authoritative reviews. Outcomes of particular interest were those reiterated as significant between multiple studies.

Data for specific complications was gleaned mostly from cohort studies^[9-20], most^[9,11,13,15,17-19] used a BMI cutoff of ≥ 30 . Some studies used more varied BMI cutoffs for their analyses^[10,12,14,16,20]. One study^[20] was not amenable to table summarization and therefore was excluded from Table 1. Of interest, the obese groups tended to be older than the nonobese groups.

Skin and soft tissue complications

Wound dehiscence and wound infection were especially common themes in studies analyzing complications by BMI category. Between studies, however, the prevalence of individual complications was variable.

Wound dehiscence occurred at a median rate of 23.8% with a mean rate of 16.2% and range of 3% to 14.3%^[15-17,19]. The highest reported rate of wound dehiscence, 36%, was noted in a study^[16] using BMI > 35 as a cutoff for their high-BMI analytic group. This may depict a gradient risk for this complication associated with rising BMI. Likewise, the lowest risks for this complication, in the 3% range, were noted in two studies^[18,19] whose obese comparator group represented a lower overall BMI distribution than other studies. Furthermore, Behzadi *et al.*^[18], did not report specific BMI ranges, but had no patients with a BMI > 35 . This issue again supports the graded impact of BMI upon certain outcomes.

Two studies^[9,19] using a cutoff BMI of 30, reported wound infection at rates of 15% to 18.2% among their obese recipients. A third study^[14], which utilized a cutoff BMI of ≥ 35 , reported far higher wound

Table 1 Post-transplant complications among obese *vs* nonobese patients

Ref.	Groups		Complication	Outcome differences	
	Obese	Nonobese		Obese	Nonobese
Singh <i>et al</i> ^[9] 1999-2002	BMI > 30 (34.1 ± 3.68) n = 33 Age 48 ± 11.1	BMI ≤ 30 (23.6 ± 3.18) n = 35 Age 43.5 ± 13.5	OR time (min)	155 ± 59	119 ± 44
			LOS (d)	13.7 ± 10	9.48 ± 4.8 (P = 0.029)
			DGF	33.3%	17.1% (P = 0.12)
			Wound infection	18.2%	0 (P = 0.01)
			Lymphocele	18.2%	2.94% (P = 0.02)
			Perinephric HTMA	12.1%	0 (P = 0.05)
			Incisional hernia	6%	3.7% (P = 0.68)
			NODAT	9%	3.7% (P = 0.41)
			1/5 yr graft surv	75%/63%	98.9/94.5 (P = NS)
			1/5 yr pt surv	87.5/79.2	98.9/95.6 (P = NS)
Cacciola <i>et al</i> ^[10] 1993-2003	BMI ≥ 35 n = 24 (Group B) Age 45 (20-61)	BMI 30-34.9 n = 90 (Group A) Age 45 (25-70)	DGF	16.5%	22% (P = NS)
			1 yr graft surv	94%	97% (P = 0.51)
			1 yr patient surv	100%	100%
Mehta <i>et al</i> ^[11] 1999-2002 Living donor	BMI ≥ 30 n = 16 Age 50 ± 16	BMI < 30 n = 37 Age 43 ± 16	Acute rejection	19%	8% (P = 0.35)
			Wound Cxn	19%	13.5% (P = 0.68)
			Other Cxns	25%	11% (P = 0.22)
			DGF	19%	2.7% (P = 0.077)
			LOS (d)	8.4 ± 7	6.4 ± 5 (P = 0.68)
			1/3 yr graft surv	LD 100/100 DD 92/75	LD 95/91 DD 94/90
			1/3 yr pt surv	LD 100/100 DD 92/83	LD 97/95 DD 96/94
Marks <i>et al</i> ^[12] 1995-2000	BMI ≥ 35 (35-56) n = 23 Age DD: 44 ± 14 Age LD: 46 ± 1	BMI ≤ 25 (17-28) (n = 224) Age DD: 48.5 ± 13 Age LD: 43 ± 13	LOS (d)	LD 10.2 ± 8.0 DD 12.9 ± 9.0	LD 6.0 ± 4.1 DD 7.8 ± 3.0
			Readmission 6 mo	LD 82% DD 92%	LD 20% DD 49%
			Mult admits 1 st 6 mo	LD 44% DD 50%	LD 21% DD 18%
			Major wound infxn	LD 44% DD 33%	LD 2% DD 4%
			Graft loss 1 yr/3 yr	6.4/42.9	5.3/7.7
			Pt death 1 yr/3 yr	7.6/46.2	3.5/11.8
			DGF	31.3%	20.5% (P = 0.253)
Grosso <i>et al</i> ^[13] 2000-2010	BMI > 30 n = 64 Age 49.1 ± 12.9	BMI ≤ 30 n = 312 Age 49.8 ± 11.1 (BMI 25-30) Age 44.9 ± 13.7 (BMI < 25)			
Schwarznaeu <i>et al</i> ^[14] 2000-2004 Living donor	BMI > 25 (28.1 ± 2.6) n = 25 Age 49.2 ± 10.9	BMI < 25 (21.4 ± 2.0) n = 56 Age 42.8 ± 13.6	1 yr graft survival	94.6%	76% (P < 0.001)
Bardonnaud <i>et al</i> ^[15] 2004-2008	BMI ≥ 30 (35.1 ± 4.35) n = 21 Age 53.3 ± 11.19	BMI < 30 (22.9 ± 3.17) n = 179 Age 46.7 ± 15.05	DGF	38% ± 0.5%	14% ± 0.34% (P = 0.004)
			Lymphocele	14.3%	4.5 (P = 0.062)
			Wound dehiscence	4.8% ± 0.22%	2.2% ± 0.15% (P = 0.485)
			(pretransplant DM)	29%	6% (P < 0.0001)
			LOS (d)	24.9	15.6 (P = 0.008)
Gusukuma <i>et al</i> ^[16] 1998-2008	BMI ≥ 35 (36.8 ± 1.7) n = 47 Age 46.5 ± 10.9	BMI < 30 (22.6 ± 3.3) n = 2822 Age 40.7 ± 12.1	1 yr graft/pt surv	93.6%/95.6%	97.7%/98.1% (P = NS)
			5 yr graft/pt surv	84.0%/89.1%	88.8%/90.5% (P = NS)
			DGF	16.7% ± 19.3%	13.5% ± 16.2% (P = NS)
			Wound dehiscence	19.1%	1.9% (P < 0.001)
			Lymphocele	6.4%	2.6% (P = 0.054)
			NODAT	36%	16.2% (P < 0.001)
Furriel <i>et al</i> ^[17] 1984-2008	BMI ≥ 30 (32.44 ± 1.86) n = 26 Age 46.08 ± 12.75	BMI < 25 (22.03 ± 1.79) n = 295 Age 41.51 ± 13.23	LOS (d)	15.9 ± 16.7	11.3 ± 11.4 (P < 0.001)
			DGF	26.9%	16.9%
			Lymphocele	7.7%	1.4%
Behzadi <i>et al</i> ^[18] 2006-2008 Age 39.8	BMI ≥ 30 (none > 35) n = 34	BMI < 30 n = 146	Wound dehiscence	11.5%	0.7%
			RAS	17.6%	2.8% (P < 0.001)
			Hematoma	47.9%	17.6% (P = 0.009)
			Wound Cxn	64.7%	9.6% (P < 0.001)
			Renal vein thromb	2%	0% (P < 0.05)
			DGF	8.8%	6.80%
Johnson <i>et al</i> ^[19] 1994-2000	BMI ≥ 30 (32.0 ± 0.3) n = 59	BMI < 30 (23.4 ± 0.2) n = 434	Lymphocele	2.9%	1.40%
			Wound breakdown	14%	4% (P < 0.01)
			Wound dehiscence	3%	0% (P < 0.01)
			Wound Infection	15%	8% (P = 0.11)

BMI: Body mass index; Cxn(s): Complication(s); DD: Deceased donor; DGF: Delayed graft function; DM: Diabetes mellitus; HTMA: Hematoma; LOS: Length of stay; LD: Living donor; NODAT: New onset diabetes after transplant; OR time: Operating time; pt: Patient; RR: Relative Risk; Surv: Survival; NS: Not significant.

infection rates of 33%-44%. Other studies^[11,18] used a more general descriptor of "wound complication", thus preventing estimates of specific outcomes among their patient populations. A smaller study noted a rate of surgical site infections following renal transplant in 108 patients of 5%; age > 60 and BMI > 30 were found to be risk factors^[21].

Anastomotic and perinephric complications: For studies reporting it, lymphocele occurred at a median rate of 7.7% among obese recipients with a mean of 9.9% and range of 2.9% to 18.2%^[9,15,17-18]. Two studies reported a higher rate of hematoma among obese recipients^[9,18]. One study^[18] reported a rate of renal artery stenosis as high as 17.6% among obese patients, accompanied by a 2% rate of renal vein thrombosis. This study group as a whole was younger than most (mean age 39.8) so it is unclear as to why these specific complications should predominate simply due to obesity. In another study, both age > 60 and BMI > 30 were found to be risk factors for lymphocele (rate of occurrence 11%)^[21].

Complications related to intrinsic allograft function

DGF was higher among obese patients with a median rate of 16.7% and a mean of 22.8% with a range of 8.8% to 38.1%^[9-11,15-18]. In a separate study, Ditunno *et al.*^[20] reported the occurrence of DGF amongst 145/521 (27.8%) recipients with a BMI < 30 compared to 20/42 (47.6%) recipients with a BMI ≥ 30. A retrospective review of all renal transplant recipients in the United Network for Organ Sharing database (2004-2009) demonstrated significant risk increase for DGF among obese patients with odds ratios (compared to BMI < 30) rising in parallel with degree of obesity - BMI 30 to 34.9: 1.34 (95%CI: 1.27, 1.42); BMI 35-39.9: 1.68 (95%CI: 1.56, 1.82); BMI ≥ 40: 2.68 (95%CI: 2.34, 3.07)^[22].

Another study determined risk of DGF as higher in obese patients, but higher still in those with BMI ≥ 35; furthermore, the rate of biopsy proven acute rejection was found to be higher in this latter group as well^[23]. Using patients with a BMI 20-24.9 as a reference group, the OR for DGF rose in parallel with degree of obesity - BMI 25-29.9: 1.08 (95%CI: 0.71, 1.65); BMI 30-34.9: 1.95 (1.16, 3.19); BMI ≥ 35: 4.49 (2.24, 9.00). A similar trend was noted for biopsy proven acute rejection - BMI 25-29.9: 0.96 (0.67, 1.38); BMI 30-34.9: 1.28 (0.83, 1.98); BMI ≥ 35: 2.43 (1.48, 3.99). The authors used BMI category at time of transplant for this analysis.

In an analysis of over 11836 transplant patients in the Scientific Registry of Transplant Recipients, and after adjusting for case mix and malnutrition-inflammation variables, Molnar *et al.*^[24] determined that pretransplant BMI remained an independent and significant predictor of DGF. Following adjustment, multivariate analysis demonstrated that for each Standard Deviation (1

SD = 6.0 kg/m²) increase from normal, the risk of DGF was increased by 35% (OR: 1.35, 95%CI: 1.27-1.45). Compared to normal (BMI 22-24.99), BMI 25-29.99, 30-34.99, and ≥ 35 had the following OR for development of DGF: 1.30, 1.42, and 2.18.

Systemic and cardiovascular complications

Two studies reported varied rates of new onset diabetes after transplant (NODAT) of 9% and 36%^[9,16]. The higher estimate comes from Gusukuma *et al.*^[16] using BMI of ≥ 35 as their cutoff. In a study of 167 renal transplant recipients^[25] NODAT developed during the 1st post-transplant year in 64 (38.2%). Using multivariate regression, the authors determined significant risk factors to be age > 50 at time of transplant (HR 2.50, 95%CI: 1.72, 3.65), waist circumference in men > 94 cm (HR 1.95, 95%CI: 1.17, 3.25) and in women > 80 cm (HR 4.50, 95%CI: 1.87, 10.86).

Of interest, a number of short-term studies have demonstrated improved glycemic control and diabetic parameters following conversion from tacrolimus (Tac) to cyclosporine (CsA) in patients with NODAT^[26-28]. However, one small study with long-term follow up suggests that the glycemic benefits associated with CsA conversion may only be short-lived^[29].

The absence in long-term incidence of NODAT between CsA and Tac based immunosuppression was further supported by a single-center study of 704 patients, nondiabetic at time of transplant (1999-2005)^[30]. BMI was, however, identified as an important risk factor. In this study, the emergence of NODAT was determined between cyclosporine based immunosuppression (*n* = 533) and then following conversion to tacrolimus (in 171 patients at a mean post-transplant time of 17.3 ± 17.7 mo) based immunosuppression. Most common reasons for conversion include rejection events or for difficulty maintaining therapeutic CsA levels) based immunosuppression. Of note, target long-term prednisone dosing in this study was 10 mg/d. Multivariate time-dependent Cox regression analysis found no difference in the adjusted 5-year risk of NODAT-free survival following conversion from CsA to Tac (87.4%) compared to CsA only groups (91.0%, *P* = 0.90). Multivariate analysis confirmed that conversion from CsA to Tac did not increase the risk for NODAT; instead, significant associations included recipient age [per year: 1.04 (95%CI: 1.02, 1.06)]; BMI at transplant [per unit increment: 1.09 (95%CI: 1.05, 1.13)]; and previous fasting glucose level [1.06 (95%CI: 1.05, 1.08)]^[30].

Length of stay (LOS) is generally higher in obese patients, with a median of 13.7 d, mean of 14.9 d, and range of 8.4 to 24.9 d^[9,11,12,15,16]. This is in comparison to a median of 9.5 d, mean of 11.32 d, and range of 6.4 to 15.6 d for the lesser BMI comparators. Authors cited emergence of DGF as a likely cause of prolonged LOS.

Elevated BMI in the setting of kidney transplantation

has been associated with increased transplant-related complications and concerns for poorer rates of graft and patient survival. In a recent analysis of 51927 adult renal transplant recipients registered to the USRDS database (1988-1997), extremes of BMI (< 18 and > 36) were significantly associated with worse patient survival and poorer graft survival - the latter independent of patient survival^[31]. The risk for graft loss by cox proportional hazard model was similar for BMI < 18: 1.213 (95%CI: 1.110, 1.326) - as it was for BMI 34-36: 1.205 (95%CI: 1.084, 1.339); and highest for BMI > 36: 1.385 (95%CI: 1.300, 1.551). Similar U-shaped outcome patterns were noted for death censored graft loss, long-term graft loss beyond 6 mo, death with functioning graft, and infectious death.

A single-center study of 1102 renal allograft recipients with baseline pre-transplant cardiac disease among 19.2% demonstrated that the 5-year cumulative incidence of a composite cardiac outcome [comprised of congestive heart failure (CHF), Atrial fibrillation, and myocardial infarction] increased significantly between the lowest and highest BMI quartiles - BMI 14.2-22.9: 8.7% (SE 2.4%); BMI 29.8-46.9: 29.3% (SE 5.4%). This increase in the composite was driven primarily by increases between 1st and 4th quartiles in CHF (3.6% vs 18.4%) and atrial fibrillation (1.0% vs 10.7%); the cumulative incidence of myocardial infarction, however, did not increase by BMI quartile^[32].

Weight gain following transplant may represent a particularly concerning risk factor. In a 20-year follow up study of a cohort of 1810 patients, a cox proportional hazards model was used with adjustment for cardiovascular risk factors to determine relative risk of death and death-censored graft failure. After multivariable adjustment, the authors found that each 5 kg/m² increment in BMI during the first year after transplant contributed a 1.23 (95%CI: 1.01, 1.50) and 1.18 (95%CI: 1.01, 1.38) additional relative risk for death and death-censored graft failure, respectively. The relative risk for mortality and graft-failure in patients with BMI > 30 was 1.39 (95%CI: 1.05, 1.86)^[33]. In a study of 292 renal transplant recipients, multivariate analysis demonstrated that an increase in BMI of > 5% contributed to a death censored hazard ratio for 1-year graft loss of 2.82 (95%CI: 1.11, 7.44)^[34].

In conflict with this finding are results from a recent study by Nicoletto *et al*^[35]. Meta-analysis of 21 studies involving 9296 patients found an association between obesity and DGF (RR: 1.41, 95%CI: 1.26, 1.57) but not with acute graft rejection. Interestingly, the association between graft-loss, death by CVD, and all-cause mortality was dependent upon transplantation era. In studies assessing 5-year survival, for example, the authors determined using univariate meta-regression that year of publication became significant. Subgroup analysis stratified by year of publication

(before or after 2003) demonstrated a difference in the association of obesity on 5-year survival - those studies prior to 2003 (RR 1.96, 95%CI: 1.55, 2.48) vs studies post-2003 (RR 1.06, 95%CI: 0.85, 1.31). Similar findings were noted for 1-year survival and graft loss at 5 years. Death by CVD was increased, but all studies evaluated predated 2003. The authors speculate the change due to modern-era (post-2000) Tac-based immunosuppression and steroid-sparing or rapid tapering based protocols compared to previous era transplants.

Chang *et al*^[36] used data from the New Zealand Dialysis and Transplant (ANZDATA) Registry to examine relationships between BMI at transplant and subsequent outcomes. 5684 patients age ≥ 16 at time of transplant (1991-2004) were included and followed until death or through 2005. Obesity was a risk factor for graft and patient survival lost significance when entered into multivariate analysis. Underweight (BMI < 18.5) status, as opposed to normal BMI (18.5-24.9), was found to be a predictor of late (> 5 years) graft loss with HR 1.70 (95%CI: 1.10, 2.64). The adverse effect of underweight status on graft survival was attributed to the likelihood that due to lesser degrees of adiposity, higher graft-kidney concentrations at a given blood level could have led to higher rates of calcineurin inhibitor nephrotoxicity^[36-39]. When analyzed as a time-varying covariate using BMI at the start of periods 0-1 years, 1-5 years, and > 5 years post-transplant, BMI ≥ 30 was not associated with poorer graft or patient survival^[36].

In a combined systematic review (of 11 studies representing 305392 participants) and meta-analysis of 4 studies, Ahmadi *et al*^[40] determined that compared to normal BMI, extremes of weight were associated with increased post-transplantation mortality risk. The hazard ratios for mortality risk were 1.09 (95%CI: 1.02-1.20), 1.07 (95%CI: 1.04-1.12), and 1.20 (95%CI: 1.14-1.23) based upon underweight, overweight, and obese BMI, respectively. The authors concluded that the "obesity survival paradox is unlikely in kidney transplant recipients since both extremes of pre-transplantation BMI are linked to higher mortality in this population".

BARIATRIC SURGERY IN RENAL TRANSPLANT RECIPIENTS

Pre-transplant patients

Given the associated technical difficulties, surgical site complications, and outcomes-related concerns, transplant programs may impose a maximal BMI eligibility threshold for transplant. To this regard, data support the efficacy of transplant facilitation through effective pretransplant weight reduction using bariatric surgery^[41,42]. In the largest of these series, laparoscopic sleeve gastrectomy (LSG) in 27 pretransplant patients

with a mean age of 57 years and mean preoperative BMI of 48.3 (range 38-60.4) underwent LSG with subsequent mean percentage excess weight loss at 1, 3, and 12 mo of 17%, 26%, and 50%^[42].

LSG involves subtotal gastric resection of the fundus and body to create a smaller tubular gastric conduit without otherwise modifying gastrointestinal nutrient flow^[42]. Despite being a restrictive as opposed to a malabsorptive procedure (such as Roux-en-Y gastric bypass or biliopancreatic diversion) postoperative nutrient deficiencies remain a concern^[43,44].

Two studies in non-transplant patients compare outcomes between LSG and Roux en Y Gastric Bypass. While overall mortality was similar, LSG is less invasive with lower morbidity rates (20.5% RYGB vs 6.5% LSG) and comparable degrees of weight loss at 6, 12, and 18 mo, while RYGB appeared to be more efficacious in terms of achieving diabetes remission^[45,46]. Another study^[46] supports similar degrees of weight loss between procedures but comparable rates of diabetes resolution; rates of resolution for hypertension and gastroesophageal reflux disease (GERD) were superior with RYGB. Given the premise of LSG, it is not surprising that GERD may actually increase postoperatively^[47].

Post-transplant patients

Accumulating data also support the safety and efficacy of bariatric surgery in reducing obesity-related morbidity in renal transplant patients. Patient selection is critical and the involvement of an experienced bariatric surgery service is crucial in pairing the appropriate procedure with the individual patient's circumstances^[48].

Long term (median of 14 mo) follow up of 8/10 renal transplant recipients following LSG demonstrated significant reduction in BMI^[49]. Median preoperative BMI was 42 (37-49); following LSG the median BMI at 6 mo and one year were 31 and 29, respectively. The median percentage excess weight loss was 54% at 3 mo, 57% at 6 mo, and 75% at 1 year. It must be noted that in 2 patients, LSG was unsuccessful or complicated. In one subject, it failed to control weight gain and subsequent conversion to biliopancreatic diversion and duodenal switch became necessary; in another, a sleeve stricture developed accompanied by nausea, vomiting, and a transient rise in creatinine. Importantly, LSG did not interfere with maintenance of immunosuppression and the associated weight loss was accompanied by improvements in both serum creatinine and urinary protein excretion.

In another series, 5 female renal transplant recipients with a mean BMI of 52.2 (range: 48-69) underwent Roux-en-Y gastric bypass (in 4) and LSG (in 1). Percent of excess weight loss at 2 years was over 50% in all patients. No postoperative complications were noted nor were alterations to immunosuppressant dosing required^[50].

In perhaps the largest series to date, Våge *et al*^[51]

present long-term outcomes data on 117 patients undergoing LSG in the post-renal transplant setting. Patients in this series had the following baseline characteristics, presented as mean (\pm SD): Age 40.3 (10.7) years, BMI 46.6 (6.0) kg/m²; type 2 diabetes was present in 23 (19.7%), hypertension in 50 (42.7%), hyperlipidemia in 14 (12.0%), sleep apnea in 15 (12.8%). Of interest, the majority of benefit had been achieved by 12 mo and remained stable for most outcomes through 24 mo follow up. These benefits included reduction in BMI to 30.3 (5.9) and 30.6 (5.6) kg/m² by 12 and 24 mo. By 24 mo, remission of the aforementioned baseline comorbidities had occurred in 80.7%, 63.9%, 75.8%, and 93.0%, respectively. Not unexpectedly, rates of gastroesophageal reflux disease increased in a statistically significant manner from 12.8% at baseline to 27.4% at 24 mo. Complications included hemorrhage (requiring transfusion) in 6 (5.1%), anastomotic leak in 2 (1.7%), abscess without leak in 1 (0.9%), and wound infection in 3 (2.6%). Of interest, alanine aminotransferase (ALT) elevations noted in 42.7% of patients at baseline resolved to rates of 4.7% and 7.4% by 12 and 24 mo. The authors attributed to this to a potential impact on rates of non-alcoholic steatohepatitis.

In an analysis of United States Renal Data System data (1991-2004) by Modanlou *et al*^[52], 188 cases of bariatric surgery were undertaken in renal allograft candidates and recipients. Thirty-day mortality after bariatric surgery was found to be 3.5% in both listed and transplanted patients. An additional 3.5% died 31-90 d postoperatively. Median excess body weight loss was estimated at 31% to 61%. Importantly, the majority of cases involved open Roux-en-Y gastric bypass, and the authors found mortality risks among these patients similar to non-renal populations. Increasing experience with bariatric surgery in the renal population and emergence of less invasive options such as LSG were raised as promising factors bearing potential for future, prospective study.

It is important to note that nutrient deficiencies often emerge following bariatric surgery, whether LRYGB or LSG. In addition to iron, folic acid, vitamin B12, and zinc deficiencies, Vitamin D deficiencies may emerge and contribute to reduced calcium absorption with secondary hyperparathyroidism^[44]. The latter is an important consideration since renal-failure mediated secondary hyperparathyroidism and disturbances in bone and mineral disorders often persists following transplant^[53]. Recently, two cases of enteric oxalate nephropathy in the renal allograft were reported as a complication of fat malabsorption resulting from gastric bypass surgery^[54].

CONCLUSION

The risk of surgical site and soft-tissue complications are increased among obese individuals as compared

to overweight or nonobese (*i.e.*, BMI < 30) recipients, as is the risk of DGF; together, these issues contribute to increased LOS. Patient and graft survival are poorer in underweight BMI recipients (*i.e.*, < 18.5), but the U-shaped survival curves applicable to extremes of BMI may also be applicable to non-transplant populations. Therefore, current studies appear to support a neutral impact of obesity upon long-term graft and patient survival^[36,40]. Increased risk of NODAT appears to be associated with age, BMI, and waist circumference. Measures of central adiposity (waist-to-hip ratio and waist circumference) in non-transplant patients appear to be strong predictors of cardiovascular mortality^[55]. The use of these measures were found to be predictors of NODAT and therefore may be useful (in addition to age, BMI, fasting blood glucose) during pre-transplant evaluation as well as following transplant for risk stratification and intervention. Bariatric surgical procedures are an option but careful patient selection and procedural considerations are warranted. Furthermore, regardless of technique, ongoing assessment for development of nutrient deficiencies is warranted. Extremes of BMI should not constitute contraindications to kidney transplant per se, but individualized risk assessment is necessary. Future areas of research should focus on reducing recognized complications associated with renal transplantation in the setting of obesity – particularly reduction of surgical site complications (*i.e.*, wound infections and lymphocele) and DGF.

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Overview of extended release tacrolimus in solid organ transplantation

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Abstract

Tacrolimus (Prograf[®], Astellas Pharma Europe Ltd, Staines, United Kingdom; referred to as tacrolimus-BID) is an immunosuppressive agent to prevent and treat allograft rejection in kidney transplant recipients in combination with mycophenolate mofetil, corticosteroids,

with or without basiliximab induction. The drug has also been studied in liver, heart and lung transplant; however, these are currently off-label indications. An extended release tacrolimus formulation (Advagraf[®], Astagraf XL[®]) allows for once-daily dosing, with the potential to improve adherence. Extended release tacrolimus has similar absorption, distribution, metabolism and excretion to tacrolimus-BID. Phase I pharmacokinetic trials comparing extended release tacrolimus and tacrolimus-BID have demonstrated a decreased maximum concentration (C_{max}) and delayed time to maximum concentration (t_{max}) with the extended release formulation; however, AUC_{0-24} was comparable between formulations. Overall extended release tacrolimus has a very similar safety and efficacy profile to tacrolimus-BID. It is not recommended in the use of liver transplant patient's due to the increased risk of mortality in female recipients. There has been minimal data regarding the use of extended release tacrolimus in heart and lung transplant recipients. With the current data available for all organ groups the extended release tacrolimus should be dosed in a 1:1 fashion, the exception may be the cystic fibrosis population where their initial dose may need to be higher.

Key words: Tacrolimus; Extended release tacrolimus; Pharmacokinetics; Pharmacoeconomics; Solid-organ transplant

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Core tip: Tacrolimus is an immunosuppressive agent to prevent and treat allograft rejection in solid organ transplant recipients. An extended release tacrolimus formulation known as Astagraf XL is now available which allows for once-daily dosing, with the potential to improve adherence. Both tacrolimus formulations have demonstrated comparable steady-state systemic tacrolimus exposure in *de novo* kidney and liver transplant recipients. The following review will address the pharmacokinetics of extended release tacrolimus,

the data in solid-organ transplantation and the pharmacoeconomic considerations of extended release tacrolimus compared to twice daily tacrolimus.

Patel N, Cook A, Greenhalgh E, Rech MA, Rusinak J, Heinrich L. Overview of extended release tacrolimus in solid organ transplantation. *World J Transplant* 2016; 6(1): 144-154 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/144.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.144>

INTRODUCTION

Tacrolimus (Prograf[®], Astellas Pharma Europe Ltd, Staines, United Kingdom; referred to as tacrolimus-BID) is an immunosuppressive agent to prevent and treat allograft rejection in solid organ transplant recipients in combination with mycophenolate mofetil (MMF), corticosteroids, with or without basiliximab induction. The drug is currently only FDA approved for kidney transplant recipients. The drug has also been studied in liver, heart and lung transplant; however, these are currently off-label indications. An extended release tacrolimus formulation (Advagraf[®], Astagraf XL[®]) allows for once-daily dosing, with the potential to improve adherence. Non-adherence with dosing has been a significant factor related to graft rejection and graft loss. Most patients receive immunosuppressants that require multiple doses a day. Patient compliance has been shown to be correlated with the number of prescribed medications taken daily; therefore, it is beneficial to simplify dosing frequency^[1]. Both tacrolimus formulations have demonstrated comparable steady-state systemic tacrolimus exposure in *de novo* kidney and liver transplant recipients^[2,3]. The following review will address the pharmacokinetics of extended release tacrolimus, the data in solid-organ transplantation and the pharmacoeconomic considerations of extended release tacrolimus compared to tacrolimus-BID^[2,3].

EXTENDED RELEASE TACROLIMUS PHARMACOKINETICS

Tacrolimus-BID is a calcineurin inhibitor which exerts its immunosuppressive effect through inhibition of interleukin-2 expression and subsequent T-lymphocyte activation^[4,5]. It has variable oral absorption and is a substrate of P-glycoprotein with metabolism through cytochrome P4503A enzymes in the liver and small intestine. Studies have demonstrated differences in tacrolimus pharmacokinetics across various ethnic groups with higher doses needed in African American and Latin American recipients^[6,7]. Therapeutic drug monitoring is essential to optimizing outcomes due to its variable bioavailability and narrow therapeutic index^[8]. Trough concentrations (C_{min}) are the standard monitoring parameter due to its correlation with overall

drug exposure (area under the curve from 0-24 h; AUC_{0-24}) and clinical efficacy.

Extended release tacrolimus is a modified release formulation, which utilizes ethylcellulose to prolong the drug release profile in the gastrointestinal tract *via* water permeation^[9]. Extended release tacrolimus has similar absorption, distribution, metabolism and excretion to tacrolimus-BID. Phase I pharmacokinetic trials comparing extended release tacrolimus and tacrolimus-BID have demonstrated a decreased maximum concentration (C_{max}) and delayed time to maximum concentration (t_{max}) with the extended release formulation; however, AUC_{0-24} was comparable between formulations (P values not available)^[4,10,11]. The differences in C_{max} and t_{max} are consistent with a prolonged release formulation. Both formulations demonstrate a diurnal variation with approximately 35% reduction in AUC following the evening dose. Consequently, extended release tacrolimus should be administered in the morning on an empty stomach to optimize absorption. Similar therapeutic trough concentrations may be used for monitoring, as a high and equivalent correlation coefficient was reported between C_{min} and AUC_{0-24} for both formulations (r = not available)^[4,10].

A 6 wk, phase II, multicenter, open-label study compared the pharmacokinetics of extended release tacrolimus and tacrolimus-BID in *de novo* kidney transplant recipients on day 1, day 14, and 6 wk post-transplant (extended release tacrolimus n = 34; tacrolimus-BID n = 32)^[12]. The AUC_{0-24} was approximately 30% lower for extended release tacrolimus on day 1; however, mean AUC_{0-24} was comparable on both day 14 and week 6 (Table 1). Trough concentrations were similar for both formulations by day 4. Similar reductions in initial AUC_{0-24} have been reported in *de novo* transplant recipients, which may necessitate an increased initial dose of extended release tacrolimus^[3,12-15]. There was a strong correlation between AUC_{0-24} and C_{min} for extended release tacrolimus and tacrolimus-BID (r = 0.83 and r = 0.94, respectively; P = not available)^[16].

A randomized, double-blind, phase III trial was subsequently performed to study the effect of pre-transplant initiation of extended release tacrolimus and tacrolimus-BID on the pharmacokinetic profiles in *de novo* kidney transplant (extended release tacrolimus n = 17; tacrolimus-BID n = 17)^[17]. The first dose of tacrolimus was administered within 12 h before reperfusion (day 0). The AUC_{0-24} was approximately 16% lower in the extended release tacrolimus group on day 1 (ratio of means 83.18%, 90%CI: 56.11%-110.25%), but reached comparable AUC_{0-24} to tacrolimus-BID on day 3 (ratio of means 102.2%, 90%CI: 76.21-128.18). The extended release tacrolimus group had a higher AUC_{0-24} compared to tacrolimus-BID on both day 7 (OR = 120.81%; 90%CI: 100.54-141.09) and day 14 post-transplant (OR = 121.24%; 90%CI: 104.29%-138.19%). Therefore,

Table 1 Comparison of pk parameters of tacrolimus administered as extended release tacrolimus and tacrolimus-BID^[12]

PK parameter	Day 1		Day 14		Week 6	
	Extended release tacrolimus (n = 34)	Tacrolimus-BID (n = 32)	Extended release tacrolimus (n = 34)	Tacrolimus-BID (n = 32)	Extended release tacrolimus (n = 34)	Tacrolimus-BID (n = 32)
Mean (SD)						
AUC ₀₋₂₄ (ng · h/mL)	231.91 (102.33)	361.49 (214.65)	363.93 (96.61)	343.69 (105.83)	331.49 (86.82)	382.60 (171.22)
C _{max} (ng/mL)	18.24 (7.63)	34.16 (13.86)	29.87 (9.61)	31.74 (12.62)	26.38 (7.30)	33.04 (13.04)
C _{min} (ng/mL)	8.25 (5.01)	10.12 (6.98)	9.64 (3.25)	10.02 (3.04)	9.60 (2.93)	12.06 (5.91)
T _{max} (h)	4.4 (4.3)	1.7 (1.0)	2.4 (1.2)	1.6 (0.9)	2.4 (1.3)	1.9 (1.3)
Mean daily dose (mg/kg)	0.189	0.185	0.203	0.19	0.175	0.164

Table 2 Equivalence comparison of pharmacokinetic parameters after conversion tacrolimus-BID to extended release tacrolimus^[19]

PK parameter	Extended release tacrolimus (n = 60)	Tacrolimus-BID (n = 60)	Ratio (90%CI) extended release tacrolimus: Tacrolimus-BID
AUC ₀₋₂₄ (ng · h/mL)	217.75	234.42	92.9% (89.9-96.0)
C _{max} (ng/mL)	15.99	21.84	73.2% (67.7-78.7)
C _{min} (ng/mL)	6.60	7.26	90.9% (87.3-94.6)

initiation of extended release tacrolimus prior to transplantation may minimize differences in exposure between formulations in the early post-transplant period. These data support the FDA-approved dosage recommendation for extended release tacrolimus in *de novo* renal transplantation (Table 1)^[9]. Frequent monitoring of trough concentrations should be implemented in order to minimize excessive exposure as evidenced by supratherapeutic concentrations.

Two additional conversion studies from tacrolimus-BID to extended release tacrolimus have demonstrated similar steady-state pharmacokinetics between formulations after a milligram-for-milligram conversion in stable kidney transplant recipients^[18,19]. Both studies used a single sequence, cross-over design with four pharmacokinetic evaluations at steady-state conditions (Table 2). These data support the conversion of tacrolimus-BID to extended release tacrolimus on a 1:1 (mg:mg) total daily dose basis. However, reductions in C_{min} and AUC₀₋₂₄ have been reported following conversion in multiple studies in various solid-organ transplant populations with a dose escalation requirement in up to 50% of recipients^[19-24]. Therefore, close therapeutic drug monitoring is warranted following conversion between formulations.

Regarding special populations, extended release tacrolimus is subject to the same renal and hepatic impairment recommendations as tacrolimus-BID. The mean clearance of tacrolimus in patients with renal dysfunction is similar to that in healthy subjects^[3]. Tacrolimus is not dialyzed to any significant extent due to its poor aqueous solubility and extensive erythrocyte and plasma protein binding. Severe hepatic impairment (mean Child-Pugh score > 10)

necessitates more frequent monitoring of tacrolimus C_{min} due to significant reduction in drug clearance and risk of accumulation. Pertinent pharmacokinetic considerations for non-renal transplant recipients are addressed in the organ-specific section.

KIDNEY TRANSPLANTATION

Extended release tacrolimus is currently only FDA approved for the prophylaxis of rejection in patients that have received a kidney transplant^[9]. One study examined extended release tacrolimus/MMF, compared to tacrolimus-BID/MMF and cyclosporine (CsA)/MMF in *de novo* kidney transplant recipients. This was a phase 3, randomized, open-label, multicenter three-arm noninferiority trial (3 arms: Extended release tacrolimus/MMF n = 214; tacrolimus-BID/MMF n = 212; CsA/MMF n = 212)^[2]. Included patients were ≥ 12 years of age who received a primary or re-transplanted deceased donor or living donor renal transplant, and received the study drug within 48 h of the transplant. Overall 668 patients were randomized and 638 patients received at least one dose and were included in the efficacy and safety analyses. Mean total daily doses were similar between the tacrolimus-BID/MMF and extended release tacrolimus/MMF groups, however slightly more patients in the extended release tacrolimus/MMF group compared to the tacrolimus-BID/MMF group had trough concentrations below target but these differences were not significant and very minimal [above target day 3: Extended release tacrolimus compared to tacrolimus-BID 19% (n = 36), 27.3% (n = 47); month 2: 5.6% (n = 10), 6.7% (n = 11); month 4: 7.5% (n = 13), 4.6% (n = 7); below target day 3: Extended release tacrolimus compared to tacrolimus-BID 30.7% (n = 58), 27.9% (n = 48); month 2: 18.2% (n = 33), 10.15% (n = 17.6); month 4: 10.3% (n = 18), 13.2% (n = 20) respectively]. Efficacy rates in both tacrolimus groups were statistically non-inferior to that in the CsA group. Kaplan-Meier estimates for 1-year patient and graft survival (extended release tacrolimus/MMF 98.6%, 95%CI: -1.6%, 3.6% and 96.7%, 95%CI: -2.7%, 4.6%; tacrolimus-BID/MMF 95.7%, 95%CI: -5.3%, 1.5% and 92.9%, 95%CI: -7.3%, 1.6%; CsA/MMF 97.6% and 95.7%) were similar among the 3 groups.

Incidence of biopsy-proven acute rejection (BPAR) at 6 mo and 1 year was significantly lower in the tacrolimus-BID/MMF group compared to the CsA/MMF group; however, there was no statistical difference between the extended release tacrolimus/MMF and CsA/MMF group. Overall extended release tacrolimus/MMF was noninferior to CsA/MMF and has a similar efficacy and safety profile to tacrolimus-BID/MMF when combined with corticosteroids and basiliximab induction^[2]. In 2014 Silva *et al*^[25] published the 4-year follow-up results to the original study. Mean trough concentrations of extended release tacrolimus and tacrolimus-BID was similar starting at 1 year ranging from 6.5–7.5 ng/mL in extended release tacrolimus and 6.1–7.8 ng/mL in tacrolimus-BID. All groups had similar efficacy reflected by patient and graft survival. In the extended release tacrolimus, tacrolimus-BID, and CsA groups patient survival was 93.8% (95%CI: 90.5%, 97.2%), 93.2% (95%CI: 89.8%, 96.7%) and 92.5% (95%CI: 88.6%, 96.3%) respectively, while graft survival was 88.1% (95%CI: 83.7%, 92.6%), 85.4% (95%CI: 80.5%, 90.4%), and 85.3% (95%CI: 80.3%, 90.4%) respectively. There was a higher rate of graft failure amongst African Americans compared to Caucasians. Graft loss for extended tacrolimus was 11.9% (19/160) in Caucasians and 19.5% (8/41) in African Americans, for tacrolimus-BID it was 10.5% (16/153) in Caucasians and 31.4% (16/51) in African Americans, and for CsA 12.3% (20/163) in Caucasians and 22.2% (8/36) in African Americans but this is consistent with 5-year data from the Scientific Registry of Transplant Recipients^[26]. Overall patient and graft survival rates were high and there was no statistically significant difference amongst groups. Of note this study included a relatively low-risk population and adherence was not evaluated^[25].

In 2010 a phase III multicenter, 1:1 randomized, parallel-group, noninferiority study that compared the efficacy and safety of tacrolimus-BID and extended release tacrolimus when combined with low dose MMF and corticosteroids without antibody induction in *de novo* kidney transplant recipients was published. The study included patients 18–65 years of age receiving a kidney transplant from a donor 5–65 years of age who were ABO compatible^[3]. Patients were excluded if they had received a previous non-renal transplant, panel reactive antibody > 50%, cold ischemic time > 30 h, uncontrolled infection or malignancy. The initial post-operative dose was 0.2 mg/kg per day for both formulations; matching placebo was taken twice daily. Overall 667 patients were randomized (tacrolimus-BID *n* = 336; extended release tacrolimus *n* = 331). The mean daily dose of extended release tacrolimus was higher than tacrolimus-BID at all time points, however whole-blood trough levels were lower in the extended release tacrolimus group at week 1 (12.8 ± 4.8 ng/mL vs 15.3 ± 5.8 ng/mL, *P* < 0.05) but comparable thereafter^[3]. This is consistent with findings from a previous phase II *de novo* study that

showed tacrolimus exposure was lower with extended release tacrolimus than tacrolimus-BID on day 1 but was similar by day 4^[3,16,21]. At 24 wk the BPAR rate was 15.8% vs 20.4% in the tacrolimus-BID and extended release tacrolimus group (*P* = 0.182). There was no correlation with early trough levels and the incidence of BPAR. Kaplan-Meier survival rates were 98.8% for both arms at week 24 and 97.5% and 96.9% at 12 mo for tacrolimus-BID and extended release tacrolimus respectively. Graft survival rates were 94.6% and 93.6% at 24 wk and 92.8% and 91.5% at 12 mo respectively. The incidence of delayed graft function, serum creatinine (SrCr) and creatinine clearance did not differ significantly between the two groups at any time point of the study. Overall this study had similar efficacy and comparable safety profile with tacrolimus-BID and extended release tacrolimus in a regimen that used low dose MMF without antibody induction in *de novo* kidney recipients^[3].

A multicenter, prospective, randomized extension study compared extended release tacrolimus to tacrolimus-BID beyond 6 mo to explore rejection, graft and patient survival^[13]. The initial study was a phase III, randomized, open-label, comparative, multicenter study in *de novo* living donor kidney transplant recipients^[27]. The initial dose of extended release tacrolimus was 0.3 mg/kg daily or 0.15 mg/kg of tacrolimus-BID. The extension of the 6-mo *de novo* study was designed as a 39-mo, single-arm follow-up to evaluate the efficacy and safety of extended release tacrolimus. A total of 124 patients were randomized. The rate of BPAR was similar between groups [19.4% extended release tacrolimus group vs 16.1% in tacrolimus-BID (*P* = 0.638)]. Forty-four patients were enrolled in the 39-mo extension study. One patient in the extended release tacrolimus group experienced BPAR at 29 mo who was treated with pulse steroids and subsequently graft function recovered. During study period 4 recipients (9.1%) were converted back to BID dosing due to skin rash, elevated SrCr without evidence of rejection, study medication prohibited and BPAR. Overall, extended release tacrolimus was shown to be safe and effective for nonsensitized kidney transplant recipients^[27].

Yang *et al*^[28] performed a 24-wk prospective, single-center, open-label, randomized trial to evaluate the safety and efficacy of switching tacrolimus-BID to extended release tacrolimus in stable renal patients. Patients were included if they were > 20 years of age, had received a kidney transplant ≥ 12 mo prior to enrollment and maintained a stable tacrolimus dose at least 12 wk before the start of the study drug. They were excluded if they had a prior organ transplant, acute rejection within the past 12 wk, malignancy after transplant, focal segmental glomerulosclerosis and SrCr > 1.6 mg/dL. Patients were randomized to either tacrolimus-BID or extended release tacrolimus and doses were converted on a 1:1 (mg:mg) basis to determine to total daily dose. Ninety-nine patients

were randomized, 50 in the tacrolimus-BID group and 49 in the extended release tacrolimus group. There were no deaths or graft losses during the study period. Two patients in the extended release tacrolimus group (4.5%) experienced acute rejection and were treated with high dose steroids and their renal function recovered. There was no significant difference in the incidence of acute rejection at week 24 between the 2 groups^[28]. Initially tacrolimus whole-blood concentrations were significantly lower in the extended release tacrolimus group, however were still in the therapeutic range. This is once again consistent with previous pharmacokinetic studies that showed slower absorption of extended release tacrolimus compared to tacrolimus-BID^[29,30]. The rate of compliance was 99.4% in the tacrolimus-BID group and 99.6% in the extended release tacrolimus group. The similarity in compliance amongst groups could be attributed to the small study population and short-term follow-up. Overall the extended release formulation can be considered as an effective alternative to current tacrolimus formulations in stable renal transplant recipients^[28].

The OSAKA trial was a phase III trial that evaluated the non-inferiority of extended release tacrolimus vs tacrolimus-BID in kidney transplantation^[31]. This was one of the largest randomized clinical trials that was conducted in kidney transplant recipients. Patients were randomized to 1 of 4 groups: Tacrolimus-BID 0.2 mg/kg per day (arm 1); extended release tacrolimus 0.2 mg/kg per day (arm 2); extended release tacrolimus 0.3 mg/kg per day (arm 3); extended release tacrolimus 0.2 mg/kg per day + basiliximab + corticosteroid bolus (arm 4) and 1214 patients received at least one dose of study drug. Extended release tacrolimus 0.3 mg/kg per day had higher trough concentrations on day 1 and 7 however, by day 14 they were similar across the board. Non-inferiority was established for efficacy failure rates between arms 1 and 2. Non-inferiority of efficacy failure between arm 3 and 1 was not established, nor was it between arms 4 and 1. The main reason for efficacy failure in all arms was graft dysfunction at week 24. The number of patients that experienced BPAR was 13.6% (42/309) in arm 1, 10.3% (31/302) in arm 2, 16.1% (49/304) in arm 3, and 12.7% (36/283) in arm 4. Overall, the efficacy of extended release tacrolimus dosing of 0.2 mg/kg per day was non-inferior to tacrolimus-BID dosing based on the same initial dosing without induction. Increasing the starting dose to 0.3 mg/kg per day did not increase efficacy; therefore, 0.2 mg/kg per day was an adequate starting dose^[31].

LIVER TRANSPLANTATION

There are several studies evaluating the pharmacokinetics, safety, and efficacy of extended release tacrolimus in liver transplant recipients. However, extended release tacrolimus is currently not FDA-approved for use in the liver transplant setting due to

an increased mortality rate in female liver transplant recipients in a *post-hoc* analysis^[9].

The first long-term liver transplant trial with extended release tacrolimus was a multicenter, randomized, double-blind, phase III study comparing the efficacy and safety of extended release tacrolimus to tacrolimus-BID^[13]. The duration of the study was 24 wk followed by an extension period to 12 mo post-transplant. The extended release tacrolimus arm ($n = 237$) received initial dose of 0.2 mg/kg per day, while the tacrolimus-BID ($n = 234$) received 0.05 mg/kg per dose given twice daily. The extended release tacrolimus arm was given a higher initial dose due to lower tacrolimus levels seen in the first few days post-transplant in a previous pharmacokinetic study^[19]. Both groups were subsequently adjusted to maintain goal trough concentrations. The primary endpoint was the rate of BPAR within 24 wk post-transplant, with an incidence of 36.3% in the extended release tacrolimus group and 33.7% in the tacrolimus-BID group ($P = 0.512$)^[13]. Furthermore, at 12 mo the extended release tacrolimus group and tacrolimus-BID group had a similar patient survival rate (89.2% and 90.8%, respectively $P = 0.535$) and graft survival rate (85.3% and 85.6%, respectively $P = 0.876$). There were no clinically relevant differences in the causes of death between the two treatment groups. In a *post-hoc* analysis, a higher mortality rate was observed in the female recipients compared with the male recipients receiving extended release tacrolimus (18.4% vs 6.8%, $P = 0.026$). There is currently no explanation for this difference in mortality. Consequently, extended release tacrolimus is not approved for use in liver transplant recipients.

The DIAMOND Study is a multicenter, 24-wk, randomized, open-label trial studying the effects of different extended release tacrolimus dosing regimens on renal function in *de novo* liver transplant recipients^[32]. There were 3 treatment arms: Arm 1 (extended release tacrolimus 0.2 mg/kg per day, $n = 295$), arm 2 (extended release tacrolimus 0.15-0.175 mg/kg per day + basiliximab, $n = 286$), or arm 3 (extended release tacrolimus 0.2 mg/kg per day delayed until Day 5 + basiliximab, $n = 276$). Estimated glomerular filtration rate (eGFR) using the four-variable Modified Diet in Renal Disease equation was significantly higher in arms 2 and 3 compared to arm 1 ($P = 0.001$ and $P = 0.047$, respectively). Additionally, there was significantly less BPAR in arm 2 compared to arms 1 and 3 ($P = 0.016$, $P = 0.039$, respectively). Overall, there were similar estimates of composite failure-free survival in arms 1-3 (72.0%, 77.6%, 73.9%, respectively, $P = 0.065$, $P = 0.726$, $P = 0.161$) and no significant difference in mortality between males and females receiving extended release tacrolimus.

A retrospective analysis of the European Liver Transplant Registry was performed to investigate long-term outcomes with extended release tacrolimus compared to tacrolimus-BID (extended release tacrolimus $n = 528$, tacrolimus-BID $n = 3839$)^[33]. Propensity

score-matched analyses were performed to minimize bias associated with differences in donor and recipient baseline characteristics. The registry data showed a significant improvement in patient and allograft survival over 3 years in patients receiving extended release tacrolimus ($P = 0.004$ and $P = 0.001$, respectively). Given the limitations of registry analysis, additional studies are needed to further validate these long-term findings.

Several prospective, observational studies have investigated the safety and efficacy of conversion from extended release tacrolimus to tacrolimus-BID in stable liver transplant recipients^[21,33-35]. All studies have shown comparable patient and allograft survival with no difference in incidence of BPAR or adverse effects. Beckebaum *et al.*^[34] also found a statistically significant reduction in nonadherence from 66% at study entry to 30.9% at 12 mo post-conversion from tacrolimus-BID to extended release tacrolimus using the "Basel Assessment of Adherence Scale to Immunosuppressives" ($P < 0.001$). The improved adherence to immunosuppression and decreased intra-subject variability in drug exposure may potentially translate into improved long-term patient and allograft survival.

Regarding extended release tacrolimus pharmacokinetics in the liver transplant population, once daily dosing has an overall similar systemic exposure as compared to the standard tacrolimus-BID regimen^[9,21,34-37]. Given the strong correlation between AUC_{0-24} and trough concentrations for extended release tacrolimus, the same therapeutic monitoring and target trough concentration range can be used for both formulations.

However, in the *de novo* liver transplant setting, systemic exposure (AUC_{0-24}) was 50% lower in extended release tacrolimus compared to equivalent doses of tacrolimus-BID. Similar trough levels between the two formulations were obtained by day 4 after implementation of dose adjustments. Consequently, initial doses for extended release tacrolimus may need to be slightly higher than tacrolimus-BID to achieve similar tacrolimus trough blood concentrations in *de novo* liver transplant recipients. The pharmacokinetic studies in stable liver transplant recipients have demonstrated a safe 1:1 daily dose conversion from tacrolimus-BID to extended release tacrolimus with close monitoring of trough concentrations^[21,34,35].

In summary, extended release tacrolimus has proven to be well tolerated with a similar safety and efficacy profile as compared to tacrolimus-BID. Extended release tacrolimus is not FDA approved for use in liver transplant recipients due to increased mortality rate in females in a *post-hoc* analysis. While the increased mortality is a concern, this finding has not been replicated in follow-up clinical trials or registry data. Extended-release tacrolimus may be particularly beneficial in improving immunosuppression compliance and subsequently long-term outcomes in

the liver transplant population, as many recipients are maintained on tacrolimus monotherapy.

HEART TRANSPLANTATION

Limited published data exists investigating the use of extended release tacrolimus in both *de novo* and established patients with heart transplants. Therefore, extended release tacrolimus is not approved for the prophylaxis of rejection in heart transplant patients in the United States or Europe^[9].

A phase II pharmacokinetic study was performed in patients that were at least 6 mo post heart transplant and were receiving tacrolimus-BID with stable levels between 5-15 ng/mL. Patients continued tacrolimus-BID study days 1-7 and were transitioned to extended release tacrolimus at 1:1 mg/d for days 8-35 of the study. Of the 85 patients enrolled, only 45 patients had complete 24 h pharmacokinetic data collected in the tacrolimus-BID and extended release tacrolimus phase necessary for analysis. The primary endpoint of the study was the comparison of the systemic exposure (AUC_{0-24}) at steady state of tacrolimus-BID to extended release tacrolimus, with a predefined acceptance range for a 90%CI of 80%-125%. The AUC_{0-24} was 219.77 ng·h/mL for extended release tacrolimus compared to 242.86 ng·h/mL for tacrolimus-BID, with a 90%CI of 86.4%-94.6%, falling within the predefined acceptable range. The AUC_{0-24} and C_{min} correlated well for both tacrolimus XL ($r = 0.94$) and tacrolimus BID ($r = 0.91$). During the study, 32.9% of the overall patients enrolled needed a dose adjustment after conversion to extended release tacrolimus. A dose increase was needed in 25.9% of patients, and 6.2% of patients required a dose decrease. No adverse events led to discontinuation during the study, and there were no reports of acute rejection, graft loss, or death. This pharmacokinetic evaluation suggests that overall exposure to tacrolimus is lower with the extended release product, with comparable correlation between trough levels and AUC_{0-24} as with tacrolimus-BID^[22].

Patients enrolled in the phase II pharmacokinetic study were given the option of continuing extended release tacrolimus in a long-term extension study. Of the 85 patients enrolled in the pharmacokinetic study, 79 patients chose to take part in the extension study that included heart, kidney, and liver transplant patients. The primary endpoint of the study was patient and graft survival, with the secondary endpoints of BPAR and safety events. Survival at four years was 92.5% in the heart transplant arm, with graft survival rate being 92.2%. Patients free from BPAR were 87% at four years. The primary reasons for study withdrawal were withdrawn consent or non-adherence to study schedule. Renal function as reflected by mean serum creatinine and creatinine clearance rates were stable across the four year study. Authors concluded that the adverse event rates seen in the study were similar to that of reported rates with tacrolimus-BID, suggesting

that extended release tacrolimus may be considered an alternative to conventionally dosed tacrolimus^[36].

As previously discussed in the article, package insert data for extended release tacrolimus suggests that patients be converted to the once daily product from tacrolimus-BID in a 1:1 ratio based on total mg/d dosing. A study of 75 heart transplant recipients were converted to extended release tacrolimus at a 25% increased dose from the tacrolimus-BID total daily dose. The retrospective analysis followed patients for 3 mo and included patients that were 61.7 ± 48.5 mo from transplant, with therapeutic troughs defined as 10-15 ng/mL within the first year following heart transplant, and 5-15 ng/mL thereafter. Two of the 75 patients (2.7%) failed to achieve therapeutic levels despite dose increases, and therefore discontinued extended release tacrolimus. Twenty-three patients (31%) required no dose adjustment following conversion, and 51 patients (68%) required one or two dose adjustments. Three patients experienced BPAR during the study period without hemodynamic compromise. Although the authors state that there were no differences in reports of glycemic control, serum creatinine, lipids, or blood pressure from pre-conversion values, these rates and values are not included in the publication. This suggests an alternative approach to conversion from conventionally dosed tacrolimus-BID to extended release tacrolimus in heart transplant recipients. The need for close monitoring of trough levels following conversion is also highlighted as 2.7% of patients were unable to achieve therapeutic levels^[38].

More recently, two studies evaluated the use of extended release tacrolimus in comparison to tacrolimus-BID in *de novo* heart transplant patients. The first followed 11 patients converted to extended release tacrolimus on post-operative day 14 from CsA, with an initial extended release tacrolimus dose of 6 mg/d. These patients were case matched to 11 patients managed with tacrolimus BID at an initial dose of 3 mg-BID. Target tacrolimus troughs in both groups were 5-8 ng/mL. Patients were followed for 36 mo with a primary composite endpoint of death, graft loss, and drug discontinuation, which occurred less often in the extended release tacrolimus arm (18.2% vs 45.54%, $P = 0.277$). Survival at three years was greater for extended release tacrolimus (90% vs 77.9%, $P = 0.291$) and more patients remained on the prescribed therapy in the extended release tacrolimus arm (90.9% vs 77.9%, $P = 0.533$). The occurrence of secondary endpoints including BPAR, malignancy, infection, and safety events did not differ between groups. The total daily dose required to achieve therapeutic trough levels was higher in the extended release tacrolimus arm (numeric values not reported). Although the safety and efficacy from this small study suggest the feasibility of extended release tacrolimus in *de novo* heart transplant recipients, the dosing strategies used to manage these patients in order to

achieve therapeutic trough levels may require further investigation^[39].

The second study evaluating extended release tacrolimus in *de novo* heart transplants randomized 19 patients, 8 to open label extended release tacrolimus and 11 to open label tacrolimus-BID. Both groups started the calcineurin inhibitor therapy on post-operative day four. Patients in the extended release tacrolimus group received initial doses of 0.5 mg/20 kg per day, with tacrolimus-BID patients receiving 0.5 mg/20 kg per dose, dosed twice daily. Initial trough targets were 8-15 ng/mL. Patients were followed for an average of 290 ± 92 d for BPAR, incidence of renal insufficiency, new hypertension, and new onset diabetes. There were no differences between the two groups for any staging of rejection throughout the follow-up period. Although total daily doses between the extended release tacrolimus group and the tacrolimus-BID group did not differ at eight and thirty days, the total daily dose of extended release tacrolimus was significantly lower than tacrolimus-BID at six months (3 ± 1 mg/d vs 6 ± 2 mg/d, $P < 0.05$). There was no difference between groups in the rate of treated hypertension or diabetes. Although a low number of patients were included in this study, this prospective analysis suggests that patients managed with extended release tacrolimus for *de novo* heart transplant may have similar efficacy and safety outcomes^[40].

The published data supporting the use of extended release tacrolimus in heart transplant recipients is limited, yet current evidence does not signal that the therapy is associated with worse efficacy or safety outcomes when compared to tacrolimus-BID. Additionally, a small study of 72 patients suggests that use of extended release tacrolimus as compared to previous regimens of tacrolimus-BID or CsA decreased rates of patient reported non-adherence measures at eight months^[41]. Further studies evaluating the use of extended release tacrolimus in heart transplant recipients is needed to define the role of the extended release product in this patient population.

LUNG TRANSPLANTATION

To date, only 2 studies evaluating extended release tacrolimus have been performed in lung transplant recipients. The studies are not outcomes based, only pharmacokinetic in nature assessing the potential for use in stable lung transplant recipients. Therefore, extended release tacrolimus is not FDA approved for the use in *de novo* lung transplantation^[9].

The first study evaluated the conversion of tacrolimus-BID to extended release tacrolimus in 19 stable lung transplant recipients. This was a phase II, open-label, single center, single arm, prospective trial. The primary outcome was a pharmacokinetic comparison of tacrolimus-BID to extended release tacrolimus on a 1:1 basis through analyzing AUC₀₋₂₄ on

both dosing regimens. Secondly, episodes of acute cellular rejection (ACR) at 6 mo and any other adverse events throughout the trial period were assessed. All patients were at least 180 d post transplantation and had stable trough levels of tacrolimus-BID ranging from 5-15 ng/mL upon entering the study. Notably, patients with cystic fibrosis (CF) or with ongoing ACR, recent ACR, or chronic rejection were excluded. All patients were receiving tacrolimus, an antimetabolite (MMF or azathioprine), and corticosteroids^[31]. Patients were converted on a 1:1 (mg:mg) basis from tacrolimus-BID to extended release tacrolimus after being stable for 30 d on tacrolimus-BID. Doses were adjusted as needed on extended release tacrolimus to maintain the previous goal concentrations of 5-15 ng/mL. Two 24 h PK curves were created: one on tacrolimus-BID and the other on extended release tacrolimus. The AUC₀₋₂₄, C_{min}, and T_{max} were then compared^[42].

The results of this trial demonstrated the mean AUC₀₋₂₄ (SD) of tacrolimus-BID was 279.8 (57.7) ng/mL per hour compared to 278.7 (52.5) ng/mL per hour for extended release tacrolimus ($P = 0.92$). No statistically significant differences were noted between the C_{max}0-24 and C_{min}0-24. The time to maximum concentrations did differ between tacrolimus-BID and extended release tacrolimus, 1.5 h vs 3 h, respectively. The AUC₀₋₂₄ and C_{min} correlated well for both products. It was noted that the mean tacrolimus-BID dose (before switching) was 4.8 ± 2.2 mg. After switching to extended release tacrolimus, the mean dose increased to 5.2 ± 2.6 on day 60, 5.4 ± 3.0 mg on day 90, and 5.6 ± 3.1 on day 180^[42].

After 6 mo, 8 patients were on the same total dose, 4 patients required a 1 mg reduction, 4 patients required a 1 mg increase, and 3 patients required more than a 1 mg increase. Throughout the study period, 4 severe adverse events occurred (lithiasic pyelonephritis, urinary sepsis, acute cholecystitis, stroke). These were not considered related to extended release tacrolimus. There were no episodes of ACR. This trial demonstrated that converting patients from tacrolimus-BID to extended release tacrolimus on a 1:1 basis provides virtually identical drug exposure when analyzed by the AUC₀₋₂₄ in the lung transplant population; however, long term outcomes are lacking^[42].

The second trial was a pharmacokinetic study. However, it included only patients with CF, who were notably excluded in the previous trial. Overall, 12 adult CF patients (7 men, 5 women) were enrolled. All patients were on a stable dose of tacrolimus BID upon entering the trial for at least 4 wk. After conversion to extended release tacrolimus on a 1:1 basis, doses were once again titrated to achieve a therapeutic trough of 10-15 ng/mL^[43].

Nine (82%) of the patients required a significant dose adjustment after conversion to extended release tacrolimus. Percentage increases ranged from 28%-66.7%. The mean (SD) daily dose of tacrolimus-BID upon enrollment was 0.17 (0.10) mg/kg per day

and this increased to 0.22 (0.12) mg/kg per day after switching to extended release tacrolimus. The mean (SD) AUC₀₋₂₄ for tacrolimus BID was 414.28 (159.43) ng · h/mL vs 388.88 (104.05) ng · h/mL for extended release tacrolimus after switching^[32]. During the study and follow up no episodes of ACR were noted. This trial demonstrated that extended release tacrolimus is a possible alternative in CF patients, however, on average they need a 28% increase in dose and the range of the increase can be up to 67%. This is in contrast with the previous study of non-CF lung transplant recipients who can safely be converted on a 1:1 basis. Long term data is still needed in CF as well with extended release tacrolimus^[43].

PHARMACOKINETIC CONSIDERATION

The effect of medication adherence to immunosuppressive therapies on risk of acute rejection and graft loss is well documented and has significant impact on graft survival^[44]. A 2004 meta-analysis evaluated the frequency of and effect of immunosuppressive non-adherence in renal transplant recipients and found non-adherent patients were 7.1 times more likely to experience graft failure than adherent patients^[34]. The most common types of nonadherence seen in the meta-analysis was missing, forgetting, or altering a dose at least once per month. A 2012 study conducted in France demonstrated an inverse relationship between the number of immunosuppressant medications and the proportion of patients with high adherence to the medications^[45]. Additional predictors of non-adherence were dosing frequency and medication regimen complexity.

Additional studies have found a link between high medication-possession ratio and lower risk of graft failure^[46]. Persistent non-compliance has been associated with increased immunosuppression and non-immunosuppression costs with persistently non-compliant patients experiencing 3-year medical costs of approximately \$33000 more than patients with excellent compliance^[36].

A 2014 study of renal transplant patients in the United Kingdom examined the budgetary impact of switching from tacrolimus-BID to extended release tacrolimus using a budget-impact model^[44]. The model assumed that patients were taking a tacrolimus dose of 0.075 mg/kg per day 1 year post-transplant and that patients were taking concurrent MMF and corticosteroids based on a 2010 study^[3]. Adherence rates were modeled after two studies, the first of which found that 88.2% of patients on extended release tacrolimus were adherent compared to 78.8% on tacrolimus-BID ($P = 0.0009$). The second study found that 11.8% of extended release tacrolimus patients were non-adherent, compared to 21.2% of tacrolimus-BID patients and that the risk of graft failure is 7.1-fold higher in non-adherent patients than in adherent patients^[46]. The model assumed that all

patients with graft failure were started on dialysis (15% peritoneal dialysis and 85% hemodialysis). Pharmacy costs were derived from the British National Formulary and dialysis costs were taken from the National Health Service tariff information.

The base-case analysis, which assumed maximum relative risk of graft failure with non-adherence found that the average cost for patients taking extended release tacrolimus was £29328 (approximately \$45750 based on a current exchange rate of 1.56) over 5 years compared to £33061 (\$51575) for patients taking tacrolimus-BID for a savings of £3733 (\$5825) per patient over 5 years. The cost savings related to extended release tacrolimus were primarily driven by lower projected rates of graft failure in this group (21.6% for tacrolimus-BID vs 18.3% for extended release tacrolimus). Decreased rates of graft failure were driven by higher adherence rates in this group (88.2% for extended release tacrolimus vs 78.8% for tacrolimus-BID). Of note, the cost of tacrolimus in the United Kingdom study was £12910 (\$20139) for extended release tacrolimus to £14467 (\$22568) for tacrolimus-BID over 5 year which amounts to a savings of £1557 (\$2430) on direct medication cost. In the United States, the per milligram price of extended release tacrolimus is approximately twice that of tacrolimus-BID and may vary depending on wholesaler price and institutional contract, which may vary significantly from institution to institution in the United States. Pharmacy cost data was derived from the British National Formulary in the United Kingdom study^[11]. Obvious differences between the United States healthcare system and the single-payer system in the United Kingdom may also limit the applicability of this analysis in the United States.

Based on the findings of the United Kingdom study, use of extended release tacrolimus may result in significant savings over 5 years when compared to immediate tacrolimus-BID. It is important to consider that these findings are predicated upon the assumption that once-daily dosing improves adherence and that improved adherence reduces the incidence of graft failure^[47].

CONCLUSION

Overall extended release tacrolimus has a very similar safety and efficacy profile to tacrolimus-BID. It is currently approved to prevent rejection in kidney transplant recipients. It is however, not recommended in the used of liver transplant patient's due to the increased risk of mortality in female recipients. There has been minimal data regarding the use of extended release tacrolimus in heart and lung transplant recipients. Currently there is no data for the use of extended release tacrolimus in multiple organ transplants, pancreas or small bowel, this is an area where further studies need to be conducted. With the current data available for all organ groups the extended release

tacrolimus should be dosed in a 1:1 fashion, the exception may be the CF population where their initial dose may need to be higher. Another important note in regards to extended release tacrolimus is that data has shown that extended release tacrolimus exposure was lower than tacrolimus-BID within the first week of transplant, however after that exposure was similar.

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Donor to recipient sizing in thoracic organ transplantation

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Abstract

Donor-to-recipient organ size matching is a critical aspect of thoracic transplantation. In the United States potential recipients for lung transplant and heart transplant are listed with limitations on donor height and weight ranges, respectively. Height is used as a surrogate for lung size and weight is used as a surrogate for heart size. While these measures are important predictors of organ size, they are crude surrogates that fail to incorporate the influence of sex on organ size. Independent of other measures, a man's thoracic organs are approximately 20% larger than a woman's. Lung size can be better estimated using the predicted total lung capacity, which is derived from regression equations correcting for height, sex and age. Similarly, heart size can be better estimated using the predicted heart mass, which adjusts for sex, age, height, and weight. These refined organ sizing measures perform better than current sizing practice for the prediction of outcomes after transplantation, and largely explain the outcome differences observed after sex-mismatch transplantation. An undersized allograft is associated with worse outcomes. In this review we examine current data pertaining to size-matching in thoracic transplantation. We advocate for a change in the thoracic allocation mechanism from a height-or-weight-based strategy to a size-matching process that utilizes refined estimates of organ size. We believe that a size-matching approach based on refined estimates of organ size would optimize outcomes in thoracic transplantation without restricting or precluding patients from thoracic transplantation.

Key words: Lung transplant; Heart transplant; Organ size; Size mismatch; Organ allocation

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Core tip: Recipients for lung transplant and heart transplant are listed with acceptable donor height and weight ranges as surrogates for organ size, respectively. While these measures are important predictors of organ size, they are crude surrogates that fail to incorporate the influence of sex on organ size. Lung size can be better estimated using the predicted total lung capacity (derived from height, sex and age). Similarly, heart size can be better estimated using the predicted heart mass (derived from sex, age, height, and weight). These refined organ sizing-measures perform better than current sizing practice for the prediction of outcomes after transplantation.

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INTRODUCTION

Donor-to-recipient size matching is a critical issue in thoracic organ transplantation^[1-7]. This topic garnered particular attention in June 2013, when a 10-year-old Pennsylvania girl with severe lung damage from cystic fibrosis needed a lung transplant (LTx)^[8]. Sarah Murnaghan was not permitted equal access to adult donor lungs because of an age restriction^[8]. Children younger than 12 years were not eligible to primarily receive adult lungs, mainly because of lung size mismatch concerns^[8].

In the United States height is used as a surrogate for lung size, and potential recipients for LTx are listed with acceptable donor height ranges^[1,9]. In heart transplantation body-weight is used as a surrogate for heart size, and recipients for HTx are listed for acceptable donor body-weight ranges^[1]. Donors falling outside the specified ranges are excluded automatically in the computerized match run process. Increasingly, evidence indicates the presence of considerable preventable pre- and post-LTx morbidity and mortality attributable to donor-recipient organ size differences that are occult in the current system due to reliance upon height or weight alone as a surrogate for organ size^[1-7,10,11]. In this review we advocate for a change in the thoracic allocation mechanism from a height-or-weight-based strategy to a size-matching process that utilizes refined estimates of organ size. We believe that a size-matching approach based on refined estimates of organ size would optimize outcomes in thoracic transplantation without restricting or precluding patients from thoracic transplantation.

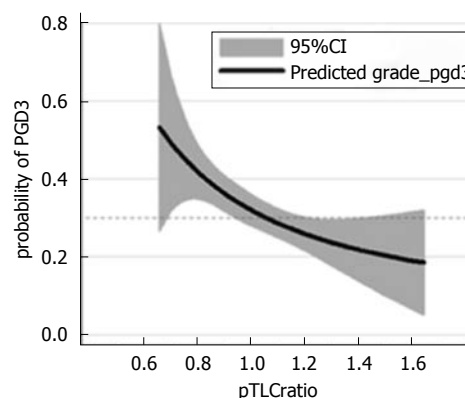


Figure 1 Lung size mismatch (the donor to recipient predicted total lung capacity ratio) is associated with the probability of primary graft dysfunction grade 3. The relationship of pTLCratio (pTLCdonor/pTLCrecipient) and predicted probability of any grade PGD grade 3 within 72 h is shown using a fractional polynomial fit with 95% CIs (gray area). Adapted with permission from Eberlein *et al*^[14]. pTLC: Predicted total lung capacity; PGD: Primary graft dysfunction.

LUNG TRANSPLANT OUTCOMES ASSOCIATED WITH SIZE-MATCHING

Primary graft dysfunction

The most prevalent complication observed immediately following LTx is primary graft dysfunction (PGD)^[12]. PGD presents with diffuse pulmonary infiltrates and hypoxia within 72 h of transplantation. PGD clinically mirrors the acute respiratory distress syndrome (ARDS) and histologic examination also shows diffuse alveolar damage, as in ARDS^[12]. Severe PGD is the primary risk factor for early mortality after LTx, and survivors of PGD are predisposed to the development of chronic rejection (bronchiolitis obliterans), which is the main barrier to long-term survival^[13]. Donor-to-recipient lung size mismatch (assessed by the donor-to-recipient predicted total lung capacity (pTLC), as a refined estimate of organ size) modulates the risk for PGD^[3,14]. In a study ancillary to the LTx outcome group (LTOG), we found that an undersized allograft was associated with a significantly increased risk of severe PGD after bilateral LTx, Figure 1^[14].

The mechanisms responsible for this association are likely multiple, but we have hypothesized that the impact of lung size mismatch on mechanical ventilation tidal volumes in the early post-LTx period could be an important factor^[14,15]. Conceptually, this is analogous to high-tidal volume ventilation when considered in terms of donor organ size^[16,17]. During the period of post-LTx mechanical ventilation hyperinflation of undersized allografts (*i.e.*, donor lungs smaller than recipient thorax) has been reported and has been linked to an increased risk of early allograft failure^[18]. In another study of early outcomes undersized allografts similarly were associated with worse outcomes, specifically increased rates of PGD, tracheostomy, and resource

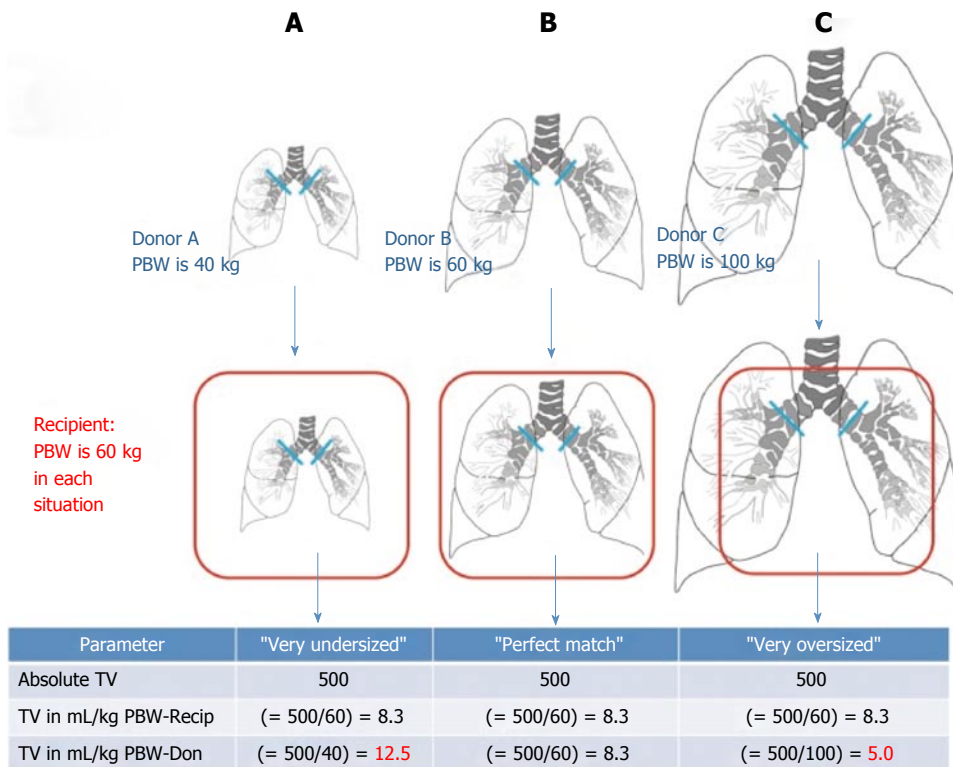


Figure 2 Conceptual graphic on the possible effect of lung size mismatch on mechanical ventilation tidal volumes expressed as mL/kg predicted body weights of the donor. Reproduced with permission from Dezuze *et al.*^[15]. Recip recipient, Don donor. PBW: Predicted body weight; TV: Tidal volume.

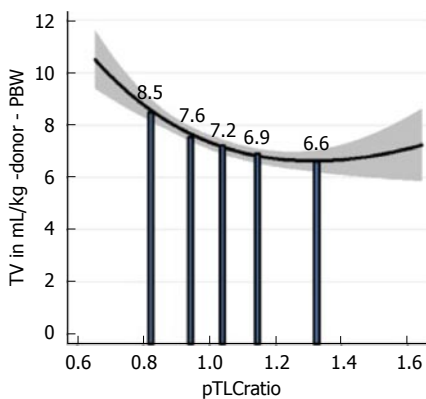


Figure 3 Lung size mismatch (predicted total lung capacity ratio) is associated with the mechanical ventilation tidal volumes at reperfusion, when the tidal volumes is related to the size of the allograft. Fractional polynomial regression of the TV in mL/kg donor-predicted body weight (PBW) plotted against the pTLCratio (pTLCdonor/pTLCrecipient). The solid vertical bars represent the mean values of the TV in mL/kg donor-PBW according to pTLCratio-quintiles. Adapted with permission from Eberlein *et al.*^[14]. TV: Tidal volumes; pTLC: Predicted total lung capacity.

utilization^[3]. Hyperinflation of significantly undersized allografts by tidal volumes set according to recipient characteristics could increase the risk of ventilator induced lung injury (VILI)^[16,17,19,20].

Several lines of evidence confirm differences in ventilator management when considered in terms of donor size. In a survey of the international LTx community, the majority of respondents reported using lung-protective mechanical ventilation after

LTx, primarily consisting of low tidal volume (TV) ventilation^[21]. Low TVs based on recipient characteristics were frequently chosen^[21]. Donor characteristics usually were not taken into consideration and frequently were not even known by the team managing the ventilator after LTx^[21]. The relationship between donor-recipient lung size mismatch and postoperative mechanical ventilation TVs was evaluated in a cohort of bilateral LTx patients, Figure 2^[15]. TV-settings were expressed as absolute values (in milliliter) and also as fractions of recipient and donor predicted body weight (PBW). Absolute TVs were comparable between subsets of patients with undersized, matched, and oversized allografts. TV-settings according to recipient-PBW were also similar. However, TV-settings according to donor-PBW were significantly different between undersized, matched, and oversized groups (11.4 ± 3.1 mL/kg-DONOR-PBW vs 9.4 ± 1.2 mL/kg-DONOR-PBW vs 8.1 ± 2.1 mL/kg-DONOR-PBW, respectively; $P < 0.05$)^[15]. Thus, during mechanical ventilation after bilateral LTx, patients with undersized allografts received significantly higher TVs compared to those with oversized allografts when TV was considered in terms of donor-PBW (as an estimate of the actual allograft size). This observation was replicated in an ancillary study to the multicenter LTOG study, Figure 3^[14].

Thus, using a refined estimate of organ size (pTLC) identified an undersized lung allograft as a risk factor for severe PGD. These data suggest that a lung-protective mechanical ventilation strategy based on

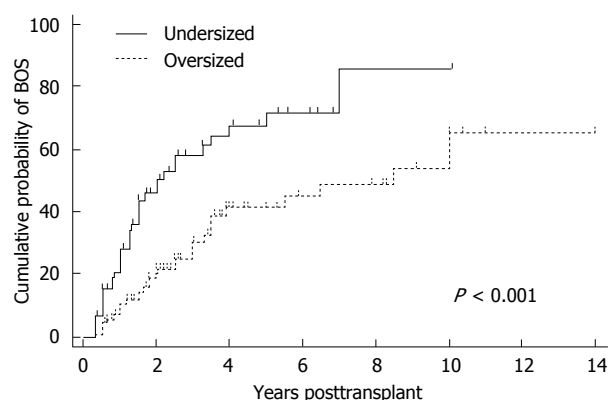


Figure 4 Kaplan Meier estimates of proportion of patients with bronchiolitis obliterans syndrome stratified by recipients of undersized or oversized donor lungs. Oversized was defined as a donor to recipient predicted total lung capacity (pTLC) ratio > 1.0 and undersized as pTLCratio ≤ 1.0 . Comparison between over- and undersized cohorts was via log-rank test. Adapted with permission from Eberlein *et al*^[6]. BOS: Bronchiolitis obliterans syndrome.

estimates of the allograft size (*i.e.*, donor-PBW) could lower the risk of PGD, especially for recipients of undersized allografts.

Airway complications

Airway complications (ACs) frequently require multiple invasive interventions and are an important cause of post-LTx morbidity^[22]. In a single center study we observed that undersized allografts were associated with a higher incidence and severity of ACs^[3]. The association between lung size mismatch and ACs suggests that a mismatch in donor-recipient airway sizes could be a risk factor for ACs. Two other studies reported findings that support the hypothesis that donor-to-recipient airway size mismatch is a risk factor for ACs. The first reported that taller recipients generally experience more frequent ACs^[23]. This was attributed to a larger recipient bronchial circumference and not to size mismatch, although neither height nor pTLC mismatch were directly evaluated in that study. The second, a large cohort study from the Cleveland Clinic transplant program, reported that in the setting of a donor-to-recipient size mismatch, obstructive ACs occurred more frequently^[24]. Similar to lung size, sex determines airway structure independent of height^[25,26]. Thus, while the pTLCratio would better capture donor-recipient lung size mismatch it may yet still underestimate the differences in airway size associated with a sex mismatch. Women tend to have smaller airway diameters than men, even when lung size is the same^[25,26]. This effect would not be fully captured in the pTLCratio, which would also not capture the effect of dysanapsis (interindividual differences in airway size in relation to lung size). Computed tomography airway dimension analysis would allow an assessment of the actual airway size mismatch between recipient and donor, but may prove more cumbersome than matching by pTLC.

Bronchiolitis obliterans

Bronchiolitis obliterans (BO) is a disease that primarily affects small airways and is characterized by progressive obstruction and subsequent loss of small airways^[27]. Bronchiolitis obliterans syndrome (BOS) is a standardized term for the clinical presentation in the absence of pathologic confirmation of BO^[27]. BOS represents the main cause of long-term mortality after LTx^[27].

Undersized allografts have been associated with an increased incidence of BOS, Figure 4^[5]. The mechanisms for this association are not clearly elucidated, but it is known that multiple lung immune and non-immune mediated injuries to the small airways are risk factors for BOS. In injured small airways, repetitive opening and closing is associated with accelerated airway epithelial cell damage, inflammation, and ultimately fibrosis.

Chest wall strapping (CWS) is a procedure that involves restricting the thorax and abdomen, forcing the subject to breathe at low lung volumes^[28]. It has been utilized to understand basic mechanisms of pulmonary physiology. CWS is conceptually similar to a mismatch between significantly oversized donor lungs transplanted into a recipient with a smaller chest cavity^[28]. CWS increases lung elastic recoil, reduces pulmonary compliance, and substantially increases maximal expiratory flows^[28]. The interactions between elastic properties of the lung parenchyma and small airways are critical for pulmonary function. CWS reduces the functional residual capacity (FRC) and leads to breathing closer to the residual volume (RV)^[28]. This is similar to observations made in donor oversizing^[11,28].

The FRC of a LTx recipient is determined by both the recipient's chest wall mechanics and the properties of the donor lung^[5,11]. A patient given an oversized allograft will likely have an FRC that is lower than the donor's FRC because of the mechanics of the relatively smaller recipient thorax, analogous to the physiology of CWS^[5,11,28]. In adults, absolute RV is determined by intrinsic characteristics of the lung (airway closure), rather than the chest wall. Thus the RV of an oversized allograft is likely large relative to the recipient's thorax. As a consequence, a patient with an oversized allograft will likely breathe at relatively low lung volumes that are closer to the RV of the allograft [that is, the expiratory reserve volume (ERV) is reduced]. This concept was evidenced in a cohort of recipients of oversized lungs in whom the pulmonary function pattern resembled that of CWS^[11]. In another group of bilateral LTx patients, an oversized allograft was, again similar to CWS, associated with higher expiratory airflows, higher FEV1/FVC-ratios, and higher flow-volume-loop slope estimates^[5]. To evaluate the physiology of the transplanted lung it is helpful to consider post-LTx allograft function in relation to donor predicted function^[5]. When flow-volume loops are analyzed in this way, oversized allografts resemble

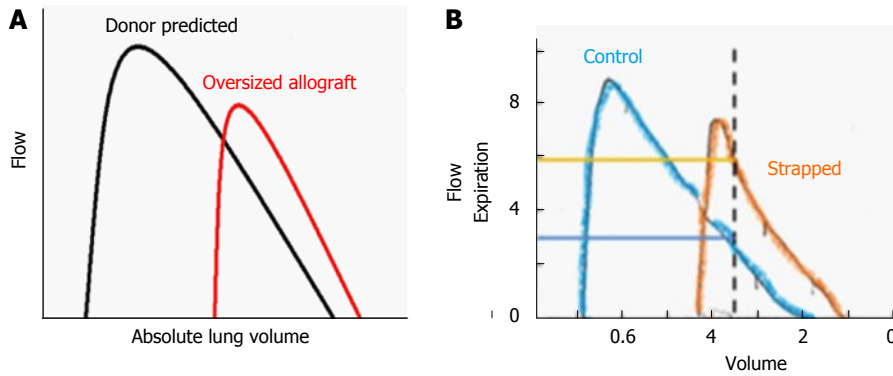


Figure 5 Oversized allograft (A) and chest wall strapping (B) analogy. A: Schematic flow volume loops according to donor predicted values (black line) and measured mean values of recipients of oversized allografts (red line) during the early post-transplant period (1-6 mo). Flows are plotted against absolute lung volume; B: Control (blue) and chest wall strapped (orange) flow volume loops are shown. Adapted with permission from Eberlein *et al.*^[5,28].

Table 1 The Surfactant system and its relation to risk factors for bronchiolitis obliterans syndrome

BOS risk factor	Effect on surfactant system
Primary graft dysfunction	Successful treatment with surfactant
Acute rejection	Type II pneumocyte destruction and surfactant disruption Rejection is associated with surfactant dysfunction Immunosuppression preserves Surfactant function
GERD - aspiration	Inactivation of surfactant
Pulmonary infection	Inactivation of surfactant

Adapted with permission from Eberlein *et al.*^[5]. GERD: Gastro-esophageal reflux disease; BOS: Bronchiolitis obliterans syndrome.

those of CWS, Figure 5^[5,28]. There is very limited information on lung compliance and lung elastic recoil pressure after lung transplantation in relation to donor-recipient size matching. In 15 recipients of bilateral LTx whose donor lungs were, on average mildly oversized, elastic recoil of the transplanted lungs was mildly increased^[29]. The likely increased elastic recoil of oversized lungs could have a beneficial effect on small airway function from the interdependence between increased elastic recoil and airways leading to greater radial distending forces on small airways and small airway dilation^[28].

A possible mechanistic explanation for the described physiology of CWS relates to the surfactant system^[5,28]. The associations between the surfactant system and risk factors for BOS are summarized in Table 1. The surfactant system shows adaptive responses to changes in lung compliance. In a model of decreased lung compliance, increases in surfactant protein and phospholipid content mediated a compensatory reduction in surface tension^[30]. Furthermore, compared with normal inflation state in the donor chest an oversized allograft would operate at lower lung volumes in the recipient and thus alveolar size would on average be reduced. Surfactant fills in the regions adjacent to infolding of the alveoli as the lung deflates to maintain a spherical inner surface.

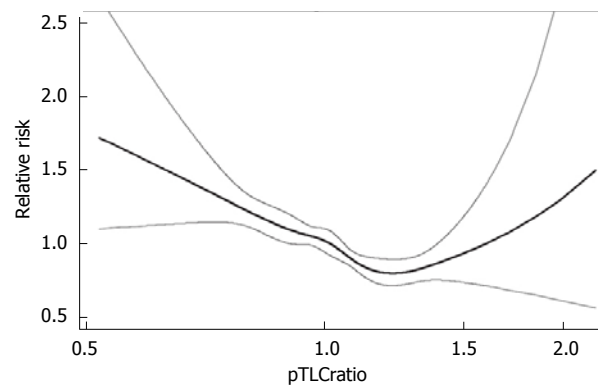


Figure 6 Impact of predicted total lung capacity ratio on the risk of death after lung transplant. Adapted with permission from Eberlein *et al.*^[6]. pTLC: Predicted total lung capacity.

Thus, a chronically underinflated lung could be expected to accumulate more surfactant.

Survival

We have shown in a series of studies that the pTLC as a more refined estimate of organ sizing performs better than height alone, and is a strong predictor of various meaningful outcomes after LTx^[3-7,10,11,31-33]. We have shown that the donor to recipient pTLCratio is an independent predictor of post-LTx survival, by addressing the following: (1) There is a non-linear association between the pTLCratio and post-LTx survival. With the pTLCratio entered as a spline there was a nonlinear association resulting in a declining risk of death with higher pTLCratio from 0.5 to about 1.3, where an inflection occurred with rising risk at higher values, Figure 6^[6]; (2) There was no significant interaction with transplant indication^[6]. Furthermore, within a single LTx indication [idiopathic pulmonary arterial hypertension (IPAH)], a condition that does not influence the size matching decision, the pTLCratio was a strong independent predictor of survival^[4]; and (3) The analysis showed that, after accounting for the pTLCratio, recipient and donor sex matching was not independently associated with death after LTx^[6,7,10].

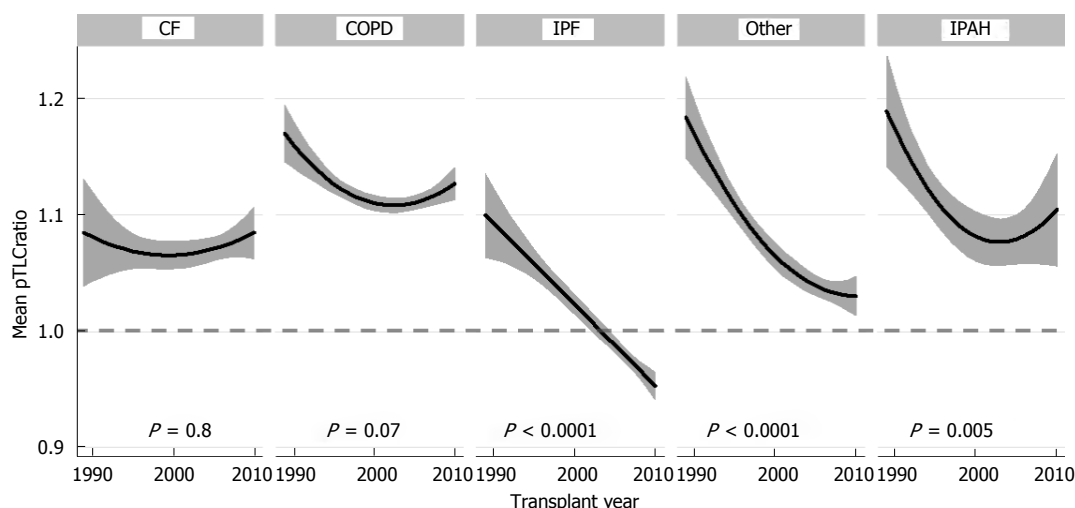


Figure 7 Mean predicted total lung capacity ratio according to transplant year stratified by lung transplant indication. Adapted with permission from Taher *et al*^[24]. pTLC: Predicted total lung capacity; CF: Cystic fibrosis; COPD: Chronic obstructive pulmonary disease; IPF: Idiopathic pulmonary fibrosis; IPAH: Idiopathic pulmonary arterial hypertension.

Thus the pTLCratio explains a previously not well understood association between worse survival and a female allograft transplanted into a male recipient (For the same donor height female lungs are on average 20% smaller than male lungs). Furthermore, in a preliminary analysis we find that the effect of race on lung size also explains the previously reported association between donor African American race and higher mortality following LTx. These associations remained significant after adjustment for all known risk factors for post LTx mortality available in the datasets, including centers and center volumes^[6].

Over the period from 1989 to 2010, the mean pTLCratio in US LTxs has decreased progressively from 1.14 to 1.04 ($P < 0.0001$)^[34]. Within diagnoses there has been temporal decline in the pTLCratio by era especially in IPF, IPAH and "Other" indications, Figure 7^[34].

Our data suggest that the secular trend to favor undersized donor lungs is ill advised. The advantage of using well matched or oversized donor lungs is supported by pathophysiological consideration that link undersized and well matched or oversized allografts to different allograft function and injury patterns.

HEART TRANSPLANT OUTCOMES ASSOCIATED WITH SIZE-MATCHING

In the setting of heart transplantation, a transplant recipient's heart is often enlarged and dysfunctional such that the size of the explant is dissociated from the workload imposed by the vascular bed. As such, the goal of size matching is to provide a donor organ that is optimally sized to be capable of sustaining the workload needed to perfuse the recipient's vascular bed - unrelated to the size of the organ removed. Currently, the only surrogate for size used in the allocation process is actual body weight^[2,35-40]. The

value of the current practice whereby offers are limited to donors within a certain weight range has been questioned in several large studies that have shown no association between outcomes and donor-recipient differences in body weight^[2,37]. Heart size varies not only in relation to body weight, but also by other factors including sex in particular^[2]. Studies of heart transplantation have consistently observed reduced survival associated with donor organ sex mismatch, particularly for male recipients of female organs^[36,40]. The mechanism of this observation has long been unknown, but a recent study examining refined measures of heart size shed considerable light on the issue^[2].

Studies utilizing cardiac MRI have provided prediction models of cardiac mass that incorporate height, weight, age, and gender. These prediction models provide estimates of heart size that differ significantly from estimates using body weight alone. For example, the predicted cardiac mass of a man and a woman both 55-year-old, 80 kg in weight, and 1.75 m tall yields a difference in predicted cardiac mass of 19%^[2]. Applying these measures again, a man would have to weigh 20 kg (25%) less than an otherwise similar woman to yield an equivalent predicted heart size^[2]. It is therefore likely that the current practice of matching donor organs to recipients based on body weight differences fails to discriminate substantial size mismatches^[2].

To evaluate whether worsened outcomes in sex mismatching are related to mismatch of organ size in heart transplantation, we performed a retrospective cohort study of 31634 donor-recipient adult HTx pairings from the United Network for Organ Sharing transplant registry^[2]. We used predictive models to calculate the predicted total heart mass (pHM) for recipient and donor pairs. By assessing organ size mismatch by calculating the percent difference between the donor and recipient

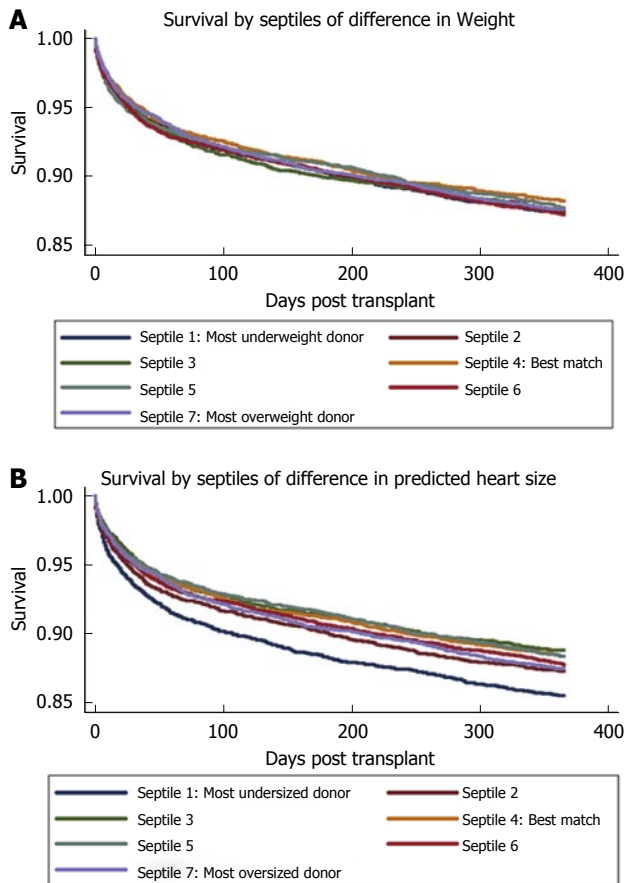


Figure 8 Unadjusted Kaplan-Meier graphs of survival, by septiles of matching by body weight (A) vs predicted total heart mass (B). Adapted with permission from Reed *et al*^[2].

pHM [= pHM recipient - pHM donor]/(pHM recipient) × 100, we found that the most undersized pHM septile experienced higher mortality during the first year post transplant (HR 1.27, $P < 0.001$)^[2]. This remained robust with very little change in the point estimate (suggesting absence of confounding) in adjusted models (HR 1.25, $P = 0.03$), Figure 8^[2]. Supporting the assertion that weight differences provide no clinically useful information, survival did not vary across septiles of weight differences, Figure 8^[2]. In univariate analysis, gender mismatch was associated with higher mortality in males. Controlling for differences in pHM eliminated this association (1 year HR, 1.00, $P = 1$). We concluded that differences in donor-recipient predicted heart sizes modulate the survival associated with donor-recipient gender mismatch and identifies donor heart undersizing as an otherwise occult and potentially preventable cause of excess mortality following orthotopic heart transplantation^[2,39].

WAIT-LIST CONSIDERATIONS

We have made the argument for both lung and HTx, that the current method for listing size preferences sub-optimally predicts outcomes after thoracic transplantation^[1]. In addition to those issues already

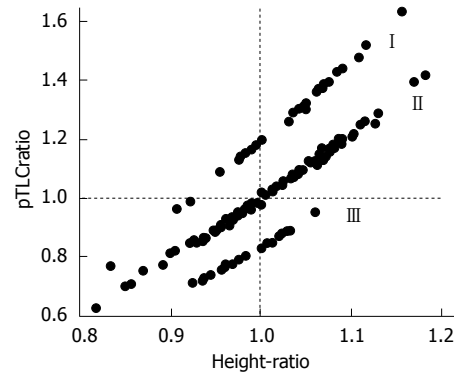


Figure 9 Relationship between predicted total lung capacity ratio and height ratio. The separation between clusters I (male donor-female recipient), II (sex matched) and III (female to male) is due to effects of sex on lung size. Adapted with permission from Eberlein *et al*^[7]. pTLC: Predicted total lung capacity.

described, the practice of limiting donor-recipient matches based on current size surrogates conceptually conveys further added morbidity and mortality based on both suboptimal matches as well as missed allocation opportunities. As mentioned previously, potential recipients for LTx are listed with acceptable donor height ranges, and recipients for HTx are listed with acceptable donor weight ranges. While these measures crudely correlate with organ size, they function particularly poorly in the setting of sex mismatch in particular. This is because a man's thoracic organs are approximately 20% larger than a woman's, Figure 9^[7].

In order to exemplify the concepts of occult suboptimal organ allocation that occur in the current system, we will present a lung recipient and three theoretical potential donors. The concept would apply similarly in the setting of HTx.

For this example, the listed transplant candidate is a 55 year old man with end stage lung disease from idiopathic pulmonary fibrosis (IPF) who is listed for LTx. Candidates for LTx with IPF are often listed for height ranges below or up to their own height, as there has traditionally been a preference towards undersizing^[34]. For this example we consider a candidate with IPF who is 170 cm tall (and has a pTLC of 6.54 L) and is listed for an acceptable donor height range from 147-170 cm, Table 2^[34].

Offer B: Appropriately identified size match

If we consider a 45-year-old male donor, who is 170 cm (and has a pTLC of 6.54 L), this would represent an appropriately identified size match and would be appropriately included in the match run for allocation to our hypothetical recipient (Table 2).

Offer C: Missed opportunity

If we then consider a 42-year-old female donor, who is 175 cm tall, this would fall outside the upper limits of the height listing range and be identified in the current system as oversized. As such, this donor would

Table 2 Hypothetical donor offers for a subject with idiopathic pulmonary fibrosis listed for lung transplantation

	Listed subject with IPF	Donor listing	Offer A	Offer B	Offer C
Age (yr)	55	12-60	42	45	42
Gender	Male	Either	Female	Male	Female
Height (cm)	170	147-170	147	170	175
pTLC (L)	6.54	3.98-6.54	3.98	6.54	5.76
pTLCratio			0.61	1.00	0.88

Adapted from Taher *et al.*^[34]. IPF: Idiopathic pulmonary fibrosis; pTLC: Predicted total lung capacity.

be automatically eliminated and would not appear in the match run for our hypothetical recipient. This example represents an incorrect assessment of size as the pTLC of the donor is actually 5.76 L - which is a smaller pTLC than the 170 cm male donor, Table 2^[34]. Furthermore, this match would represent a pTLCratio of 0.88, which although undersized, would likely represent an acceptable match (Table 2).

Offer A: Inappropriately undersized

If we finally consider an offer from a donor who is a 147 cm tall female, we can see that in the current system this would fall within acceptable parameters and would enter into the match run and potentially be allocated to our hypothetical recipient. While the height difference falls within the lower limit of the acceptable height range listed, the pTLCratio of 0.61 reveals the organ to be markedly undersized with outcomes predictably suboptimal. This would represent a failure of the current system to identify and possibly avoid an inappropriately undersized match (Table 2).

Not only is this hypothetical candidate not receiving by lung size (pTLC) well matched donor lung offers; but in addition we have shown in a series of studies, that it is not necessary to avoid oversizing. On the contrary, we have shown that a higher donor to recipient pTLCratio, suggestive of an oversized allograft, is associated with improved survival after LTx, irrespective of indication. Thus oversizing, up to a point, should not be avoided and is an important additional means of increasing the chance of receiving an appropriately sized donor offer^[6].

However it has been shown that short stature is associated with increasing wait list times and increased risk of death on the wait list. Since the implementation of the Lung Allocation Scoring (LAS) system, characteristics of candidates on the wait list have changed to include a sicker group of patients with a greater proportion of LAS diagnoses group D (restrictive lung diseases)^[9]. As a consequence, wait-list mortality rates are again rising despite higher wait-list transplant rates compared to the pre-LAS era. Potential LTx-recipients with short stature and small thoracic cavities have longer waiting times on the LTx list, as donor lungs considered to be size-appropriate are particularly limited^[41]. This often affects patients with cystic

fibrosis and pulmonary fibrosis. In both groups, LTx can become an urgent issue when significant disease exacerbations occur, and in this setting in particular patients are at high risk for wait list mortality. Higher acuity at the time of LTx is in turn associated with decreased survival. It would thus seem logical to consider a change to thoracic organ allocation to incorporate better estimates of organ size. Rather than relying on a donor height range for lung allocation, it would be logical to express sizing preferences in terms of an acceptable donor pTLC range.

CONCLUSION

Donor-to-recipient organ size matching is a critical aspect in thoracic transplantation. We advocate for a change in the thoracic allocation mechanism from a height-or-weight-based strategy to a size-matching process that utilizes refined estimates of organ size. Studies examining the impact of refined estimates of organ size suggest that there is considerable preventable pre- and post-LTx morbidity and mortality attributable to organ size differences that are occult in the current system due to reliance upon height (in LTx) and weight (in HTx) alone as a surrogate for organ size. The current allocation system also misclassifies a proportion of well-matched organs as inappropriately sized, and thus fails to optimally match available organs to the highest-priority appropriate recipients. Further studies simulating the impact of this proposed organ allocation change will hopefully provide the foundation for a change in the United States (UNOS/OPTN), and consequently improve donor lung utilization with resulting reductions in post-LTx complications and graft failure rates.

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Heparin-induced thrombocytopenia in solid organ transplant recipients: The current scientific knowledge

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(HIT), an immune mediated prothrombotic adverse drug effect. Transplant recipients are frequently exposed to heparin either due to the underlying end-stage disease, which leads to listing and transplantation or during the transplant procedure and the perioperative period. To review the current scientific knowledge on anti-heparin/PF4 antibodies and HIT in transplant recipients a systematic PubMed literature search on articles in English language was performed. The definition of HIT is inconsistent amongst the publications. Overall, six studies and 15 case reports have been published on HIT before or after heart, liver, kidney, and lung transplantation, respectively. The frequency of seroconversion for anti-PF4/heparin antibodies ranged between 1.9% and 57.9%. However, different methods to detect anti-PF4/heparin antibodies were applied. In none of the studies HIT-associated thromboembolic events or fatalities were observed. More importantly, in patients with a history of HIT, reexposure to heparin during transplantation was not associated with thrombotic complications. Taken together, the overall incidence of HIT after solid organ transplantation seems to be very low. However, according to the current knowledge, cardiac transplant recipients may have the highest risk to develop HIT. Different alternative suggestions for heparin-free anticoagulation have been reported for recipients with suspected HIT albeit no official recommendations on management have been published for this special collective so far.

Key words: Heparin-induced thrombocytopenia; Heparin-induced thrombocytopenia; Heparin; Organ; Transplantation

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Abstract

Exposure to heparin is associated with a high incidence of immunization against platelet factor 4 (PF4)/heparin complexes. A subgroup of immunized patients is at risk of developing heparin-induced thrombocytopenia

Core tip: Heparin-induced thrombocytopenia (HIT) II is a life-threatening complication of heparin therapy. Transplant recipients frequently are exposed to high doses of heparin before, during, and after transplantation. This review gives a systematic overview

on the current scientific knowledge and existing publications on anti-platelet factor 4/heparin antibodies and HIT in transplant candidates and recipients.

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INTRODUCTION

Heparin plays a pivotal role in peri-operative anticoagulation therapy to prevent thrombosis and thromboembolism^[1].

During the course of the underlying disease, nearly all patients who finally undergo solid organ transplantation, are exposed to prophylactic or therapeutic dose heparin (e.g., dialysis due to endstage renal disease; cardiac assist devices because of heart failure). During organ perfusion for procurement and within the transplant procedure heparin is used to prevent formation of blood clots.

Heparin application entails several risks for the transplant recipients who need careful observation to prevent additional morbidity and mortality. Heparin interferes with platelets. It may directly activate platelets, causing a mild, reversible decrease in platelet counts, so-called heparin-induced thrombocytopenia (HIT) type I. In contrast to clinically irrelevant HIT type I, immune mediated HIT type II is of major clinical importance^[1]. If not recognized early during its development this relevant adverse reaction of heparin paradoxically triggers potentially lethal venous and arterial thromboses. Clinical manifestation of HIT type II is very heterogeneous^[1]. Therefore, HIT type II should be considered in every patient who develops thrombocytopenia, thrombosis, embolism, vascular obliteration, or skin necroses during heparin therapy.

HIT type II is caused by IgG antibodies binding with complexes of negatively charged heparin molecules and a positively charged, soluble platelet protein platelet factor 4 (PF4). When several of these antibodies bind with PF4/heparin complexes, immune complexes are formed that activate platelets *via* the platelet Fc-receptor. Activated platelets provide the catalytic surface for enhanced thrombin generation, which is the reason for an increased risk for thrombosis^[2], especially when other risk factors for thrombosis are present.

Enzyme linked immunosorbent assay (EIA) can detect the anti-PF4/heparin antibodies underlying HIT. However, in the context of HIT, only anti-PF4/heparin IgG antibodies are relevant, as IgM and IgA antibodies cannot bind to the platelet Fc receptor and can therefore not induce platelet activation with subsequent

thrombin generation^[3,4]. Platelet activating antibodies can be identified by functional assays such as serotonin release assay (SRA)^[5,6] and heparin induced platelet activation assay (HIPA)^[2,7,8]. This stepwise emergence of seroconversion (EIA), activating antibodies (SRA/HIPA), thrombocytopenia, and HIT II associated thrombosis (HIT thrombotic syndrome: HITTS) has previously been illustrated as an "iceberg model of HIT" (Figure 1)^[4,9-11]. As only a minority of anti-PF4/heparin antibodies induces HIT, the diagnosis of HIT requires both, clinical and serological findings^[4,7].

Unfortunately, a major criterion of HIT, a platelet count decrease by more than 50%, is not very specific after major surgery due to a frequent post-operative decrease in platelet counts for surgery-related reasons. However, HIT occurs typically between day 5 and 14 after starting heparin treatment and is often associated with new thrombosis. Taking these criteria together, the diagnosis of HIT becomes likely if the platelet count decreases by > 50% between days 5 and 14 after starting heparin treatment, especially if accompanied by new thrombotic complications. Basically, patients receiving heparin need routine laboratory controls of platelet counts to detect an emerging thrombocytopenia and HIT II^[7,12]. To this day, no screening procedure exists to detect patients at risk of HIT II. In case of suspected HIT II it is important to stop heparin application immediately, initiate laboratory investigations, and switch to a heparin-free anticoagulation regimen such as danaparoid, lepirudin, argatroban, or fondaparinux^[12].

In daily clinical practice the 4Ts score (Table 1) has been repeatedly shown to serve as a reliable tool to assess the individual probability of HIT II^[7,12-14]. A high 4T score together with a positive functional assay are regarded as being confirmatory for HIT. A negative PF4/heparin EIA rules out HIT with very high likelihood. However, a positive PF4/heparin EIA on its own is not very informative. Therefore, according to the "classic" definition of HIT an intermediate to high pretest probability and detection of platelet activating, heparin-dependent anti-PF4/heparin IgG antibodies (EIA + SRA/HIPA) are required for a reliable diagnosis of HIT. Less stringent criteria often lead to an inappropriate change to alternative, heparin-free anticoagulation, which causes both an increased risk of bleeding and increased treatment costs^[15,16]. Most importantly, this overdiagnosis may lead to patients being delisted from the transplant list.

In regard to disease specific impacts on HIT, comprehensive and reliable data mainly exist based on patient series from cardiac surgery^[17], orthopedic surgery^[18], and vascular surgery^[19,20]. However, reports and systematic studies on HIT in solid organ transplant recipients are rare and inconclusive.

In this review we give a systematic overview of the current scientific knowledge about anti-PF4/heparin antibodies and HIT in patients undergoing organ

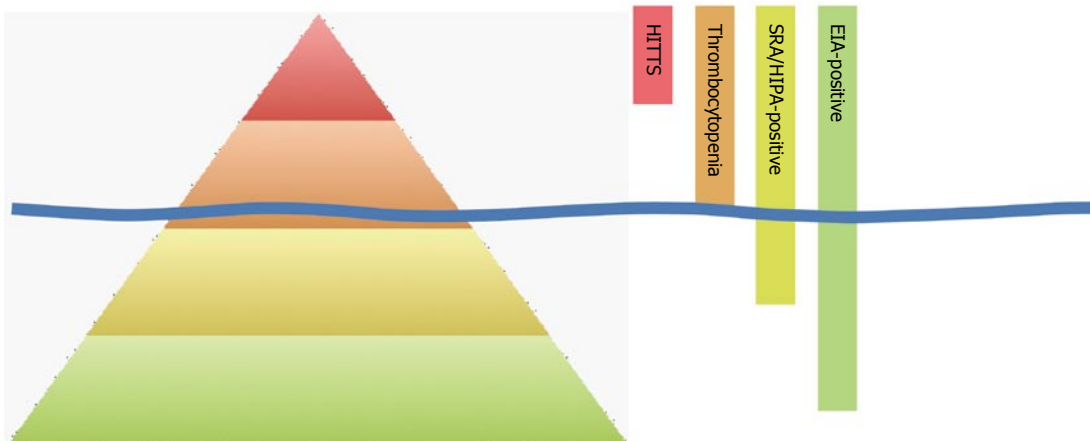


Figure 1 The frequency of antibody seroconversion, activating heparin-induced thrombocytopenia antibodies (serotonin release assay/heparin induced platelet activation assay), thrombocytopenia, and clinically manifest heparin-induced thrombocytopenia thrombotic syndrome are illustrated as an “iceberg”^[4,9,10]. The waterline indicates the threshold between positive laboratory findings and clinical appearance of HIT. HIT: Heparin-induced thrombocytopenia.

Table 1 The 4Ts scoring system^[62]

Parameter	2 points	1 point	0 points
Thrombocytopenia	Platelet count drop > 50% and platelet nadir \geq 20 g/L	Platelet count drop 30%-50% or platelet nadir 10-19 g/L	Platelet count drop < 30% or platelet nadir < 10 g/L
Timing of platelet count drop	Onset on days 5-10 or platelet count drop \leq 1 d and previous heparin exposure < 30 d ago	Onset on days 5-10 but platelet count drop not clear (e.g., missing counts); onset after day 10 of heparin therapy or drop \leq 1 d and previous heparin exposure 30-100 d ago	Platelet count drop \leq 4 d after beginning of heparin therapy and no previous heparin exposure
Thrombosis and sequelae	New proven thrombosis; skin necrosis; acute systemic reaction after heparin application	Progressive or recurrent thrombosis; suspected thrombosis; non-necrotizing skin lesions	None
Other causes of thrombocytopenia	Apparently none	Possible	Definite

Probability of HIT II: 1-3 points: Low; 4-5 points: Intermediate; 6-8 points: High. HIT: Heparin-induced thrombocytopenia.

transplantation and discuss appropriate diagnostic and therapeutic strategies for transplant physicians.

RESEARCH STRATEGY

The authors independently performed a systematic PubMed literature search on articles published in English. The following keywords were used: Transplantation AND heparin-induced thrombocytopenia OR HIT antibodies OR HIT disease OR HIT II OR anti-PF4/heparin. The search was performed on May 31st, 2015. In addition, the authors' libraries and the Internet were searched. The following medical subject headings were used: Heparin-induced thrombocytopenia after heart OR lung OR liver OR pancreas OR kidney OR organ transplantation; risk factors in transplantation; and HIT development. Papers deemed relevant by the authors were retrieved.

RESULTS

Transplant recipients frequently are multimorbid patients with major diseases of the cardiovascular, the

hematologic, the coagulation, and the endocrinologic systems, which each can trigger thrombocytopenia. This is why relevant side-effects of drugs, thrombocytopenia associated to the underlying disease, sepsis, disseminated intravascular coagulation, and post transfusion purpura always have to be considered in every individual case of thrombocytopenia in ICU patients^[21]. However, a platelet count drop is also well known to occur after major surgery and extracorporeal circuitry such as heart-lung machine or cell saver[®] autotransfusion^[22]. Drug-induced immune thrombocytopenia has been reported for calcineurin inhibitors^[23], mycophenolate, and anti-thymocyte globulin (ATG)^[24,25]. Therefore, other syndromes and diseases have to be taken into consideration within the postoperative setting after solid organ transplantation to carefully distinguish between physiologically and pathologic thrombocytopenia such as HIT II.

DISCUSSION

As noted in the introduction, the combined clinical and laboratory proof of HIT II has to be performed

according to the “classic definition of HIT”^[4,15,16] to avoid overdiagnosis. Unfortunately, the diagnosis of HIT is difficult in critically ill patients as both leading symptoms of HIT (thrombocytopenia and thrombosis) are not specific^[21]. Although the absence of anti-heparin/PF antibodies has a high negative predictive value to exclude HIT, it is not sufficient to detect these antibodies without further satisfying the stepwise criteria (Figure 1) including the 4Ts pretest clinical score^[13,14] for the diagnosis of HIT^[7,9-11,15].

Our literature research revealed six studies, nine case reports, and six case series on anti-PF4/heparin antibodies or HIT in solid organ transplant candidates and recipients. Detailed data on different organ transplants, type of study, number of patients investigated, performed laboratory diagnostics, time of HIT investigation, and the clinical course and outcome of the recipients are provided in supplementary Table 1.

THORACIC ORGANS

Heart transplantation

The treatment of seriously ill cardiac patients is a demanding challenge for the interdisciplinary team of physicians. The risk for HIT is proposed to be high due to high doses of heparin used in cardiac surgery and a vast release of PF4 from platelets because of the platelets’ contact to cardiopulmonary bypassing^[26].

Patients with a history of HIT who need extracorporeal circulation within a surgical procedure require careful planning of anticoagulation therapy. Respective considerations on HIT prevention have been published in several case reports^[27-35] and are explicitly discussed in the guidelines published by both the American College of Physicians (ACCP)^[12] and the British Society of Hematology^[36].

In prospective studies a relevant discrepancy was observed between detection of anti-PF4/heparin antibodies (EIA positive: 27%-50% of the patients) and the capability of these antibodies to activate platelets (SRA or HIPA positive: 7%-40% within the EIA positive patients)^[26,37,38]. The development of clinically relevant HITTS was reported to range between 1% and 3%^[39] and is therefore considerably smaller in regards to the high rate of seroconversion. An investigation on HIT in pediatric patients revealed a comparable frequency of 1%-2%^[17,40].

According to the ACCP guidelines heparin is recommended for anticoagulation during cardiopulmonary bypass in patients with a history of HIT provided that anti-heparin/PF4 antibody testing is negative at the time of surgery^[12]. This advice is based on the fact that an anamnestic response and antibody production cannot emerge that fast to develop fulminant HITTS^[41,42]. Nevertheless, for all cases of proven HIT (defined as positive antibody detection plus thrombocytopenia) several alternative regimens have been published^[43] starting with strategies to adjourn surgery through to complex heparin-free combination therapies.

However, cardiac transplant surgeons have to draw on the latter regimens because heart transplantation cannot be deferred. Selleng *et al.*^[44] addressed this complex situation in candidates awaiting heart transplantation and defined the state of regenerating platelet counts but still detectable anti-PF4/heparin IgG antibodies in EIA as “subacute HIT”. When platelet-activating antibodies were not detectable by the functional assay HIPA, the authors demonstrated that heart transplantation can be performed despite using heparin for anticoagulation without serious complications. Furthermore, the article provides useful recommendations and structured strategies for choosing perioperative anticoagulation in recipients with a positive history of HIT^[44].

However, these patients already are under critical surveillance of transplant physicians and hematologists and receive an adapted anticoagulation therapy because of known anti-heparin/PF4 antibody seroconversion before transplant. The true challenge for transplant physicians is rather the sufficiently early recognition of a de-novo HIT development or postoperative reactivation within the complex clinical setting of a just transplanted recipient. This differentiation is rather difficult because on the one hand many cardiac patients have long-term heparin therapy (LMWH) and on the other hand postoperative thrombocytopenia can usually be ascribed to reasons other than HIT^[45]. This is why a scoring system comparable to the 4Ts system was developed to assess the HIT probability after cardiopulmonary bypass surgery^[46]. Heart transplant recipients should be monitored with the same due skill, care and diligence as other cardiac surgery patients. For these patients routine screening is not recommended^[7,12,47]. However, HIT laboratory diagnostic should be started immediately in every case of intermediate or high risk in the 4Ts system^[12,17].

Having cognizance of a general HIT incidence of 1% to 3%, Hourigan *et al.*^[48] performed a retrospective analysis on cardiac transplant recipients. Overall, thrombocytopenia was found in 26 of 46 patients. Thrombocytopenia was the decisive factor to initiate anti-PF4/heparin antibody testing using EIA. Antibodies were detected in 11 recipients, but in 10 cases seroconversion had already occurred before transplantation. Therefore, these patients also have to be assigned to the above-mentioned population with HIT development due to heparin application during the pre-transplant period. Only one patient who suffered from CMV pneumonitis was suspected for HIT 10 mo after transplant. However, the limitation of Hourigan *et al.*^[48] study is that no functional assay on platelet activating antibodies was performed to meet the “classic” definition of HIT development. This liberal definition of HIT, which is only based on thrombocytopenia and a positive result in EIA, might explain the high frequency of HIT as reported in their retrospective study. Nevertheless, Hourigan *et al.*^[48] recognized thromboembolic events in 5 EIA-positive

patients (45% of the EIA-positive and 11% of all investigated patients) but unfortunately they failed to promptly perform a functional test to confirm the true evidence of HIT. Furthermore, thromboses occurred exclusively before heart transplantation and therefore were non-transplant related anyway. Interestingly, the authors reported on no significant difference in mortality between EIA-positive and EIA-negative patients on the one hand and EIA-positive patients and those patients with thromboses on the other hand, respectively.

Hassan *et al.*^[49] performed the most comprehensive study on HIT in transplant recipients and they consider the mentioned potential of overdiagnosis^[15,16]. The authors therefore consistently distinguished between "HIT antibody positivity" (4Ts score > 3 points and EIA positive) and "HIT" (plus positive SRA). A total number of 2587 transplant patients (thoracic and abdominal organs) from one center were retrospectively evaluated. Due to unexpected thrombocytopenia HIT was initially assumed in approximately 10% of the patients. Therefore, the 4Ts scoring system pretest probability was calculated and anti-heparin/PF4 EIA was subsequently performed. Seroconversion was observed in 1.9% of all investigated patients. Compared to the investigation of Hourigan *et al.*^[48], this study mainly reports on antibody detection after transplantation. SRA verification was performed in 29% (14/48) of the seroconverted patients and revealed positive results in 11 of 14 cases (78%). Assuming that 78% of all antibody positive patients were SRA positive, the frequency of HIT (suspicious 4Ts test and both EIA and SRA positive) would be 1.5% in the whole investigated population. The study actually revealed "HIT" according to the authors' definition in 3.6% of the heart recipients and 0.9% of the lung recipients. Interestingly, thromboembolic events were found in 23% of all the anti-heparin/PF4 antibody positive patients and in 2.4% of the cardiac graft recipients, respectively. However, no thrombotic event was observed in recipients with low 4Ts scores and no single case of HIT-associated death was revealed in this comprehensive analysis^[49].

Both analyses are limited due to their retrospective single center design and the difficulties to generalize these results to the heterogeneous transplant population^[48,49].

Lung transplantation

No data are available besides the results by Hassan *et al.*^[49] (see heart transplantation).

ABDOMINAL ORGANS

Kidney transplantation

Kidney transplant recipients have a high frequency of pretransplant heparin exposure due to dialysis. Therefore, an increased risk of HIT-associated syn-

dromes and complications could be assumed in this collective. Strict heparin exposure can only be avoided in those candidates who are either planned for preemptive transplantation or who perform CAPD.

There are four case reports on anti-PF4/heparin antibodies and HIT in renal transplantation up to the present day. However, according to the recommended criteria for manifest HIT disease (HITS) no report fulfills the "classic" criteria as the 4Ts pretest score was not performed^[50-53], no functional test on the activating potential of the EIA-positive anti-PF4/heparin antibodies was further analyzed in either SRA or HIPA^[51,52], or was even SRA-negative^[50]. In two cases the renal graft was lost due to proven thrombosis^[50,51] but the association with HIT cannot be determined because of the inadequate diagnostic approach. One case report^[53] addresses an adolescent patient with end-stage renal disease who performed thrombocytopenia after eight months of repeated heparin exposure during dialysis, which is untypical for HIT. Even though both anti-PF4/heparin EIA and SRA were positive, the patient did not have a manifest thromboembolic event, had not been transplanted at that time, but showed additional major procoagulatory disorders potentially accountable for thrombocytopenia and thrombosis. The authors reported on a heparin-free hirudin-based perioperative anticoagulatory regimen and successful kidney transplantation, which could serve as recommendation in cases of (suspected) HIT.

Liver transplantation

Chronic end-stage liver disease is frequently associated with coagulation disorders and secondary thrombocytopenia due to portal hypertension and hypersplenism^[54]. These preexisting disorders in liver transplant candidates make clinical recognition of HIT difficult because a significant drop in the platelet count according to the 4Ts system's definition tends to be rather small when the baseline value is already reduced below the normal range. This is why a reactive thrombocytopenia in the postoperative course of a liver transplant recipient may easily mislead the accountable physicians to assume HIT, prompt HIT testing, and impetuously change anticoagulation to a heparin-free protocol with all its risks and side-effects. Therefore, the assessment of the clinicopathological syndrome of HIT is especially demanding in liver transplantation. Both clinical findings in recipients and published data have to be questioned carefully with regards to the correct adherence to the "classic" definition of HIT to avoid overdiagnosis.

In literature, three case reports and four studies have been published within this field so far. Unfortunately and as criticized before, the inadequately implemented stepwise diagnostics and evidence of "classic" HIT^[15,16] displays a substantial problem in interpretation of the results from these data. All three

case reports concern liver transplant recipients with a history of anti-PF4/heparin antibody seroconversion^[55,56] or proven HIT^[57] before transplantation. In these reports no data are available regarding HIT-antibodies after transplant.

Amongst the comprehensive studies on postoperative HIT-antibodies after liver transplant a retrospective study on 205 recipients revealed only 1.95% anti-PF4/heparin antibody positive (EIA) patients but information on the number of patients tested through EIA is missing^[58]. No single case of HIT-associated thrombosis or thromboembolism was found after liver transplantation in this study though the definition of HIT rather meets a "liberal" definition of HIT compared to the suggested "classical" iceberg model^[7,9-11,15].

In a prospective series of 52 living donor liver transplant recipients, Kaneko *et al.*^[59] investigated anti-PF4/heparin antibody seroconversion starting before surgery until three weeks after transplant. This study revealed a low incidence of antibodies (5.6%), no detection of antibodies in two patients with postoperative thrombosis, and no proof of HIPA-positive antibodies in two patients with suspicious postoperative platelet courses. However, recipients with anti-PF4/heparin antibodies in EIA did not develop thrombosis despite continuation of heparin therapy. These findings could mostly be confirmed by the results of the two studies we performed on anti-PF4/heparin antibodies after liver transplantation.

In a first retrospective analysis the authors evaluated the incidence of anti-PF4/heparin antibodies in patients undergoing liver transplantation^[60]. The analysis revealed a remarkably high frequency of anti-PF4/heparin antibody seroconversion in 30.4% of the recipients. However, none of them developed HIT-associated thromboembolic complications within the characteristic period between day 5 and 14 after the beginning of heparin therapy. In a univariate and multivariate analysis of potentially causative factors for antibody production the authors ruled out suspected impact from cell saver® autotransfusion, transfusion, and postoperative dialysis. The only trigger that could be identified in multivariate analysis and binary logistic regression was patient's age with a cutoff at 59 years in chi-square testing and an increased risk for patients of 59 years and older. Unfortunately, due to the retrospective character of the analysis the authors could not further distinguish between antibody subclasses (IgG, IgA, and IgM) and their activating features in SRA or HIPA.

Therefore, Bakchoul *et al.*^[61] initiated a prospective cohort analysis on 38 consecutive deceased donor whole organ liver transplant recipients. In their study, patient sera were investigated for the different anti-PF4/heparin antibody subclasses, their activating power in HIPA, thrombocytopenia, and HIT-associated thromboembolic events according to the "classic"

definition of HIT^[15,16] until post-operative day 21.

Antibody testing in subclass-specific EIA directly before surgery revealed pre-existing seroconversion of 13.2% (IgG), 7.9% (IgA), and 57.9% (IgM), respectively. Interestingly, 80% of the recipients with pre-operative anti-PF4/heparin antibodies presented decreasing titers after transplantation and none of them developed HIT^[61]. These data confirm previous recommendations that liver transplant candidates with a history of positive HIT-testing but without activating features should not be excluded from the waiting-list^[57,58,61].

After surgery 15.2% of the recipients developed de-novo IgG antibodies and two of the recipients (6.1%) showed activating IgG-antibodies in HIPA^[61]. Overall, none of the liver transplant recipients developed HITTS in their systematic study. Furthermore, recipients who were clinically suspected to suffer from HIT according to 4Ts pretest clinical scoring system^[7,12-14] did not develop platelet activating antibodies in HIPA^[61]. Therefore, HIT can be assumed to be very unlikely in these recipients^[4]. This observation raises the question whether the 4Ts system is suitable to estimate the probability of HIT without restrictions in transplant recipients. The 4Ts scoring system has not been investigated in this special subgroup of patients so far.

Heparin-free anticoagulation is difficult to monitor in critically ill patients and entails a relevant risk of bleeding complications. According to the reported findings^[59-61], changing anticoagulation to a heparin-free regimen should be reconsidered in liver transplant recipients with non-activating anti-PF4/heparin antibodies^[61].

Pancreas transplantation

No data are available.

CONCLUSION

Due to repeated and usually high-dose heparin application before and after transplant surgery, HIT could be expected to occur frequently in organ recipients. Furthermore, standardized organ procurement procedures use heparin for donor anticoagulation, which causes an inevitable exposure of the recipient to heparin. This review questions the assumption of a relevant role of HIT in these patients according to present investigations.

First, the "classic" definition of HIT needs to be established as a common basis to allow for convincing and comparable results of research. Second, clinicians need to distinguish carefully between data on HIT before and after transplantation.

Several publications reported on uneventful cases of heparin re-exposure of transplant patients with a positive history of HIT, when anti-heparin/PF4 antibodies were not detectable in EIA anymore. Different heparin-free anticoagulation regimens were given (hirudin, bivalirudin, lipirudin^[53,56,57]) but

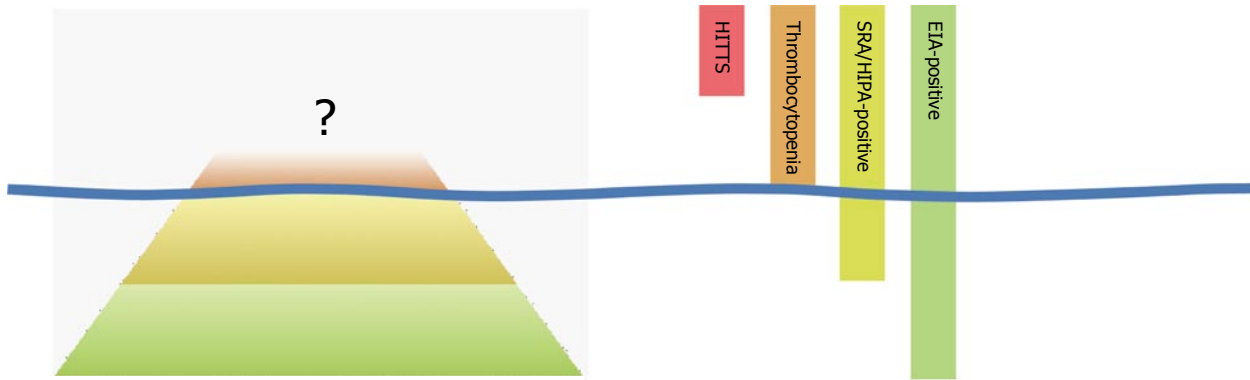


Figure 2 Modified iceberg model of the assumed frequency of antibody seroconversion, activating heparin-induced thrombocytopenia antibodies (serotonin release assay/heparin induced platelet activation assay), thrombocytopenia, and clinically manifest heparin-induced thrombocytopenia thrombotic syndrome according to the current knowledge on heparin-induced thrombocytopenia in solid organ transplant recipients. HIT: Heparin-induced thrombocytopenia.

the recipients had one inevitably heparin exposure during surgery due to the usage of UFH during organ procurement. These reports consistently confirm the hypothesis that the risk of early-onset HIT after heparin re-exposure is small after cessation of heparin more than 100 d prior to surgery^[53,56,57].

According to the current knowledge as depicted in this review we suggest that: A patient with a history of HIT more than 100 d ago and negative anti-heparin/PF4 EIA and SRA/HIPA can be re-exposed to heparin during surgery for organ transplantation; organs from donors treated with heparin can be transplanted to these patients; organs rinsed with heparin can be transplanted to these patients; and patients with a history of HIT need not be delisted from the waiting-list.

To this day, only few systematic investigations on HIT in solid organ transplant recipients (after transplantation) have been published. Thereof, most data exist on anti-PF4/heparin antibody seroconversion after liver transplantation. The most conclusive studies consistently report on no HIT-associated thromboembolic events despite anti-PF4/heparin antibodies in EIA between 1.9% to 57.9% and continuation of heparin therapy^[49,59-61].

Available research shows that on the one hand immunosuppressed solid organ transplant recipients are capable to develop anti-PF4/heparin antibodies, and on the other hand apparently do not suffer from HIT according to the "classic" definition and as displayed in the iceberg model^[9-11]. These findings could potentially be displayed carefully in an adjusted iceberg model with a broad basis below the waterline but apparently only little mass and no summit above (Figure 2). Until now research has not provided any reliable information on clinically apparent HIT in this special cohort, which is displayed by the question mark in the depiction. Nevertheless, we point out that this illustration has to be handled with care as strong evidence from comprehensive prospective trials is missing.

Routine screening for anti-PF4/heparin antibody seroconversion is not recommended to avoid an increase in false-positive results with unnecessary change of anticoagulation^[7,12,47,49]. The true incidence of HIT after solid organ transplantation and its morbidity and mortality appears to be rather low^[49,59-61]. Nonetheless, cardiac transplant recipients possibly have the highest risk of developing HIT among transplanted patients in general^[49].

In the absence of large prospective studies, no conclusive recommendations on the acute therapeutic management of HIT-suspected recipients can be provided besides switching to heparin-free anticoagulation.

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Imaging-based diagnosis of acute renal allograft rejection

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Abstract

Kidney transplantation is the best available treatment for patients with end stage renal disease. Despite the introduction of effective immunosuppressant drugs, episodes of acute allograft rejection still endanger graft survival. Since efficient treatment of acute rejection is available, rapid diagnosis of this reversible graft injury is essential. For diagnosis of rejection, invasive core needle biopsy of the graft is the "gold-standard". However, biopsy carries the risk of significant graft injury and is not immediately feasible in patients taking anticoagulants. Therefore, a non-invasive tool assessing the whole organ for specific and fast detection of acute allograft rejection is desirable. We herein review current imaging-based state of the art approaches for non-invasive diagnostics of acute renal transplant rejection. We especially focus on new positron emission tomography-based as well as targeted ultrasound-based methods.

Key words: Acute allograft rejection; Imaging; Positron emission tomography; Ultrasound; Magnetic resonance imaging; Single photon emission computed tomography; Kidney transplantation; Renal

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Core tip: Kidney transplantation is the best available treatment for patients with end stage renal disease. For diagnosis of rejection, invasive core needle biopsy of the graft is currently considered as the "gold-standard". As biopsies carry the risk of significant graft injury, a non-invasive, specific and fast tool screening the whole graft for acute rejection is desirable. We herein review current imaging-based state of the art approaches for non-invasive diagnosis of acute kidney allograft rejection, focussing particularly on new positron emission tomography-based as well as targeted ultrasound-based methods.

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INTRODUCTION

Kidney transplantation (KTx) is the favorable treatment for patients suffering from end stage renal disease (ESRD)^[1]. Although modern immunosuppressive regimens offer good patient and graft survival rates, acute rejection (AR) after KTx remains a serious problem significantly limiting both graft and patient survival^[2,3].

Therefore, early detection and treatment of AR is necessary. To date, renal biopsy is the "gold-standard" to diagnose AR, but might jeopardize allograft recipients due to its invasive character.

Thus, non-invasive techniques for detection of AR are desired. During the last decades, medical imaging techniques have improved tremendously. Novel methods do not only focus on structural details, but also visualize functional processes.

This review focuses on the current non-invasive imaging techniques to detect AR which might replace renal biopsies in the future.

ULTRASOUND

Sonographic allograft examination is part of the standard care of transplanted patients. This procedure detects allograft swelling, morphological changes, abatement of corticomedullary differentiation, alterations of echogenicity and distinctive structures such as medullary pyramids; renal blood circulation can be analyzed by means of Doppler ultrasound and contrast-enhanced ultrasound examination. While the method is cost-effective and widely available, it still has considerable limitations in sensitivity and specificity for the diagnosis of AR.

New approaches might overcome these caveats. The resistive index (RI) is a noninvasive method using the vascular resistance and elastic compliance to evaluate the function of the allograft. Unfortunately, the RI measured in the allograft is influenced by systemic parameters like the vascular compliance, pulse pressure, heart rate and rhythm. Due to progressing arteriosclerotic processes of the vascular system, older recipient age is the strongest determinant for a higher RI^[4]. Higher RIs are also associated with antibody-mediated rejection and acute tubular necrosis in index biopsies^[4], and RIs of 0.8 or higher are associated with decreased patient survival^[4,5]. However, data on the correlation between RI and allograft outcome are unequivocal^[4-6].

Recently, another non-invasive index for the

prediction of AR has been developed on the base of contrast-enhanced ultrasonography (CEUS). It includes CEUS factors such as rising time, time to peak and delta-time among regions of interest^[7].

Acoustic radiation force impulse imaging (ARFI) assesses tissue elasticity and was utilized to identify AR in a small series of 8 patients. ARFI-values were elevated by more than 15% in patients undergoing AR, when compared to other causes of allograft damage^[8]. However, the method has not been evaluated by others and is not used in clinical routine yet.

An experimental but promising procedure is the use of microbubbles targeting T-lymphocytes. The accumulation of T cells during AR can be visualized *via* microbubbles coupled to anti-CD3 antibodies (Figure 1)^[9]. The method allows differential diagnosis of AR with high specificity.

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is another non-invasive method to evaluate kidney allograft function. MRI is based on the detection of signals from hydrogen nuclei or protons changing their magnetic behaviour in response to altered magnetic fields in the MRI system, and can reveal various tissue characteristics, including intrinsic MR properties like the relaxation times T_1 and T_2 ^[10]. An important advantage of MRI is the high spatiotemporal resolution, which allows the precise visualization of anatomical structures as well as functional assessment of the graft. MRI allows the detection of distinctive features of vascular and interstitial structures, thereby discriminating between different mechanisms of renal allograft injury such as AR or acute tubular necrosis (ATN)^[11]. In the field of nephrology, various MRI techniques can be used to visualize different pathophysiological processes^[10].

Dynamic contrast enhanced MRI (DCE MRI) is a common MRI method involving the use of a contrast agent. DCE MRI using gadolinium-based contrast agents is also termed MR renography (MRR). The contrast agents are freely filtered at the glomeruli but are not secreted or reabsorbed in the tubules. Therefore they can optimally be used to quantify renal perfusion, glomerular filtration rate (GFR) and tubular function, which helps to distinguish between AR and ATN^[11]. The assessment involves the measurement of cortical and medullary blood flow within the graft after administration of contrast agent. In contrast to normal grafts, the cortical and medullary blood flow is significantly reduced in grafts experiencing AR. The predominantly reduced medullary blood flow seems to be characteristic for AR and helps to differentiate between AR and ATN^[12].

Identification of and discrimination between various mechanisms of allograft damage is also possible by using a tracer kinetic renal model which determines the mean transit time (MTT) of a tracer through the

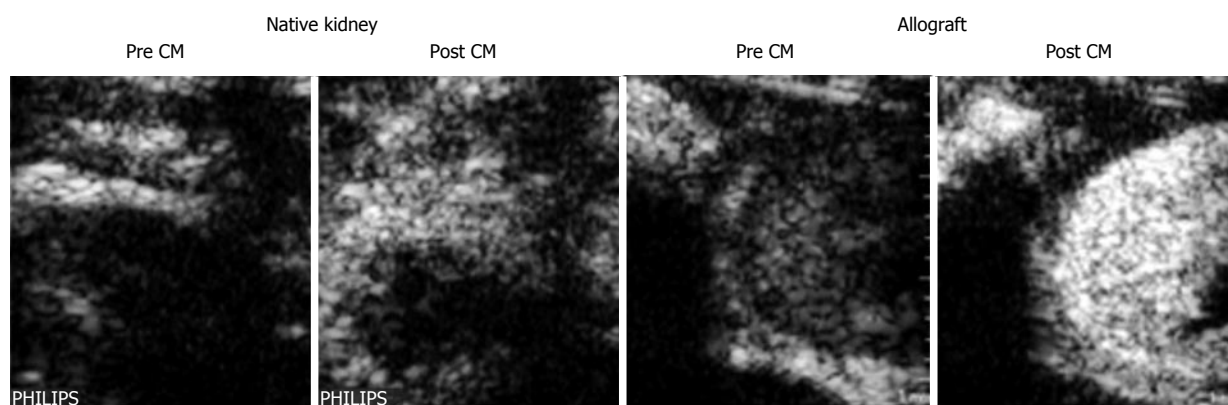


Figure 1 Representative ultrasound images of an allogeneically transplanted (aTX) rat kidney (graft) and its native control kidney (native) on day 4 post surgery. Depicted are examples of transversal images taken before (pre CM) and 15 min after (post CM) tail vein injection of anti-CD3-antibody labeled microbubbles. CM: Contrast media/microbubbles conjugated to anti CD3 antibody.

different compartments of the kidney^[13]. However, although differences in the fractional MTT values between normal grafts or grafts undergoing AR or ATN have been observed, substantial overlaps among these groups and with healthy control kidneys exist. Moreover, the rare but characteristic risk of gadolinium-induced nephrogenic systemic fibrosis needs to be considered^[14].

Another MRI technique which is independent from contrast agent usage is diffusion-weighted MRI (DWI MRI). DWI MRI depends on the signal decay that is induced by the relative diffusion-based displacement of water molecules, which can be quantified by calculating the so called apparent diffusion coefficient (ADC). The ADC is influenced by the tissue microstructure and does not account for directionality of molecular motion. To address this issue of anisotropic diffusion properties due to the radial orientation of main anatomic structures like vessels and tubules, the more sensitive diffusion tensor imaging (DTI) has been applied^[15]. DTI allows the assessment of the fractional anisotropy (FA) of tissues, thereby considering the directionality of diffusion. Recently, the role of diffusion-weighted MRI for differentiation between AR and ATN was discussed, and new automated segmentation protocols might be helpful^[16].

The differentiation between AR and ATN might also be possible by applying blood-oxygen level-dependent (BOLD) MR^[17-19]. This method utilizes the paramagnetic effects of deoxyhemoglobin. Deoxyhemoglobin is increased in tissues with lower oxygen concentration and shortens the transverse relaxation time constant $T2^*$. Inversely, the apparent relaxation rate, $R2^*$ ($= 1/T2^*$), is elevated. Therefore, BOLD MR can serve as a non-invasive technique to evaluate the renal parenchymal oxygenation concentration. In kidneys displaying AR, a significantly lower medullary $R2^*$, corresponding to a higher oxygenation, was observed compared to ATN^[18,20].

Arterial spin labeling (ASL) MRI is another approach to assess allograft function especially for longitudinal

perfusion evaluation. ASL MR utilizes arterial blood as an endogenous contrast agent. Inflowing blood is selectively labeled by altering its longitudinal magnetization to have an opposite magnetization compared to the destination tissue. The difference between a labeled image (tag) and a non-labeled image (control) can be used to determine tissue perfusion. ASL MR has successfully been applied to examine native and transplant kidneys. ASL studies using a flow sensitive alternating inversion recovery (FAIR-ASL) scheme (for details see^[21]) revealed a significant lower overall or medullary perfusion in allografts when compared to healthy kidneys for subjects with $eGFR > 60$ mL/min per 1.73 m² or with $eGFR < 60$ mL/min per 1.73 m² respectively^[22]. Also, a significant lower cortical perfusion in renal grafts with acute decrease in renal function was observed when compared to allografts with good postoperative and long-term function^[23].

Given the need for non-invasive diagnosis of renal inflammation, several studies used nanoparticles to detect specific immune cells or immune proteins in the kidney (for review see^[24]). In the context of renal transplantation, Hauger *et al.*^[25] and Chae *et al.*^[26] reported successful usage of super magnetic iron oxide (SPIO) particle-loaded macrophages to differentiate between various causes of graft failure. Accumulation of iron particles in the kidney during AR was shown 3 and 5 d after application, respectively. Unfortunately, non-phagocytic cells such as T-cells generally have a low labeling efficiency and poor contrast agent incorporation, which limits cellular MR imaging *in vivo*. Recently, Liu *et al.*^[27] reported a new synthesized class of MRI contrast agent, IOPC-NH₂ particles, for labeling of T-cells in allograft rejection in a rat model of heart-lung transplantation. This technique might represent an approach for potential clinical translation of MRI-based tracking of non-phagocytic cells, such as T- and B-lymphocytes.

Various MRI techniques including BOLD, DWI and ASL have been combined in several longitudinal

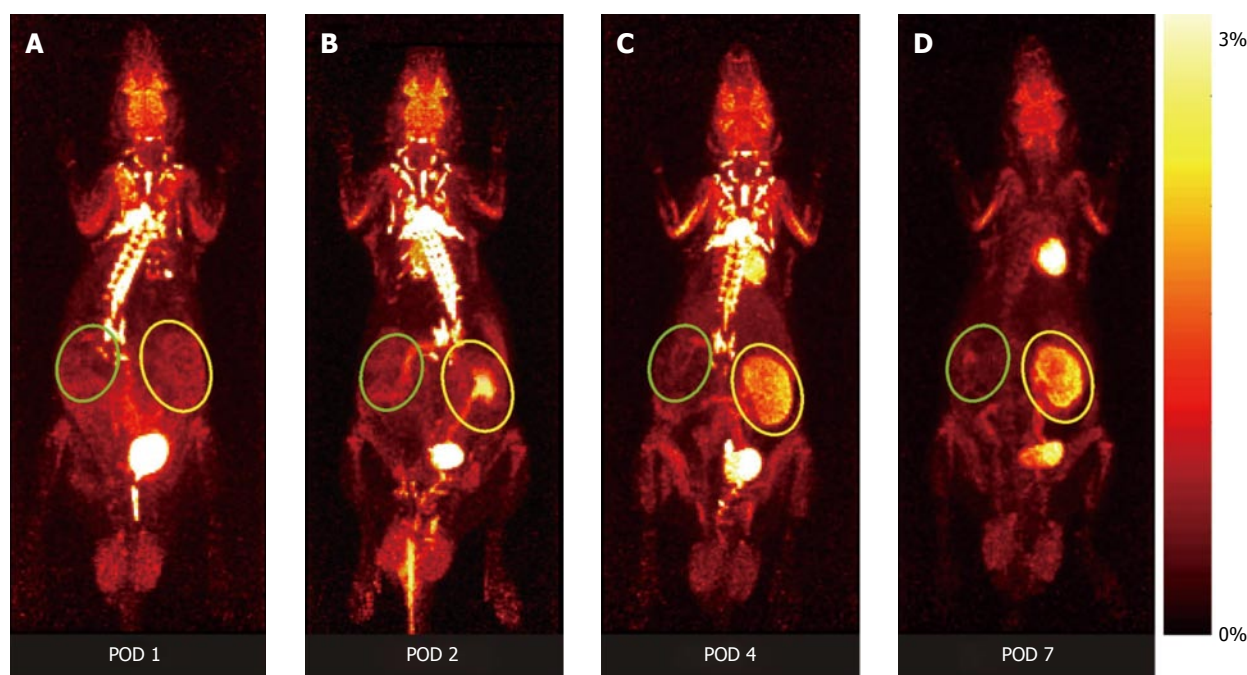


Figure 2 Representative positron emission tomography-images of dynamic whole body acquisitions of a series of an allogeneically transplanted rat [postoperative day 1 (A), 2 (B), 4 (C), and 7 (D)], after tail vein injection of 30 MBq ^{18}F -fluorodeoxyglucose (maximum a posterior projection, 180 min pi). While the allograft undergoing rejection shows distinct enhancement of ^{18}F -FDG (yellow circle) the native control kidney without rejection does not (green circles). Figure taken from^[44]. POD: Postoperative day; FDG: Fluorodeoxyglucose.

studies, but case numbers were low and results were contradictory^[28,29]. Further longitudinal studies with larger sample sizes are needed to determine the value of the different MR techniques for the evaluation of long-term allograft function.

POSITRON EMISSION TOMOGRAPHY

Positron emission tomography (PET) is an imaging procedure based on the detection of internal radiation. After administration of an intravenous radioactive tracer, gamma rays emitted by the tracer are recorded by an external detector system called gamma camera. PET enables whole body visualization with high intrinsic sensitivity and provides high specificity although only very low concentrations of the tracer are needed^[30,31]. The method offers a spatial resolution of 3-5 mm and generates 3D images^[32]. Metabolic and cellular processes like pH-changes, apoptosis, inflammation and infection can be visualized^[33].

The use of ^{18}F -fluorodeoxyglucose (FDG) for scintigraphic detection of glucose metabolism was published in 1978^[34] and became the mainly used radionuclide in PET. After injection of the tracer, ^{18}F -FDG enters the cell using glucose transporters like GLUT1. ^{18}F -FDG acts like a glucose analogue and correlates with the metabolic activity of the cell. After phosphorylation of ^{18}F -FDG, it cannot be further metabolized and is entrapped in cells with a high metabolism. The biodistribution of ^{18}F -FDG can be assessed by PET^[35]. ^{18}F -FDG-PET is a well-established method used in clinical diagnostic. However, PET

with glucose-based radionuclides is not specific for a particular disease and needs to be evaluated in the clinical context. For example, the uptake of ^{18}F -FDG depends on the presence of glucose transporters which are upregulated under several conditions, like inflammation and tumor genesis. The application field of PET has extended over the last years, and ^{18}F -FDG-PET has successfully been used in many pathological processes like cancer^[36-38], vasculitis^[39], fever of unknown origin^[40], asthma^[41], cystic fibrosis^[42], and organ transplantation^[43-46].

Recently, our group was able to non-invasively assess renal function by ^{18}F -fluoride clearance and to monitor graft inflammation by ^{18}F -FDG^[43,47]. This PET method allows the visualization of molecular and cellular processes characteristic for AR, e.g., the assessment of metabolic activity of recruited leucocytes, hypoxia cell death, as well as allograft function. The pattern of the ^{18}F -FDG-uptake during AR indicates a state of increased metabolism, driven by inflammatory cells (Figure 2). The specific distribution pattern of cell activity allows the discrimination of AR from other pathological conditions in both a rat renal transplantation model and in transplanted patients^[44,48]. Despite specific signals in kidney allografts undergoing AR, the clearance of ^{18}F -FDG has to be taken into account. ^{18}F -FDG signals derived from urinary tracer remnants within the urinary pelvis can be avoided by extending the time between the application of the tracer and the PET procedure, or by simply using ^{18}F -FDG labelled T-cells^[44,49]. As ^{18}F -FDG uptake by renal allografts immediately decreases after

successful treatment of AR, the method might also be used to monitor treatment efficacy^[43].

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

Single photon emission computed tomography (SPECT) is another nuclear imaging-based method for the detection of AR in kidney allografts. Similar to PET, SPECT provides functional rather than morphological data, but while PET captures an indirect signal (pairs of gamma rays resulting from annihilation of the emitted positrons with electrons) SPECT directly measures gamma radiation from the deployed radioisotopes. Although PET provides higher spatial resolution^[32], better sensitivity and better quantification, SPECT is still the most commonly used technique. Beside its high availability and the wide range of adequate radionuclides, the cost-effectiveness is a noteworthy advantage of SPECT^[50]. Regarding the available tracers used to visualize metabolic processes as well as cellular and molecular events, the generally longer half-lives of SPECT radionuclides are of additional advantage, as they better correspond to the duration of the investigated biological processes. Common markers in SPECT are ¹¹¹In, ⁶⁷Ga, ¹²³I and ^{99m}Tc, the latter offering the broadest application spectrum because of its relatively simple production, availability and optimal decay characteristics compared to the rather unstable and short-lived PET tracers^[51]. However, the more complex incorporation process of ^{99m}Tc into a molecule which is impeded by involvement of chelating moieties and possible steric hindrance needs to be mentioned. Thus, thorough definition and characterization of the respective processes to be examined is necessary in order to choose the appropriate tracer.

The broad application field of SPECT imaging in numerous diseases has continuously expanded during the last years. Existing technologies have been optimized and new, more sophisticated approaches have been evolved. Particular in oncology, lots of different strategies have been introduced facilitating SPECT-based diagnosis and therapeutic monitoring in oncological patients^[52-54]. Moreover, processes like tissue injury, cell death or angiogenesis in cardiac and pulmonary diseases^[55-57], as well as specific bacterial infections^[58], inflammation severity in rheumatoid arthritis^[59] and neurological disorders^[60-62] can be detected and monitored with increasing precision.

According to the various pathophysiological mechanisms involved in AR after kidney transplantation, different markers for SPECT imaging have been developed during the last decades. The general principles of detecting the diverse pathophysiological processes and their implementation in PET-based diagnosis have already been discussed above. Many of these processes can be assessed by SPECT as well.

As early as in 1976, George *et al.*^[63] were able to

visualize kidney allograft rejection using ^{99m}Tc-sulfur colloid, which accumulates in areas of fibrin thrombi in acute and chronic rejecting allografts.

As leukocyte recruitment plays a crucial role in allograft rejection, many attempts to label various cell lines *ex vivo* and *in vivo* have been made. Common markers used for radiolabelling white blood cells in SPECT are ^{99m}Tc-HMPAO or ¹¹¹In-oxine^[64-66]. Compared to ¹⁸F-FDG, these markers are more stable, have a longer half-life time and therefore should be used for sustained biological processes^[67]. Labeling efficiency and viability of the marked cells are additional concerns. Whereas the labeling rate of ¹⁸F-FDG is only about 60%, ¹¹¹In-oxine and the PET marker ⁶⁴Cu exhibit are more efficient and have labeling rates of approximately 80%. Viability of the cells was shown to be comparable within the first four hours for ¹¹¹In-oxine, ^{99m}Tc-HMPAO, ⁶⁴Cu and ¹⁸F-FDG, while a significant decline of cell survival was observed after 24 h^[68]. Regarding kidney transplantation, the use of ^{99m}Tc-HMPAO-labeled mononuclear cells has been shown to differentiate between rejection and ATN^[69].

Different ^{99m}Tc-, ¹¹¹In- or ¹²³I-labeled antibodies binding to cell surface markers of different immune cells, like CD3, CD4, CD20 or CD25 have been developed for *in vivo* imaging (for review see^[31]). Detection of AR in kidney transplantation is possible by using ^{99m}Tc-OKT3, a mouse monoclonal antibody against the CD3 complex, which targets T cells, natural killer cells and natural killer T cells^[70]. Side effects of this antibody due to its immunogenicity have been eliminated by using a humanized form, ^{99m}Tc-SHNH-visilizumab^[71,72]. Further studies are needed to evaluate its utility in diagnosing AR.

A high-affinity radiolabelled ligand binding to FPR1, a leukocyte receptor which is involved in chemotaxis and inflammatory responses, has recently been reported as a novel method to detect leukocyte accumulation in inflammation. FPR1 is upregulated during inflammation, and the ^{99m}Tc-labeled FPR1 antagonist cFLFLK-NH₂ has been shown to bind to FPR1 without interfering with the inflammatory processes^[73].

Sharif-Paghaleh *et al.*^[74] published a reporter gene mediated method of radiolabelling regulatory T cells with Technetium-99m pertechnetate (^{99m}TcO₄⁻) *in vitro* and *in vivo*, enabling the precise visualization of the cells as long as they are vital. This method might become a useful tool in the transplant setting as well.

Besides accumulation of immune cells, complement activation is another mechanism which plays an important role in the pathophysiology of transplantation. Recently Sharif-Paghaleh *et al.*^[75] successfully demonstrated non-invasive imaging of complement activation following ischemia-reperfusion injury (IRI) in a model of cardiac transplantation, using ^{99m}Tc-recombinant complement receptor 2 (^{99m}Tc-rCR2). As IRI and complement activation *per se* are involved in transplant rejection and complement inhibitors have been developed as a therapeutic option, this principle

could be a useful tool to identify tissue damage after transplantation, to allow patient risk stratification and to monitor the effects of therapeutic interventions.

SPECT imaging can also be applied for monitoring of allograft function. While static imaging using ^{99m}Tc -dimercaptosuccinic acid (DMSA) can visualize functioning kidney tissue and anatomical abnormalities^[76,77], dynamic imaging with ^{99m}Tc -diethylenetriaminepentaacetic (DTPA) or ^{99m}Tc -mercaptoacetyltriglycine (MAG3) further allows detection of AR and discrimination from ATN^[78-81].

DISCUSSION

Although core needle biopsy of the kidney allograft is still the gold standard to discriminate causes of renal injury, imaging of immunological processes offers promising, novel and non-invasive possibilities. As perfect imaging depends on severity of rejection, imaging-based methods still suffer from low sensibility^[82]. Currently, PET and SPECT are able to discriminate ATN from AR. Unfortunately, differentiation between different forms of AR, namely acute antibody mediated rejection (ABMR) and T cell-mediated rejection (TCMR), has not been tested sufficiently in preclinical imaging studies so far. As both entities are treated differently, the discrimination between both is of high clinical relevance. Identification and assessment of discriminating targets like T cells (TCMR) or C4d (ABMR) might support further differential diagnostics. The ultrasound visualization of T-cells by use of microbubbles coupled to anti-CD3 antibodies is a first approach for specific diagnostics of TCMR^[9]. MRI-based assessment of IOPC-NH2 labeled T-cells is based on the same principle and has been shown to be useful for the detection of rejection of a heart-lung transplant^[27]. New biomarkers, like cell free DNA, microRNA, chemokines, clusters of differentiation or tubular injury markers that correlate with AR, might provide additional information. Unfortunately, most of these markers are time-consuming, expensive and do not distinguish between subclinical tubulitis, BK virus infection and different forms of AR. Nevertheless, some of these approaches, like a combination of monitoring urinary CXCL10:creatinine ratio and donor specific antibodies, might significantly improve the noninvasive diagnosis of ABMR^[83]. An approach involving the use of biomarkers as well as non-invasive imaging, might improve sensitivity as well as specificity for the detection of renal allograft AR.

CONCLUSION

Non-invasive methods for specific diagnosis of AR and surveillance monitoring of the allograft are highly desired. Advances in technology and tracer development provide new diagnostic options. At present most of the promising new imaging technologies are still used at a pre-clinical stage, but represent very useful research tools on the way into clinical use. Future

studies in human allograft recipients are needed to fully support these methods for clinical routine.

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Immunosuppressive potency of mechanistic target of rapamycin inhibitors in solid-organ transplantation

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Abstract

Mammalian target of rapamycin, also known as mechanistic target of rapamycin (mTOR) is a protein kinase that belongs to the PI3K/AKT/mTOR signaling pathway, which is involved in several fundamental cellular functions such as cell growth, proliferation, and survival. This protein and its associated pathway have been implicated in cancer development and the regulation of immune responses, including the rejection response generated following allograft transplantation. Inhibitors of mTOR (mTORi) such as rapamycin and its derivative everolimus are potent immunosuppressive drugs that both maintain similar rates of efficacy and could optimize the renal function and diminish the side effects compared with calcineurin inhibitors. These drugs are used in solid-organ transplantation to induce immunosuppression while also promoting the expansion of CD4+CD25+FOXP3+ regulatory T-cells that could favor a scenery of immunological tolerance. In this review, we describe the mechanisms by which inhibitors of mTOR induce suppression by regulation of these pathways at different levels of the immune response. In addition, we particularly emphasize about the main methods that are used to assess the potency of immunosuppressive drugs, highlighting the studies carried out about immunosuppressive potency of inhibitors of mTOR.

Key words: Everolimus; Immunosuppression; Mechanistic target of rapamycin inhibitor; Rapamycin; Tolerance

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Core tip: Inhibitors of mechanistic target of rapamycin (mTOR), rapamycin and its derivative everolimus, have been used as immunosuppressive drugs during the last decade. Several reviews have been written on the use of these drugs compared to classical calcineurin inhibitors, however few has been reviewed about

immunosuppressive potency of such compounds. Our aim is to summarize the principal studies about potency of the immunosuppressants, highlighting the studies carried out with inhibitors of mTOR.

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INTRODUCTION

The elucidation, at the molecular level, of T-cell-mediated rejection, explained by the three-signal model of lymphocyte activation, has facilitated the development of novel immunosuppressive drugs (Figure 1). Advances in immunosuppressive therapy have had a great impact on the evolution and success of solid-organ transplantation. Rejection responses after transplantation can be minimized by optimally matching major histocompatibility complex (MHC) antigens, by administration of drugs that generally suppress the immune system, or by inducing a state of tolerance^[1]. With the introduction of newer immunosuppressive pharmacological agents, the incidence of acute cellular allograft rejection has decreased to low levels, and one and five-year patient survival rates are approaching 85% and 68%, respectively, with a 10-year survival closer to 50%^[2].

Immunosuppressive drugs can be classified into two categories: Biologic agents, such as polyclonal and monoclonal anti-lymphocyte antibodies; and pharmacological or small-molecule drugs, such as corticosteroids and inhibitors of nucleotide synthesis, calcineurin inhibitors or mammalian target of rapamycin inhibitors (mTORi) (Table 1 and Figure 1)^[1,3]. These drugs are used in combinations that are intended to maximize immunosuppression while reducing the adverse effects of each individual drug^[4].

Calcineurin inhibitors (CNI), such as tacrolimus and cyclosporine, have become the cornerstone of immunosuppressive therapy in solid organ transplantation^[5]. Their use resulted in lower rejection rates and improved short-term patient and allograft survival rates. However, long-term improvements in graft survival have been more difficult to achieve with these drugs. The main reason for this observation is that prolonged CNI exposure is associated with nephrotoxicity^[6], neurotoxicity^[7], increased risk for cancer^[8], metabolic complications^[9], and hypertension^[10], which are an important cause of long-term morbidity and mortality. Nevertheless, the limitation in the long-term survival of patients with transplantation depends on other factors

not directly related to the immunosuppression, such as recurrence of basal disease and death with a functioning graft for reasons beyond to the own transplantation. Reducing CNI exposure is the main strategy to lower these adverse events, for example combining immunosuppressants with different mechanism of action to minimize the adverse events while maintaining immunosuppressive efficacy.

The mTORi, such as rapamycin and its derivate everolimus, are powerful nonnephrotoxic agents with a different toxicity profile respect to CNI, specially affecting to a gastrointestinal, respiratory and hematological level, in addition to a different mechanism of action than CNI. Meanwhile CNI block the production of proinflammatory cytokines such as IL-2 and, subsequently, inhibition of T-cell activation, mTORi reduce T-cell activation later in the cell cycle by blocking growth-factor-mediated cell proliferation in the cellular response to alloantigen^[11,12] (Figure 1). The distinct mechanism of action and favorable nephrotoxicity profile has led to mTORi-containing regimens being developed with the aim of minimizing, eliminating, or avoiding exposure to CNI, although many trials failed because of the high incidence of antibody-mediated rejection^[13].

Rapamycin is an immunosuppressive drug that was approved by the United States Food and Drug Administration (FDA) in 1999 and by the European Medicines Agency (EMA) in 2000 as an immunosuppressive agent for renal transplantation patients once its T-cell suppression characteristics were recognized^[14]. Later, everolimus was approved in 2003 for the prophylaxis of organ rejection in kidney and heart transplant recipients in many European countries, followed by FDA approval for kidney transplantation in 2010^[15]. Everolimus was developed to improve the pharmacokinetic profile of rapamycin. At position 40 of the rapamycin molecule, everolimus has a covalently bound 2-hydroxyethyl group that provides a pharmacokinetic advantage, conferring faster absorption and a shorter half-life in comparison to rapamycin^[16,17]. These properties allow everolimus to be formulated as an oral agent, while maintaining immunosuppressive and anti-neoplastic activities similar to rapamycin^[18,19]. In addition, unlike rapamycin, no loading dose is required for everolimus, and the twice-daily dosing schedule enables accurate dose adjustments^[20].

In this review, we summarize some of the main methods that are used to assess the potency of immunosuppressive drugs, highlighting the studies about immunosuppressive potency of mTORi.

ROLE OF mTOR IN THE IMMUNE RESPONSE AND EFFECTS OF mTORi IN THE IMMUNE SYSTEM

mTOR is a protein kinase involved in the signal 3

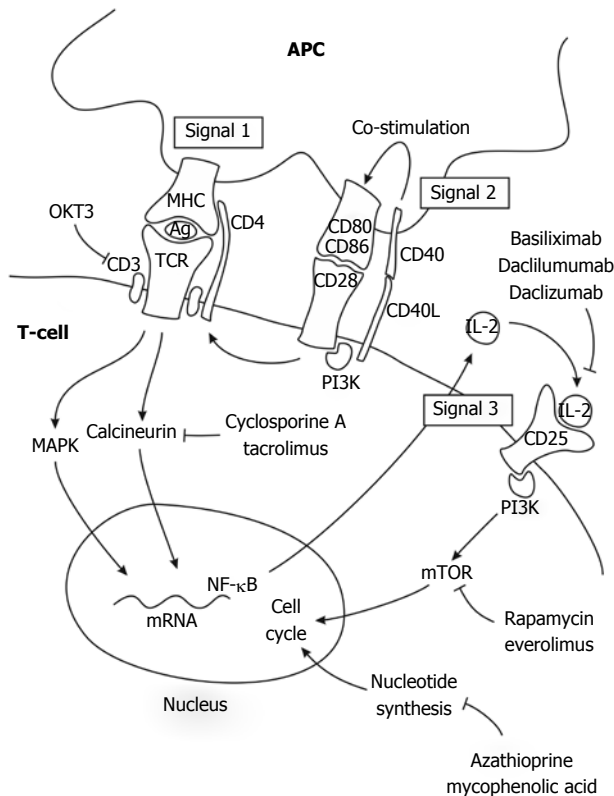


Figure 1 Three-signal pathway of lymphocyte activation and targets of inhibitory agents. The elucidation of lymphocyte activation pathways has facilitated the development of novel immunosuppressive drugs. At the molecular level, T-cell-mediated rejection is explained by the three-signal model of lymphocyte activation. Signal 1 occurs when alloantigen-bearing APCs engage alloantigen-reactive naïve and memory T-cells and trigger their activation; alloantigen recognition is transduced through the TCR-CD3 complex. Signal 2 occurs when CD80 and CD86 on the surface of APCs engage CD28 on T-lymphocytes, providing T-lymphocyte co-stimulation. Together, signals 1 and 2 activate several signal transduction pathways, including the calcium-calcineurin pathway, the MAPK pathway, and the NF- κ B pathway, which in turn, trigger the expression of many cytokines. Several of these cytokines (IL-2, IL-4, IL-7, IL-15, and IL-21) induce proliferation (signal 3) through PI3K and mTOR pathways. Ag: Antigen; APC: Antigen-presenting cell; MAPK: Mitogen-activated protein kinase; MHC: Major histocompatibility complex; mTOR: Mechanistic target of rapamycin; NF- κ B: Nuclear factor kappa B; PI3K: Phosphatidylinositol 3-kinase; TCR: T-cell receptor.

pathway of lymphocyte activation^[3] (Figure 1). More specifically, mTOR belongs to the PI3K pathway, which is involved in several fundamental cellular functions such as cell growth, proliferation, and survival. The mTOR protein interacts with several proteins to form two distinct complexes: mTOR complex 1 (mTORC1) and 2 (mTORC2)^[21]. Both complexes share the catalytic mTOR subunit, mammalian lethal with Sec13 protein 8 (mLST8), DEP domain-containing mTOR-interacting protein (DEPTOR), and the Tti1/tel2 complex. Furthermore, mTORC1 is composed uniquely of regulatory-associated protein of mTOR (RAPTOR) and the proline-rich AKT substrate 40 kDa (PRAS40). By contrast, mTORC2 uniquely contains the scaffolding protein rapamycin-insensitive companion

of mTOR (RICTOR), mammalian stress-activated map kinase-interacting protein 1 (mSIN1), and the protein observed with RICTOR 1 and 2 (PROTOR1/2)^[21]. Located adjacent to the kinase domain of mTOR is the FKBP12-rapamycin-binding (FRB) domain^[22].

mTORC1 participates in the translocation and synthesis of cell-cycle regulating and ribosomal proteins, as well as the synthesis of lipids that are required for proliferating cells to generate membranes^[23-25]. However, mTORC2 activates protein kinase B (AKT), which is the central mediator of the PI3K pathway and promotes cell growth and survival *via* several mechanisms^[26] (Figure 2).

In addition, mTOR has an important role as a central regulator of the immune response, functioning as a central node in a signaling cascade that directs the integration of diverse environmental inputs in the immune microenvironment. mTOR regulates the function of diverse immune cell types, including dendritic cells, B cells or regulatory and effector T-cells^[27-30].

mTORi (rapamycin and everolimus) are immunosuppressive drugs that interact with and inhibit mTOR, but only when it is part of mTORC1 and not mTORC2^[21]. These drugs bind to the cytosolic protein FKBP12. This complex binds to the FRB domain of mTOR, which blocks the ability of RAPTOR to bind to mTOR, thereby inhibiting formation of mTORC1^[31]. However, prolonged treatment with rapamycin has also revealed the inhibition of mTORC2 signaling^[32]. Rapamycin mediates immunosuppressive effects through multiple immune cell types and processes. Inhibition of mTOR by rapamycin suppresses the immune response by preventing cell cycle progression from G1 to S phase, thereby blocking proliferation^[33]. In addition, rapamycin can promote T-cell anergy independently of the inhibition of proliferation even in the presence of TCR activation and co-stimulation by CD28 and IL-2^[34,35].

Rapamycin inhibits the ability of dendritic cells to mature into APCs that can strongly stimulate T-cells. Immature dendritic cells promote the expansion of regulatory T-cells while concomitantly suppressing conventional T-cell responses by inducing T-cell anergy and apoptosis, thus promoting tolerance to the graft^[36]. Furthermore, rapamycin has beneficial effects on the survival and proliferation of regulatory T-cells^[37]. Many studies have confirmed the beneficial effects of rapamycin or everolimus on regulatory T-cell biology^[38-40]. By contrast, CNI impair the number, function and phenotype of regulatory T-cells, potentially acting as a barrier to the achievement of host tolerance to an allograft^[38,39,41]. However, this issue is controversial, because some studies have shown how CNI does not affect or improve the expansion of Treg^[42,43]. Likewise, everolimus can inhibit humoral responses both directly, by suppressing B cell proliferation and differentiation, and indirectly, by suppressing T-cell help^[44,45].

Table 1 Classification of biological and pharmacological immunosuppressive agents^[1,3]

Biologic immunosuppressive agents	Function
Lymphocyte-depleting agents	
Monoclonal anti-CD20 (rituximab)	Depletion of B-cells
Monoclonal anti-CD52 (alemtuzumab)	Depletion of T-cells, monocytes, macrophages and natural killer cells
Monoclonal anti-CD3 (OKT3)	Interference with signal 1 in T-cells
Anti-thymocyte globulin	Interference with signals 1, 2 and 3 in T-cells
Non-lymphocyte-depleting agents	
Anti-IL-2 receptor (basiliximab, daclizumab)	Inhibition of T-cell proliferation and signal 3
Belatacept	Inhibition of signal 2 in T-cells (competition with CD28 for CD80/CD86 binding) inhibiting T-cell co-stimulation
Daclizumab	Inhibition of signal 2 in T-cells (binds to CD25, the alpha subunit of the IL-2 receptor) preventing IL-2-induced T-cell activation
Pharmacological drugs	Function
Corticosteroids	Inhibition of cytokine transcription by APCs
Azathioprine	Inhibition of nucleotide synthesis, blocking lymphocyte proliferation
Mycophenolic acid	Inhibition of nucleotide synthesis, blocking lymphocyte proliferation
Calcineurin inhibitors (cyclosporine A, tacrolimus)	Inhibition of signal 2 transduction in T-cells [inhibits calcineurin <i>via</i> cyclophilin (cyclosporine A) or <i>via</i> FKBP12 (tacrolimus)], blocking IL-2 transcription
FK778 (manitimus)	Inhibits dihydro-orotate dehydrogenase, interrupting <i>de novo</i> pyrimidine synthesis, thereby acting on both B-cells and T-cells beyond the early S phase of the cell cycle, differentially from calcineurin inhibitors
mTOR inhibitors (rapamycin, everolimus)	Inhibition of signal 3 transduction in T-cells (inhibits mTOR), preventing IL-2-induced T-cell proliferation

APC: Antigen-presenting cell; IL-2: Interleukin-2; mTOR: Mammalian target of rapamycin.

METHODS TO MEASURE IMMUNOSUPPRESSIVE POTENCY. SCIENTIFIC EVIDENCE FOR THE IMMUNOSUPPRESSIVE AND IMMUNOREGULATORY POTENCY OF mTORi IN TRANSPLANTATION

No standardized methods are available to measure the immunosuppressive potency of drugs that are used to improve transplantation outcomes. To date, routine clinical use of immunosuppressive drugs has relied on blood concentration measurements (pharmacokinetics) rather than on biologically relevant analysis of drug effects on immune-cell function (pharmacodynamics)^[46,47]. However, several methods are used to evaluate and monitor the pharmacodynamics of immunosuppression in transplantation in the context of research studies^[48]. Some of these methods include changes in lymphocyte markers, measure of cytokine levels, soluble CD30 or intracellular ATP.

The immunosuppressive potency of mTORi, such as rapamycin and everolimus, has been evaluated in several studies using various methods. The studies can be categorized into three groups: Studies that examined inhibition of T-lymphocyte proliferation, studies that analyzed inhibition of B-lymphocyte proliferation, and studies that evaluated immunoprotective capabilities.

Measurement of changes in T-cell subsets: Inhibition of T-lymphocyte proliferation

Fluorescent-activated cell sorting (FACS) analysis can

be used for the quantification of T-lymphocyte subsets. This simple and sensitive method involves sorting and quantification of lymphocyte subsets by fluorescent labelling of cell surface markers. Using this approach, reductions in the number of regulatory T-cells have been reported in kidney transplant recipients in which recipients were treated with CNI compared with those patients treated with rapamycin^[49]. One study that investigated inhibition of T-lymphocyte proliferation evaluated the pharmacodynamics of everolimus at varying doses (0.75-10 mg) when combined with cyclosporine A and prednisolone in human renal transplant recipients^[50]. T-lymphocytes isolated from peripheral blood one day before everolimus treatment (baseline), 1 d after and 21 d later, were stimulated *in vitro* using monoclonal anti-CD3 antibodies. Lymphocyte proliferation was measured by cell viability through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In contrast to placebo, T-cell proliferation was significantly reduced by a single dose of everolimus by 2-6 h, but had returned to baseline values by 10 h. In addition, lymphocyte proliferation of everolimus-treated patients decreased significantly on day 1 after everolimus intake by 25.4% ($P < 0.05$), and on day 21 by 53.3% ($P < 0.01$) compared to placebo. Patients receiving a placebo showed no meaningful changes in lymphocyte proliferation rates over the whole study period. By day 42, 21 d after the last everolimus intake, decreased lymphocyte proliferation returned to baseline values. Moreover, everolimus reduced the production of IL-10 from supernatants of peripheral blood mononuclear cells, as measured by enzyme-linked immunosorbent

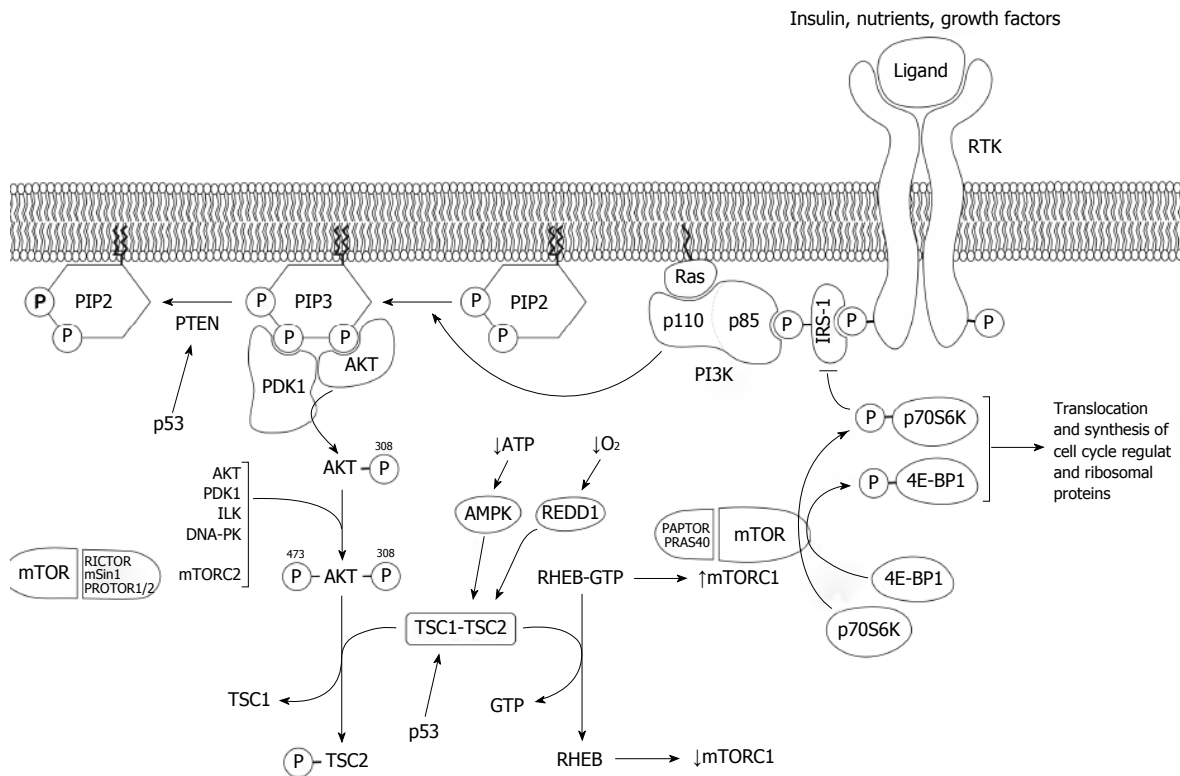


Figure 2 PI3K/AKT/mTOR signaling pathways. PI3K is activated by growth factor stimulation through RTK. The regulatory subunit of PI3K, p85, binds directly to phosphotyrosine residues on RTK and/or adaptors, such as the IRS-1. This binding relieves the intermolecular inhibition of the p110 catalytic subunit of PI3K by p85 and allows it to move toward PI3K to the plasma membrane where its substrate, PIP2, resides. The catalytic subunit can also be activated by activated RAS, which binds directly to p110, and by G protein-coupled receptors. PI3K phosphorylates PIP2 to produce PIP3. In addition, the tumor suppressor PTEN dephosphorylates PIP3 to PIP2, thereby regulating PI3K-dependent signaling in a negative manner. Following PIP3 formation, PDK1 and AKT bind to PIP3 through its pleckstrin homology domains into close proximity at the cell plasma membrane. PDK1 activates AKT by phosphorylating AKT at threonine 308. After phosphorylation, AKT is fully activated by the subsequent phosphorylation at serine 473 by several protein kinases such as PDK1, the complex mTORC2, or AKT itself. AKT phosphorylates TSC2, thereby inhibiting the GTPase activity of the TSC1-TSC2 dimer, and the GTP-binding protein RHEB remains in its active GTP-bound state, causing a rise in mTORC1. In the mTORC1 complex, mTOR phosphorylates p70S6K and 4E-BP1, leading to an increase in the translation and synthesis of cell cycle-regulating and ribosomal proteins. Activated p70S6K also participates in a negative feedback loop, reducing the activation of the PI3K pathway through the phosphorylation and subsequent inhibition of IRS-1. 4E-BP1: eIF4E-binding protein; AMPK: AMP-activated kinase; AKT: Protein kinase B; IRS-1: Insulin-like growth factor-1; p70S6K: 70 kDa ribosomal protein S6 kinase; PDK1: Phosphoinositide-dependent kinase 1; PI3K: Phosphatidylinositol 3-kinase; PIP2: Phosphatidylinositol 4,5-bisphosphate; PIP3: Phosphatidylinositol 3,4,5-trisphosphate; PTEN: Phosphatase and tensin homolog; mTOR: Mechanistic target of rapamycin; RAPTOR: Regulatory-associated protein of mTOR; REDD1: Factor protein regulated in the development of DBA damage response 1; RHEB: RAS homolog enriched in brain; RICTOR: Rapamycin-insensitive companion of mTOR; RTK: Receptor tyrosine kinase; TSC1-TSC2: Tuberous sclerosis protein 1 and 2.

assay (ELISA), by 23.7% on day 1 ($P < 0.05$) and 62.2% on day 21 ($P < 0.01$) in renal-allograft recipients compared to baseline. It is believed that IL-2 induces expression of IL-10^[51]. Thus, mTORi interfere with IL-2-dependent signal transduction and inhibit IL-10 expression.

Another study investigated the *in vitro* effects of several doses of everolimus and intravenous immunoglobulin, widely used for treatment of autoimmune and systemic inflammatory disorders^[52], on induction of lymphocyte proliferation [by two-way mixed lymphocyte reaction (MLR)] and apoptosis (by terminal deoxynucleotidyltransferase dUTP nick-end labeling and annexin V assays)^[53]. Everolimus and intravenous immunoglobulin alone each inhibited cell proliferation in a dose-dependent manner: Everolimus decreased it from 16% to 67%, and intravenous immunoglobulin from 12% to 66%. In addition, intravenous immunoglobulin induced apoptosis in B and T-cells, but everolimus

did not. The study concluded that everolimus is a potent inhibitor of immune cell proliferation but does not act additively or synergistically with intravenous immunoglobulin under the *in vitro* conditions used in the study.

A prospective study determined whether systemic signatures of immunoregulation are promoted by switching liver transplant patients from treatment with the CNI tacrolimus to rapamycin^[41]. The investigators argued that immunosuppression withdrawal from CNI is possible in only approximately 20% of all liver transplant recipients. However, mTORi such as rapamycin appear to be more immunoregulatory than CNI and might promote a tolerant state to enable withdrawal. Several assays were conducted before and after converting to rapamycin treatment. Flow cytometry revealed a significant increase in the number of regulatory T-cells in peripheral blood mononucleated cells (PBMC) and in bone marrow, and

in the number of regulatory dendritic cells in PBMC after conversion. Immunohistochemical analysis of liver biopsy showed that the ratios of FOXP3:CD3 and CD4:CD8 were higher following conversion to rapamycin treatment, with an increase the proliferation of new or existing FOXP3+ cells. Both tacrolimus and rapamycin treatment were associated with inhibition of lymphocyte proliferation as measured by an MLR, although only tacrolimus suppressed regulatory T-cells generation. Finally, 289 novel genes and 22 proteins, some of which have been implicated in immunoregulatory pathways, were expressed after conversion to rapamycin treatment. The study concluded that conversion from tacrolimus to rapamycin treatment increases the number of systemic regulatory T-cells and regulatory dendritic cells, and induces an immunoregulatory proteogenomic signature in liver transplant recipients.

Another study evaluated the capacity of FK778 administered either alone or in combination with tacrolimus, rapamycin or everolimus, to inhibit the clonal expansion of T-lymphocytes and the expression of lymphocyte-activation antigens^[54]. FK778 is a malononitrilamide which has been found to prevent acute allograft rejection in multiple experimental transplantation models^[55]. Cell proliferation was assessed by ³H-thymidine incorporation in whole blood cultures stimulated with concanavalin A, whereas the effect on the alloresponse in a MLR, and the expression of lymphocyte surface antigens by flow cytometry. All four of the drugs showed a high capacity to inhibit lymphocyte proliferation in a dose-dependent manner, and FK778 had an additive effect when combined with the other three immunosuppressive drugs that is similar to that found in mycophenolic acid combinations. Furthermore, FK778 inhibited the expression of lymphocyte surface antigens that have been implicated in activation, co-stimulation and apoptosis of T-cells. The authors suggested that these combinations appear promising, especially the combination of FK778 and mTORi for transplant patients with renal failure, because they are non-nephrotoxic.

In another study, the potency and efficacy of different concentrations of cyclosporine A and tacrolimus, rapamycin and mycophenolate mofetil, administered alone or in combination, were analyzed to develop a human whole blood assay for flow cytometric assessment of T-cell function, proliferation and the expression of surface antigens^[56]. Whole cell cultures were stimulated with concanavalin A and then analyzed by flow cytometry to detect lymphocyte proliferation and activation by bivariate expression of proliferating cell nuclear antigen (PCNA)/DNA content and T-cell-surface activation markers such as CD25, CD95 and CD154. Rapamycin alone had the most potent effect on proliferation of the drugs used in the study, followed by tacrolimus, cyclosporine A

and mycophenolate mofetil, as rapamycin required a lower dose than the other drugs to achieve the same inhibition. In particular, rapamycin showed a synergistic effect on proliferation and activation marker expression when added to cyclosporine A at various concentrations. Rapamycin also synergistically inhibited proliferation and activation marker expression when combined with low concentrations of tacrolimus. However, when combined with high concentrations of tacrolimus, rapamycin acted antagonistically. Rapamycin combined with mycophenolate mofetil further increased the inhibition of lymphocyte function compared to treatment with either drug alone.

Inhibition of B-lymphocyte proliferation

As antibody-secreting plasma cells can develop from B-cells with or without the help of T-cells in response to donor antigens^[57], it is imperative to understand the mode of drug action during B-lymphocyte differentiation (*i.e.*, independent of drug effects on T-cells). Therefore, B-lymphocytes are therapeutic targets for immunosuppressive drugs. However, although T-cell assays such as the MLR (to measure proliferation) and ELISPOT (to measure cytokine production) have been well established, the B-cell responses have been more difficult to measure.

A study analyzing the effect of sotrastaurin (a protein kinase C inhibitor for the prevention of transplant rejection and treatment of psoriasis), mycophenolic acid or everolimus assessed proliferation, apoptosis, CD80/CD86 expression, and immunoglobulin and IL-10 production in primary stimulated B-cells *in vitro*. Additionally, B-cells were co-cultivated with pre-activated T-cells with anti-CD28 monoclonal antibody to evaluate the effects of these immunosuppressive drugs on T-cell-dependent immunoglobulin production^[44]. Everolimus and mycophenolic acid but not sotrastaurin strongly inhibits B-cell functions in a dose-dependent manner, but all three agents decreased T-cell-dependent immunoglobulin production. The study concluded that although sotrastaurin can affect B-cell function only indirectly by suppressing T-cell help, everolimus and mycophenolic acid can inhibit humoral responses both directly and indirectly.

The effects of everolimus, mycophenolic acid, or prednisolone were analyzed in a three-step *in vitro* culture system developed to promote the proliferation and differentiation of peripheral CD19+ B-cells into plasma cells that produce IgG antibodies^[45]. The inhibitory effect of everolimus, mycophenolic acid, and prednisolone on cell proliferation was examined in each step of a three-step culture model. This culture model consisted of: B-cell activation (step 1, days 0-4), plasmablasts generation (step 2, days 4-7), and plasma cell generation (step 3, days 7-10). On day 10, IL-10 production was analyzed by ELISA and cell proliferation by flow cytometry analysis. Although both everolimus and mycophenolic acid efficiently

suppressed cell proliferation and differentiation in step 1, everolimus suppressed B-cell differentiation in step 2. IgG production on day 10 was significantly suppressed by everolimus, mycophenolic acid, and prednisolone, but not cyclosporine. These results suggest that suppression of IgG production by plasma cells could avoid antibody-mediated rejection facilitated by donor-specific antibodies, thus precluding one of the main causes of acute or chronic allograft dysfunction that leads to graft loss. However, these results were obtained from *in vitro* assays and so this hypothesis must be validated in clinical settings.

Immunoprotection

We have described the evidence that mTORi inhibit lymphocyte proliferation and cytokine and antibody production, but mTORi also induce other important immunomodulatory effects. As discussed above, mTORi selectively promote the expansion of regulatory T-cells, which may contribute to the immunoprotective effects of mTORi^[37,58-60]. In this section, we review studies indicating that mTORi protect transplant recipients against cytomegalovirus infection and disease, which is a major complication in transplant recipients, and how they aid in DNA repair, thereby lowering cancer risk.

A review explained how mTORi may increase immunity against cytomegalovirus infection^[61]. Specifically, activation of mTOR in host cells is essential for cytomegalovirus to propagate viral proteins successfully, even under conditions that normally block mTOR activity^[62]. A recent study investigated why patients treated with an mTORi are protected against cytomegalovirus disease, even while graft rejection is prevented^[63]. The study was conducted among renal transplant recipients who were treated with prednisolone, cyclosporine A, and mycophenolate sodium for the first 6 mo after transplantation, followed by double therapy with prednisolone and everolimus, prednisolone and mycophenolate sodium, or prednisolone and cyclosporine A. All patients tested cytomegalovirus-seropositive before transplantation. The study observed a significant increase in cytomegalovirus-specific effector-type CD27-CD8+ and CD28-CD27-CD4+ T-cell counts in patients treated with everolimus, but not among those treated with the other drugs. Furthermore, everolimus strongly inhibited allo-responses *in vitro*, whereas it did not affect cytomegalovirus-specific responses. Cyclosporine A and mycophenolate sodium dose-dependently reduced virus-specific proliferation, although less effectively as the allo-responses. Another study investigating cardiac transplant recipients treated with everolimus and cyclosporine, or mycophenolate mofetil and cyclosporine, achieved similar results related to cytomegalovirus infection^[64]. Patients in this study treated with the everolimus regimen had a significantly lower incidence of any cytomegalovirus event, infection

or cytomegalovirus syndrome, than patients treated with the other regimen.

Other study compared the effect of rapamycin on CD8+ T-cells responding to a graft vs a pathogen using a transgenic mice system in which the same monoclonal TCR transgenic T-cells responded to a bacterial pathogen infection or a skin graft^[65]. Whereas treatment with rapamycin increased the antigen-specific CD8+ T-cell response to the pathogen, the same T-cell population did not show an enhanced response in the context of a graft.

The results of another study in mice treated with rapamycin have suggested that antigen-specific T-cells responding to a pathogen express CD62L, which is associated with the development of a memory phenotype, whereas antigen-specific T-cells responding to a graft do not express this marker^[66]. These results suggest that the conditions under which T-cells are stimulated can profoundly modify the impact of rapamycin on antigen-specific T-cell responses. The mechanism underlying this effect might be linked to the ability of rapamycin to enhance fatty acid oxidation in responding T-cells, and to reduce glucose utilization, a change that has been shown to be crucial for an effector-to-memory transition in CD8+ T-cells^[67]. Thus, minimizing the generation of memory cells by treatment with an mTORi could decrease graft rejection responses, and indirectly promote an environment where tolerance could be established.

CONCLUSION

In this review, we have discussed how the mTORi rapamycin and everolimus mediate a potent immunosuppression while concomitantly promoting the expansion and survival of CD4+CD25+FOXP3+ regulatory T-cells after transplantation, which could help to induce tolerance to the graft. However, although the tolerogenic properties of mTORi have been well demonstrated in rodent transplant models, they have not been shown to induce regulatory T-cell-mediated tolerance in humans. The pathogen-activated pro-inflammatory response in humans, which is enhanced by mTOR inhibition, may counterbalance the tolerogenic potential of regulatory T-cell expansion. Future immunomodulatory protocols based on mTORi should combine other immunomodulatory molecules to limit the capacity of mTORi to promote anti-pathogen responses while further supporting regulatory T-cell expansion and stability.

Our review of methods used to quantify the potency of immunosuppressive agents indicates that the available options are not yet sufficiently sensitive for that, or their utility is supported by only a few studies. Until better approaches are developed, a combination of methods may be the most effective way to accurately quantify the potency of immu-

nosuppressive agents. However, from the studies on immunosuppressive potency it can be deduced that mTORi are immunosuppressive drugs with significant power similar to that of CNi.

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Pentamidine in *Pneumocystis jirovecii* prophylaxis in heart transplant recipients

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Abstract

Despite advances in transplantation techniques and the quality of post-transplantation care, opportunistic infections remain an important cause of complications. *Pneumocystis jirovecii* (*P. jirovecii*) is an opportunistic organism, represents an important cause of infections in heart transplantation patients. Almost 2% to 10% of patients undergoing cardiac transplantation have *Pneumocystis pneumonia*. Prophylaxis is essential after surgery. Various prophylaxis regimes had been defined in past and have different advantages. Trimethoprim/sulfamethoxazole (TMP/SMX) has a key role in prophylaxis against *P. jirovecii*. Generally, although TMP/SMX is well tolerated, serious side effects have also been reported during its use. Pentamidine is an alternative prophylaxis agent when TMP/SMX cannot be tolerated by the patient. Structurally, pentamidine is an aromatic diamidine compound with antiprotozoal activity. Since it is not effectively absorbed from the gastrointestinal tract, it is frequently administered *via* the intravenous route. Pentamidine can alternatively be administered through inhalation at a monthly dose in heart transplant recipients. Although, the efficiency and safety of this drug is well studied in other types of solid organ transplantations, there are only few data about pentamidine usage in heart transplantation. We sought to evaluate evidence-based assessment of the use of pentamidine against *P. jirovecii* after heart transplantation.

Key words: Pentamidine; Prophylaxis; Trimethoprim; Heart transplantation; *Pneumocystis pneumonia*; *Pneumocystis jirovecii*; *Pneumocystis carinii*

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Core tip: Trimethoprim/sulfomethoxazole (TMP/SMX), the first-line drug for pneumocystis pneumonia prophylaxis following heart transplantation, is well tolerated, however; serious side effects have also been reported during its use. Pentamidine is an alternative prophylaxis agent when TMP/SMX cannot be tolerated following solid organ transplantations. Although there are various studies evaluating the efficiency and safety of pentamidine in these groups, merely reports were found about its usage in heart transplantation recipients. This review aims to evaluate the use of pentamidine against *Pneumocystis jirovecii* following heart transplantation.

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INTRODUCTION

Infection is a major determinant of survival among many others in patients undergoing cardiac transplantation^[1,2]. *Pneumocystis jirovecii* (*P. jirovecii* or *P. carinii*), an opportunistic organism, represents an important cause of infections in this group of patients. The objective of the present review was to provide a comprehensive and evidence-based assessment of the use of pentamidine against *P. jirovecii*, which is a potential threat in patients undergoing cardiac transplantation who require very close monitoring during all stages of the peri-operative care.

OPPORTUNISTIC PULMONARY INFECTIONS IN PATIENTS UNDERGOING CARDIAC TRANSPLANTATION

Despite advances in transplantation techniques and the quality of post-transplantation care, opportunistic infections remain an important cause of complications. As compared non-respiratory infections, pneumonia represents a more serious threat when one considers its incidence and severity. A classification scheme for pneumonia based on the temporal occurrence proposes that pneumonia within the first post-transplant period is referred to as nosocomial, while those occurring between post-transplant months 1 and 6 are considered opportunistic, and those occurring thereafter can be considered as community-acquired pneumonia. Despite this general classification scheme, certain specific patient groups experience an increased risk of opportunistic infections even 6 mo after the procedure^[3-6].

Other than the bacterial infections, *Aspergillus*

spp, *Candida* spp, CMV, *Nocardia* spp and PCP represent the causative organisms that are most frequently associated with pulmonary disease. Invasive pulmonary aspergillosis is a serious condition with high mortality^[7], and introduction of the lipid formulations of amphotericin B, echinocandins, and novel azole antifungals resulted in an increased chance of successful treatment in patients with this condition^[8].

Mycobacterium tuberculosis is a bacterial agent and infections caused by this organism are closely related with demographic characteristics of the patient groups. Globally, *Mycobacterium tuberculosis* has been reported to occur in 0.35% to 15% of the cases undergoing solid organ transplantation^[9]. This organism may be expected to play a greater role in the future both in the community in general and in immunocompromised individuals in particular (particularly in Anatolia and Europe), considering the mass migrations and conflicts influencing the populations across the Middle East region. In areas with high endemicity, the potential for prophylaxis may be evaluated using purified protein derivative (PPD) or QuantiferON tests in high-risk individual^[10].

Pneumocystis carinii (*P. carinii*) was initially described in rats and humans. This organism has been re-named as *P. jirovecii* in honor of the Czech parasitologist Otto Jirovec in order to differentiate other variants of *Pneumocystis* found in other species from this organism, which was first described in 1976 in humans^[11]. Although initially thought to be a protozoan, further studies ascertained that it is actually a yeast-like single cell fungus^[12]. Although the International Code of Nomenclature for Algae, Fungi, and Plants (ICNafp) recommended the use of the name with two "i"s, i.e., *P. jirovecii*, for academic publications, currently *P. jiroveci*, *P. jirovecii* and *P. carini* are frequently used synonymously^[13]. The term PCP is widely accepted as the acronym for pneumocystis pneumonia.

This organism is ubiquitous in the nature. The probable route of transmission is through respiration. The infection caused by this organism takes the form of diffuse bilateral pneumonitis with a mortality of 90% to 100% and 35% for untreated and treated cases, respectively. The clinical course is closely associated with the age of the patients. Most common signs and symptoms associated with the disease include tachypnea, cough, and hypoxia resulting from pneumocyte injury.

THE INCIDENCE OF PCP IN PATIENTS UNDERGOING CARDIAC TRANSPLANTATION

Almost 2% to 10% of patients undergoing cardiac transplantation have PCP^[14-18]. The divergence in the reported figures reflects the differences between centers and populations examined. Also, there may be

an increased frequency and severity of PCP in centers where seasonal clustering of *P. jirovecii* is observed^[17].

The incidence of PCP may vary depending on the type of the immunosuppressive treatment administered after transplantation. Recent evidence suggests that after the introduction of the effective immunosuppressor mycophenolate mofetil (MMF) there has been a decrease in the frequency of PCP, despite the absence of data involving cardiac transplant patients^[19-21]. For instance, Oz *et al*^[20] showed a decreased incidence of PCP with MMF in rat models of immunosuppression. Virus-free Sprague Dawley rats were immunosuppressed by tacrolimus, sirolimus, dexamethasone and/or MMF in study models and no PCP development was observed in any of the rats treated with MMF. Another team of investigators led by Husain *et al*^[21] reviewed 4 separate clinical studies in which patients received MMF, and found no cases of PCP in patients receiving MMF among a group of 1068 subjects. In contrast, 1.8% of the patients who did not receive MMF had PCP. Although the exact mechanisms of this protective effect conferred by MMF are unknown, blockade of the replication of the microbial genetic material at one step of microbial growth has been proposed. In contrast with these positive findings for MMF, Arichi *et al*^[22] suggested that administration of MMF may represent a risk factor for PCP in patients undergoing renal transplantation due to strong immunosuppression.

Cardenal *et al*^[23] compared 72 CT patients with a group of subjects representative of the normal population during an average follow up duration of 5 years and showed a similar frequency of PCP in both groups. While the causative agent was associated with opportunistic infections, it was associated with subclinical infection in the normal subjects^[23].

MECHANISM OF PNEUMOCYSTIS JIROVECI INFECTION

Currently two different hypotheses have been put forward to explain how *P. jirovecii* may lead to development of an infectious disease in cardiac transplant patients while not causing any infections despite common presence in healthy individuals. According to the first hypothesis, after the initial infection (primary infection) with *P. jirovecii*, the organisms enter a latent phase in the pulmonary tissue and are activated after immunosuppression as to cause PCP^[24]. The strongest piece of evidence for this hypothesis comes from the detection of antigens against this pathogen in healthy young individuals^[25]. On the other hand, several studies found no evidence of this pathogen up to one year after PCP^[26]. The second hypothesis proposes that the pathogen that is associated with *P. jirovecii* infection is actually of exogenous origin. A low incidence of PCP during the initial months where immunosuppression is

most severe as well as a prolonged duration of time between the transplantation and occurrence of PCP are supportive of the second hypothesis. Currently there is no conclusive evidence, both for the first hypothesis proposing a latent source of infection, and for the hypothesis offering a more likely explanation of an exogenous source.

REQUIREMENT FOR PROPHYLAXIS

Regardless of the source of *P. jirovecii* infections, currently no consensus exists on the need for primary prophylaxis (PP) in all solid organ transplantations^[27]. On the other hand, most authors advocate the use of PP in CT patients^[28]. In a Vancouver based study involving patients undergoing a variety of different solid organ transplantation procedures (657 kidney, 436 liver, 44 kidney/pancreas, 104 lung and heart/lung), prolonged prophylaxis has been recommended on the basis of the occurrence of late PCP more than 1 year after post-transplantation^[29].

In studies where it has been reported that there may be no need for prophylaxis in a variety of patients with immunosuppression, a recommendation to administer selective prophylaxis has been made, in addition to drawing attention to the possibility that PCP may have a more severe clinical course^[30]. When one considers studies reporting occurrence of PCP even under trimethoprim/sulfamethoxazole (TMP/SMX) prophylaxis, the need for prophylaxis in CT patients becomes even more important^[31].

Among patients undergoing cardiac transplantation, those receiving MMF may be considered as those with the least need of PCP prophylaxis. As mentioned earlier, the anti-microbial properties of MMF, the mechanisms of which have not been clearly elucidated, and the supporting evidence, though few in number^[20,21], suggest that prophylaxis may not be necessary in this patient group. Yet, there is no consensus regarding the use of prophylaxis in this patient group.

AGENTS USED FOR PROPHYLAXIS

One of the first agents utilized for PP for *P. jirovecii* was TMP/SMX. It is one of the most commonly used agents for this indication since 1988, when it was first introduced for use in PP. While in the initial years, a recommendation to use TMP/SMX for the first 3 or 13 mo was made, after 1997 the recommended duration of prophylaxis has been extended as to include a prophylaxis of several years to life-long prophylaxis^[19]. TMP/SMX has been shown to reduce the risk of PCP by more than 90%^[32]. This agent is also effective against listeriosis and toxoplasmosis^[32-36]. Although it is generally accepted that the incidence of PCP is reduced after one year, cases with late-onset PCP have also been reported. Majority of these cases occurred during phases of acute rejection^[29,32]. Some authors have advocated more prolonged use of TMP/SMX in

association with this condition^[28].

Except for some isolated reports, numerous studies have established the efficacy and safety of TMP/SMX prophylaxis^[23,37,38]. Generally, although TMP/SMX is well tolerated, serious side effects have also been tolerated during its use^[39,40]. Some of the side effects may be associated with its mechanism of action involving the folate metabolism. Agents that may be administered through non-systemic routes such as the inhalational route instead of this agent are warranted, particularly in patients undergoing bone marrow transplantation who are prone to adverse effects involving the myeloproliferative system.

After year 2000, ataviquone has been introduced for *P. jirovecii* prophylaxis in patients who were not considered suitable for TMP/SMX or pentamidine prophylaxis. This agent is not only effective for protection against *P. jirovecii*, but also against *Toxoplasma gondii*. Alternatively, oral combinations of pyrimethamine and sulfadoxine or agents such as dapsone may be utilized^[19].

PENTAMIDINE IN PROPHYLAXIS

Although pentamidine was originally used for the treatment of trypanosomiasis and leishmaniasis in 1930s, it was first licensed in 1950s. Goa *et al*^[41] was the first to provide evidence for its efficiency against PCP in 1987. Structurally, pentamidine is an aromatic diamidine compound with antiprotozoal activity. Since it is not effectively absorbed from the gastrointestinal tract, it is frequently administered *via* the intravenous route. It may cause mild and generally reversible nephrotoxicity or hypoglycemia, while pancreatitis represents its most common side effect. Nephrotoxicity may cause acute allograft dysfunction, particularly in renal transplant patients^[42]. Hypotension, hypocalcemia, and cardiac dysrhythmia are other side effects that can be observed. A patient developing torsades des pointes during inhaled pentamidine treatment in a renal transplant patient has also been reported^[43]. These side effects may be assumed to occur less frequently during inhaled use. Due to its potent efficacy against pneumocytosis and toxoplasmosis, it has been included in the 2013 Model List of Essential Medicines issued by the World Health Organization (WHO).

In patients who cannot tolerate TMP/SMX due to side effects after cardiac transplantation, pentamidine is an alternative agent and is frequently administered through inhalation at a monthly dose of 150 mg or 300 mg. It is diluted with 6 mL of water for preparation and is administered *via* a 20 min nebulization. During the administration, the patient has to be positioned in the sitting position and the patient should perform a deep inspiration after each 4 to 5 normal inspiratory activity^[44]. The device that has been reported to be most commonly used in for the delivery of the inhalational drug is Respirgard II nebulizer (Marquest,

Englewood, Colo, United States). Once or twice monthly dose-regimens do not differ significantly in terms of efficacy^[45]. Administration of bronchodilators with nebulizer prior to the procedure may allow better tolerance of the drug by reducing cough and bronchospasm. Due to its method of administration, some patients may require hospitalization. The terms used to describe the inhalational treatment in literature include "inhaled", "aerolized", or "nebulized" treatment.

As compared to studies in liver transplant patients^[46-51], studies examining the role of pentamidine in PCP prophylaxis in patients undergoing cardiac transplantation are relatively scarce in number. Except for Altintas *et al*^[52], who showed safe use of inhaled pentamidine in a cardiac transplant patient developing allergic reaction to TMP/SMX, no other studies in this patient group have been identified in the literature. In that study, due to the absence of established guidelines regarding the route and dosage of administration of pentamidine in CT patients, the use of this agent in that patient was based on the use in other patient groups with immunosuppression^[53,54]. Since the publication this study in 2011, no other studies have been published. The scarcity of reports may be due to the fact that PCP occurs at a relatively low frequency in CT patients after introduction of the widespread use of TMP/SMX as well as due to the generally good safety profile of TMP/SMX.

When the use of pentamidine in other patient groups with immunosuppression is examined, it is evident that intravenous route is also used for its administration. In certain centers, intravenous PCP prophylaxis is used, generally after the hematopoietic stem cell transplantation in children or adolescents^[55], and initial results with this route of administration suggest that pentamidine may be used as a first-line therapy. In the study by Kim *et al*^[56], it was considered as a safe second-line agent after TMP/SMX in a similar patient population. Again, in a study involving patients undergoing bone marrow transplantation, the authors recommended that inhaled pentamidine may be used as a second-line agent based on positive results with this agent^[57]. On the other hand, Vasconcelles *et al*^[58] found high rates of failure with inhaled pentamidine in bone marrow transplant patients.

CONCLUSION

Despite an ever decreasing incidence of PCP in cardiac transplant patients, in patients who are unable to receive treatment with TMP/SMX for PP, there is a need for effective second-line agent(s). In the absence of large-scale studies in CT populations, pentamidine distinguishes itself as a safe and effective potential second-line agent based on the results in other patient groups with immunosuppression. In a specific patient group such as those undergoing CT, large-scale studies are warranted to establish reliable therapeutic algorithms.

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Hematopoietic stem cell transplantation for auto immune rheumatic diseases

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Abstract

Stem cells have their origins in the embryo and during the process of organogenesis, these differentiate into specialized cells which mature to form tissues. In addition, stem cell are characterized by an ability to indefinitely self renew. Stem cells are broadly classified into embryonic stem cells and adult stem cells. Adult stem cells can be genetically reprogrammed to form pluripotent stem cells and exist in an embryonic like state. In the early phase of embryogenesis, human embryonic stem cells only exist transiently. Adult stem cells are omnipresent in the body and function to regenerate during the process of apoptosis or tissue repair. Hematopoietic stem cells (HSC) are adult stem cells that form blood and immune cells. Autoimmune responses are sustained due to the perennial persistence of tissue self autoantigens and/or auto reactive lymphocytes. Immune reset is a process leading to generation of fresh self-tolerant lymphocytes after chemotherapy induced elimination of self or autoreactive lymphocytes. This forms the basis for autologous HSC transplantation (HSCT). In the beginning HSCT had been limited to refractory autoimmune rheumatic diseases (AIRD) due to concern about transplant related mortality and morbidity. However HSCT for AIRD has come a long way with better understanding of patient selection, conditioning regime and supportive care. In this narrative review we have examined the available literature regarding the HSCT use in AIRD.

Key words: Transplant related mortality; Hematopoietic stem cell transplantation; Systemic sclerosis; Stem cell therapy; European Group for Blood and Marrow Transplantation

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Core tip: Hematopoietic stem cell transplantation for the management of autoimmune rheumatic diseases has come a long way. It is being recognized as a viable option in severe autoimmune diseases, in particular for systemic sclerosis.

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INTRODUCTION

Stem cells have their origins in the embryo and during the process of organogenesis, these differentiate into specialized cells which mature to form tissues. In addition, stem cells are characterized by ability to indefinitely self renew. Stem cells are broadly classified into embryonic stem cells and adult stem cells. Adult stem cells can be genetically reprogrammed to form pluripotent stem cells and exist in an embryonic like state. In the early phase of embryogenesis, human embryonic stem cells only exist transiently. Adult stem cells are omnipresent in the body and function to regenerate during the process of apoptosis or tissue repair. Hematopoietic stem cells (HSC) are adult stem cells that form blood and immune cells.

Embryonic stem cells have great promise as they have the capability to replenish every functioning cell in the human body. Uncontrolled replication of embryonic stem cells leads to teratomas. Embryonic stem cell biology is subject to ethical controversy. Currently there are no Food and Drug Administration (FDA) approved embryonic stem cells based therapies available for clinical use. There are several clinical trials ongoing exploring use of human embryonic stem cell based therapies in regenerative medicine. HSC are blood and immune cells that have their origin from adult stem cells. HSC can be isolated from the umbilical cord, peripheral blood or the bone marrow^[1].

Manifestations of autoimmune rheumatic diseases (AIRD) are heterogeneous in which the etiology is compounded by genetic risks, racial differences and infection triggered oligoclonal lymphocyte responses. As a result of multitudes of external insult, there is interference in the signal responses that sustain immune tolerance to normal tissues. Breakdown of these signals leads to activation of effector cellular mechanism and subsequent self-tissue destruction in a self-propagating manner^[2]. Autoimmune responses are sustained due to the perennial persistence of tissue auto antigens, which often do not get destroyed. The treatment response is, hence; often generalized and most patients indeed have a relapsing and remitting

course. Better understanding of mechanisms involved in immunopathogenesis and of effector cells have lead to the acceptance of aggressive modalities of treatment namely hematopoietic stem cell transplantation (HSCT) which resets the host immune system^[3]. Immune reset is a process leading to generation of fresh self-tolerant lymphocytes after chemotherapy induced elimination of self or auto reactive lymphocytes. This forms the basis for autologous HSCT.

Extensive preclinical animal transplantation experiments lead to HSCT (Figure 1) as a therapeutic option for patients with severe autoimmune diseases began in the late 1990s. In the beginning, the use of HSCT had been limited to refractory diseases due to concern about transplant related mortality and morbidity. Later it became clear that transplant related mortality and morbidity is a function of the disease state^[4] and conditioning regimen^[5]. The conditioning regimens included either myeloablative or nonmyeloablative. High dose chemotherapy and total body irradiation (myeloablative regimen) together with stem cell support ensures a complete replacement of the entire bone marrow compartment, hence abolishing the entire tumor cell load. Marrow failure is life threatening if HSC are not reinfused. Reduced doses of chemo radiotherapy constitute the nonmyeloblastic regimen. This leads to lymph ablation and marrow cells are invariably preserved such that the incidences of a lethal failure is minimized even without HSC reinfusion. However, treatment related marrow suppression could be minimized using autologous stem cell support. The significant reduction in the treatment related mortality and morbidity following the use of non myeloablative regimens over myeloablative regimens, makes it a more viable option for the treatment of autoimmune diseases (natural history is relapsing and remitting) compared to malignant diseases^[1].

The major advantage of HSCT for autoimmune diseases is the ability to achieve an "immune reset", i.e., the ability to eliminate the autoimmune T cell clones and alter the natural history of the disease. The major disadvantages of HSCT for autoimmune disease are the added toxicity of the high dose chemotherapy or radiation used as part of conditioning regimen.

The use of HSCT has been reported for various AIRD. Long term data is available from the European Group for Blood and Marrow Transplantation (EBMT) registry^[6,7] (Table 1), clinical trials in systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) with maximum data available in patients with SSc. Isolated reports are available for remission of some other AIRD such as ankylosing spondylitis. In this narrative review we have appraised the available literature on HSCT use in AIRD.

SEARCH STRATEGY

For the purpose of present narrative review, the

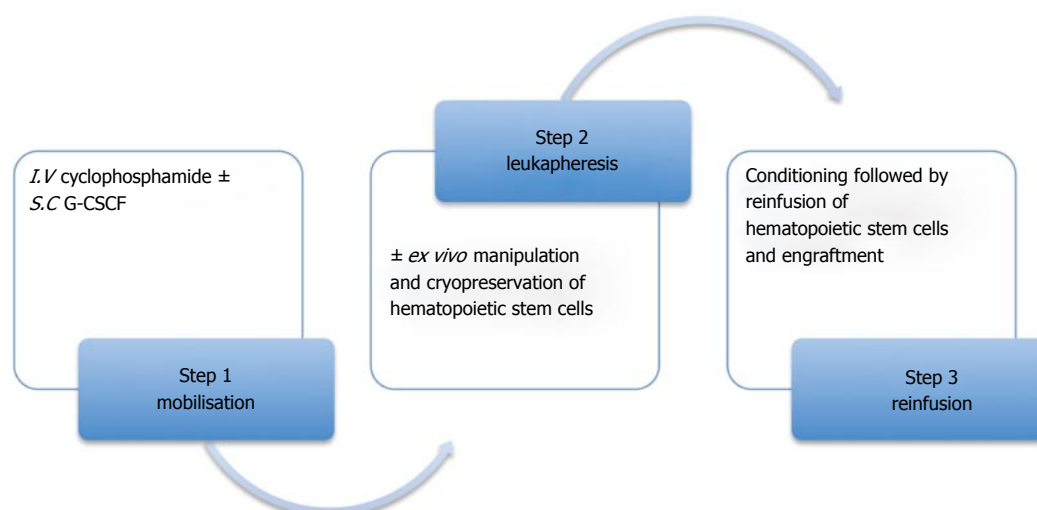


Figure 1 Stems cells are harvested from the peripheral blood, bone marrow or umbilical cord. Step 1: Chemomobilization, involves use of chemotherapeutic agents name cyclophosphamide together with cytokines (G CSF) which have a synergistic effect on increasing the stem cell repertoire; Step 2: Leukapheresis, which involves *ex vivo* collection of large volumes of centrifuged blood products till target CD34⁺ cells are achieved and the isolated stem cells are cryopreserved with the use of dimethylsulfoxide; Step 3: Reinfusion of the cryopreserved stem cells preceded by conditioning chemotherapeutic ± radiation regimens.

Table 1 Summary of European Group for Blood and Marrow Transplantation registry experience^[6,7]

Disease	Number	Mean age at Tx (yr)	TRM (100 d)	5 yr progression free survival	5 yr overall survival	Death due to disease	Deaths due Tx
Systemic sclerosis	175	41	6%	55%	76%	23	12
SLE	85	28	11%	44%	76%	5	11
Rheumatoid arthritis	89	42	1%	18%	94%	0	2
JIA	65	11	11%	52%	82%	2	7

Tx: Transplant; TRM: Transplant related mortality; SLE: Systemic lupus erythematosus; JIA: Juvenile idiopathic arthritis.

search strategy included screening of primary sources MEDLINE (1990 to date) using the PubMed interface, as well as secondary sources, the Embase, Cochrane Library, Best evidence and Clinical evidence without any time limits. Appropriate combinations of search terms including "autoimmune", "stem cell transplantation", "rheumatic diseases", "hematopoietic" and the names of individual known musculoskeletal disorders were used with limits "(English, human)". Relevant keyword variations for different databases were used. This was supplemented by a manual search of bibliographies of these articles and of previously published reviews.

HSCT IN SSc

SSc is a fibrotic disease characterized by extensive dermal and visceral organ involvement. There is phenotypic difference in the disease subsets, which are classified, as diffuse and limited depending upon the degree of skin involvement, which is semi objectively, measured by the modified Rodnan's score (mRSS). The extent of skin fibrosis portends the degree of visceral involvement, which has a direct bearing on the long term mortality and morbidity in these patients. The higher the skin score, the presence of

cardiac, renal or pulmonary involvement increases the mortality to 40%-50% in the next 5 years^[8-12].

HSCT has been explored as a therapeutic option in the treatment of SSc with its first case dating back to 1997. Since then numerous Phase I/II trials have done. The long-term data from the EBMT registry has shown encouraging results with respect to improvement in skin score and stabilization of lung functions and pulmonary hypertension together with improvement in functional status^[6,7,13] (Table 1). Three randomized control trials namely - ASTIS^[14]: A phase 3 trial (Autologous Stem cell Transplantation International Scleroderma trial); ASSIST^[15]: A phase 2 trial (Autologous non-myceloablative hematopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for SSc) and SCOT^[16]: A phase 3 (US multicenter Scleroderma: Cyclophosphamide or Transplantation) exists which have evaluated the efficacy of HSCT in Scleroderma (Table 2). SCOT completed the recruitment of patients in May 2011 and some of the results are expected soon.

Most of the data available for HSCT in SSc has shown a significant improvement in skin scores in patients and moderate improvement in FVC and DLCO. In the ASSIST trial^[15], 19 patients with SSc

Table 2 Randomized control trials of hematopoietic stem cell transplantation in systemic sclerosis

Trial name	Patients	Controls	Number	Outcome	TRM	Comments
ASTIS ^[14]	mRSS 15 for disease duration 4 yr, mRSS 20 if disease duration is 2 yr; and major organ involvement	IV CYC	156 (79 HSCT, 77 CYC)	5 yr survival: 52% (40 patients) in CYC; 70% (55 patients) in HSCT	10.01%	At 2 yr: significantly better event free survival, mRSS, EuroQol. HAQ; decline in creatinine clearance and increase in FVC/VC Median follow up 5.8 yr
ASSIST ^[15]	mRSS 14 with internal organ involvement or coexistent pulmonary Involvement if mRSS was < 14	IV CYC	19 (10 HSCT, 9 CYC)	HSCT: all improved; CYC: 8 progressed	None	Small study, 7/8 that progressed in CYC group switched to HSCT. All HSCT patients (including switches) had significant improvement in mRSS and FVC and TLC Follow up 2 yr
SCOT ^[16]	mRSS > 16, significant visceral organ involvement, disease duration < 4 yr	IV CYC	75	Not reported	-	Recruitment completed, yet to be published. Identical regimen to ASTIS except total body irradiation in HSCT

mRSS: Modified Rodnan skin score; IV: Intravenous; CYC: Cyclophosphamide; HSCT: Hematopoietic stem cell transplantation; HAQ: Health Assessment Questionnaire.

and organ involvement were randomized to HSCT ($n = 10$) or monthly cyclophosphamide for 6 mo ($n = 9$). Eight/nine patients on monthly cyclophosphamide progressed vs none for HSCT group within the first year after randomization. Seven patients underwent HSCT after evidence of progression on monthly cyclophosphamide. For 11 patients who underwent HSCT and had follow up for at least 2 years there was significant improvements in mRSS ($P < 0.0001$) and FVC ($P < 0.03$) compared to baseline. This trial was closed early and there were no deaths reported in either arm.

In ASTIS trial 156 patients with SSc and heart, lung or kidney involvement were randomized to HSCT ($n = 79$) vs monthly cyclophosphamide ($n = 77$) for 12 mo. During the first year there were more events (death and irreversible organ failure) in the HSCT group, 13 (16.5%) vs 8 (10.4%) in the cyclophosphamide group. However during the second the cumulative events were similar in two groups 14 (17.7%) vs 14 (18.2%). By 4 year the cumulative events in HSCT group 15 (19%) were less than cyclophosphamide group 20 (26%).

HSCT IN SLE

SLE is a prototype autoimmune disease characterized by a wide array of autoantibodies with myriad clinical presentations. Major organ involvement and persistent disease activities are predictors of poor outcome^[17]. Treatment response varies in population subsets owing to the genetic composition and racial differences^[18]. Hormonal influences in the adult and pediatric patients of SLE further add to the heterogeneity of the disease manifestations. Immunosuppressive therapy is often protracted for adequate disease control and to minimize organ damage in patients with very high disease activity. These are however, associated with significant treatment-related morbidities. Prolonged uses of corticosteroids and repeated flares requiring

higher doses of immunosuppressant, inadequate responses have resulted in unfavorable long-term disease free outcomes or drug free intervals^[19].

In a trial by Burt *et al*^[20], non-myeloablative HSCT in refractory SLE showed significant advantages of HSCT in terms of progression free survival and alleviation of nephritic symptoms in patients with SLE. HSCT in SLE showed promising results with respect to the SLEDAI score and the serological markers with increasing 5-year progression free survival. There was a stabilization of the nephritic disease with disappearance of APLA titers in a majority^[20]. A follow up study using third generation "rituximab sandwich" conditioning regimen (cyclophosphamide, rabbit ATG and CD20 monoclonal antibody rituximab) is ongoing^[21]. In EBMT too, positive trends in progression free and overall survival were noted (Table 1)^[6].

HSCT IN RA

RA is characterized by progressive joint destruction due to the formation of an inflammatory pannus, which erodes the synovial cartilage and the surrounding bone. The manifestations include articular symptoms like pain and morning stiffness and as the disease progresses extra-articular manifestations like pulmonary fibrosis, vasculitis and eye disease may occur.

With the advent of biologics and early aggressive DMARD therapy, adequate control and a possibility of remission has been possible in early disease. Despite aggressive modalities, some patients are resistant to therapy. Functional disabilities as assessed by Health Assessment Questionnaire (HAQ) and persistence of inflammation in multiple joints are prognostic indicators for a poor survival.

HSCT in RA dates back to 1997. Pilot studies have shown that sustained remission responses were short lived for up to 6-12 mo which was followed by reintroduction of DMARD's/anti TNF therapy. This was due to the failure to completely obliterate the synovial

T cell repertoire following a HSCT. However, following HSCT there was a better response to biologic and non-biologic DMARDs supporting the immunomodulating effect of HSCT. There has been variable success of HSCT in RA but the results have not been encouraging as compared to diseases like SSc^[22-24] (Table 1).

The success of HSCT is measured in terms of progression free survival and disease free survival both being the highest in-patient with SSc and RA as compared to other AIRD. Though the results for RA in terms of overall survival rates have been approximately 98%^[6], the ability to maintain a sustained ACR 70 response was low with only 28% achieving a progression free survival at the end of 3 years for such an expensive therapy.

HSCT IN JUVENILE IDIOPATHIC ARTHRITIS

Juvenile idiopathic arthritis (JIA) is a deforming joint disease in children a majority of them have a protracted clinical course as with a failure to respond to conventional DMARD's and biologicals^[25,26] and this causes severe morbidity with significantly impaired quality of life. Increased mortality is often due to disease, and from drug toxicities, especially in patients with systemic JIA^[27,28]. Published data from the EBMT registry showed transplant related mortality in 7 out of 65 patients of JIA and 52% and 85% of the patients having 3 year progression free and overall survival rates respectively^[6] (Table 1).

HSCT IN VASCULITIS

The experience with HSCT in patients with severe primary systemic vasculitis (PSV) as published in case reports and from EULAR and EBMT-databases gives some evidence that HSCT might be an effective treatment option in refractory cases of PSV and related diseases^[29]. In 15 transplanted patients of different forms of vasculitis with an overall response rate of 93% (46% complete and 46%) partial responses were observed^[29].

HSCT IN OTHER AIRD

HSCT has been tried in other AIRD such as polymyositis/dermatomyositis, Sjogrens syndrome, psoriatic arthritis^[30] and ankylosing arthritis^[31]. However, the experience is limited to only few patients to allow any generalisable conclusions.

FACTORS RESPONSIBLE FOR GOOD OUTCOME IN HSCT

Several factors determine the sustained clinical remissions or even cure in the treatment of AIRD namely: (1) type and stage of the autoimmune disease; (2)

type of transplant allogenic vs autologous^[32]; and (3) conditioning regimen (non-myeloablative vs myeloablative)^[33]. The EBMT data suggests that in addition to the influence of original diagnosis; age less than 35 years and HSCT performed after December 2000 were associated with a higher progression-free survival^[6]. The original diagnosis was a strong determinant of overall survival (highest in RA and lowest in SSc); other factors associated with a better overall survival were the centers' experience, the use of peripheral blood stem cells, and a disease duration longer than the median before HSCT^[6].

The best results with HSCT have been reported for patients with SSc and SLE, whereas for RA it was associated with a higher rate of relapses. Restricted synovial T cell repertoire^[34] and T cell responses to a variety of microbial antigens and self-antigens such as type II collagen epitopes are probably the reasons for higher rate of RA relapses in patients who have undergone HSCT. With the advent of biologicals, over the years the use of SCT for RA has become almost obsolete due to the failure of suppression of the synovial T cells.

In SSc, overall there has been a statistically significant improvement in the mRSS and the pulmonary function tests whereas in SLE, the results have been encouraging with higher rates of renal remission.

CONCLUSION

Treatment of AIRD has been revolutionized over the last two decades with increasing use of biological agents and HSCT in refractory diseases. Careful selection of patients, especially in those with SSc and SLE for HSCT offers long-term progression free and overall survival. Though, till date no one therapy has offered complete remission from these diseases due to multifactorial etiology of this disease along with various external factors also play a role in the progression of these diseases.

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Basic Study

Interaction between castanospermine and immunosuppressant and cyclosporin A in rat cardiac transplantation

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Author contributions: Hibberd AD designed the study; Clark DA carried out the experiments; Mcelduff P analysed the data, justified the statistical tools used and constructed the figures; Hibberd AD, Clark DA, Trevillian PR and Mcelduff P all contributed to the interpretation of the data analyses; Hibberd AD and Mcelduff P drafted the manuscript; Hibberd AD, Clark DA, Trevillian PR and Mcelduff P all provided critical intellectual comment about the manuscript; all authors reviewed and approved the final manuscript.

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Data sharing statement: Technical appendix and dataset are available from the corresponding author at adrian.hibberd@hnehealth.nsw.gov.au.

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Abstract

AIM: To investigate the interaction between castanospermine and cyclosporin A (CsA) and to provide an explanation for it.

METHODS: The alkaloid castanospermine was prepared from the seeds of *Castanospermum australe* consistently achieving purity. Rat heterotopic cardiac transplantation and mixed lymphocyte reactivity were done using genetically inbred strains of PVG (donor) and DA (recipient). For the mixed lymphocyte reaction stimulator cells were irradiated with 3000 rads using a linear accelerator. Cyclosporin A was administered by gavage and venous blood collected 2 h later (C₂). The blood levels of CsA (Neoral) were measured by immunoassay which consisted of a homogeneous enzyme assay (EMIT) on Cobas Mira. Statistical analyses of interactions were done by an accelerated

failure time model with Weibull distribution for allograft survival and logistic regression for the mixed lymphocyte reactivity.

RESULTS: Castanospermine prolonged transplant survival times as a function of dose even at relatively low doses. Cyclosporin A also prolonged transplant survival times as a function of dose particularly at doses above 2 mg/kg. There were synergistic interactions between castanospermine and CsA in the prolongation of cardiac allograft survival for dose ranges of CsA by castanospermine of (0 to 2) mg/kg by (0 to 200) mg/kg (HR = 0.986; 95%CI: 0.981-0.992; $P < 0.001$) and (0 to 3) mg/kg by (0 to 100) mg/kg (HR = 0.986; 95%CI: 0.981-0.992; $P < 0.001$) respectively. The addition of castanospermine did not significantly increase the levels of cyclosporin A on day 3 or day 6 for all doses of CsA. On the contrary, cessation of castanospermine in the presence of CsA at 2 mg/kg significantly increased the CsA level ($P = 0.002$). Castanospermine inhibited mixed lymphocyte reactivity in a dose dependent manner but without synergistic interaction.

CONCLUSION: There is synergistic interaction between castanospermine and CsA in rat cardiac transplantation. Neither the mixed lymphocyte reaction nor the metabolism of CsA provides an explanation.

Key words: Cardiac transplantation; Castanospermine; Cyclosporin A; Positive interaction; Mixed lymphocyte reaction

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Core tip: The authors have established that a biological, castanospermine, interacts with cyclosporin A (CsA) in a synergistic manner when prolonging the survival of cardiac allografts in inbred rats. They suggest that the explanation is not its effect on the mixed lymphocyte reaction nor interference in the metabolism of CsA but rather an inhibition of migration through the basement membrane of the vasculature. They suggest that its effect on heparanase in mononuclear cells and heparan sulphate in the allograft should now be studied. This immunosuppressant holds promise of safe dose reduction of CsA but further assessment of its safety remains.

Hibberd AD, Clark DA, Trevillian PR, Mcelduff P. Interaction between castanospermine an immunosuppressant and cyclosporin A in rat cardiac transplantation. *World J Transplant* 2016; 6(1): 206-214 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/206.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.206>

INTRODUCTION

Transplant recipients are at risk from the adverse

effects of immunosuppressive agents for the duration of the transplant and beyond. All immunosuppressive agents currently used create adverse effects; this includes cancer^[1], infection^[2], nephrotoxicity^[3] and diabetes mellitus^[4]. Hence there is an ongoing need to improve immunosuppressive agents and treatment regimes. One method of managing the adverse effects of cyclosporin A (CsA), a common maintenance immunosuppressive agent, is the addition of a second agent that interacts synergistically with it: This allows reduction in the dose of CsA (thus reducing the risk of adverse effects) while maintaining the overall immunosuppressive effect provided the second agent is well tolerated.

Glycoproteins are essential components of the cell as they are used to construct receptor ligand combinations, membranes and cytokines. Castanospermine disrupts their construction by competitively inhibiting glucosidase 1 and 2. It is a biological found in the Moreton Bay Chestnut Tree. In general construction of glycoproteins takes place in the endoplasmic reticulum and the Golgi apparatus. In the endoplasmic reticulum the oligosaccharide is bound to the polypeptide carried on polysomes^[5]. Here it is then refined by removal of glucose by glucosidase 1 and 2, removal of mannose by mannosidase 1 and glycosylation by N acetyl transferase. After moving to the Golgi it is further refined by removal of mannose by mannosidase 2 and glycosylation by N acetyl transferase. Hence the mannose-6-phosphate receptor may be disrupted and the transport of glycoproteins impaired. Overall some glycoproteins become dysfunctional. It is interesting to note that work to date has shown CAST is immunosuppressive and anti-inflammatory: Cardiac allograft rejection^[6], thyroid allograft rejection^[7], autoimmune encephalomyelitis^[8] and chemically induced arthritis^[9] are all mitigated.

When developing new immunosuppressive molecules the emphasis has been upon two major targets; the T and B cells. But allograft rejection has other sites that are open to therapeutic intervention including lymphocyte binding to the vascular endothelium and cell migration through the basement membrane of the allograft vasculature. The basement membrane which contains heparan sulphate proteoglycan (HSPG) perlecan^[10] protects islet clusters against autoimmune destruction; this protection is broken by heparanase secreted by mononuclear cells which cleaves heparan sulphate from the HSPG^[11] thus allowing cell entry. By effecting the membrane expression of adhesion molecules on both lymphocytes and endothelial cells CAST reduces the binding of the two cell types^[12]. It may also impair the production of heparanase by MNCs and the degradation of extracellular matrix by endothelial cells^[13]. Hence it may conserve the structure of HSPG in the basement membrane of the allograft vasculature and thus protect against rejection. These mechanisms of action are different from those of CsA,

to our knowledge, and therefore warrant investigation as a strategy to reduce the adverse effects of CsA. To date an immunosuppressive agent that conserves the function of allograft basement membrane (and also prevents the binding of alloreactive cells to the endothelium) is not in clinical use.

Hence in this study we aimed to determine if there is a synergistic interaction between castanospermine (CAST) and CsA. If so we aimed to provide an explanation for it.

MATERIALS AND METHODS

Rat strains

The inbred rat strains PVG (RT1^c) (donor) and DA (RT1^a) (recipient) were used to study cardiac allograft survival and the mixed lymphocyte reaction (MLR); DA rats were used to study the blood levels of CsA. The rats were housed under standard conditions in the Animal House of the Faculty of Health Sciences, University of Newcastle, Australia.

Rat heterotopic cardiac transplantation

Heterotopic cardiac transplants were done using a published technique^[14]. Cardiac function was assessed daily by abdominal palpation and transplant electrocardiography. The end point of cardiac transplant survival was defined as the last day of palpable heart beating. Care of all rats in this study complied with the Animal Research Act 1985 (NSW, Australia). The protocols were designed to minimise pain and discomfort to the animals. Animals were acclimatised to laboratory conditions (22 °C, 12 h cycle of light and dark, 50% humidity, ad libitum access to food and water) for a minimum of 1 wk prior to experimentation. Intragastric gavage administration was carried out with conscious animals, using curved gavage needles appropriate for animal size (250-300 gm body weight: Gauge 16, 100 mm). All transplanted rats were given post-operative analgesia (Carprofen 4 mg/kg every 12-24 h subcutaneously). They were euthanized by approved carbon dioxide asphyxiation when survival reached 100 d or when the heart stopped beating confirmed by electrocardiography prior to tissue procurement.

Castanospermine

This indolizidine alkaloid is extracted from the seeds of *Castanospermum australe* (the Australian Moreton Bay Chestnut) by a standard technique yielding purity $\geq 99.5\%$ ^[13]. For the studies on cardiac transplant survival it was administered by Alzet osmotic pumps (Alza Corporation, Palo Alto, United States) at doses of 50, 100, 150, 200 or 300 mg/kg per day by constant subcutaneous infusion (10 μ L/h) from day 1 until day 6 when the pump was removed. For the studies of CsA blood levels, CAST was delivered by osmotic pumps at 100 mg/kg per day or 200 mg/kg per day

from day 1 until day 6 when the pump was removed. The control was a pump filled with 0.9% saline and removed at day 6. For studies on the MLR, CAST was dissolved in RPMI medium 1640 (Trace Biosciences, Sydney, Australia) supplemented with 10% foetal calf serum (FCS, Trace Biosciences, Sydney, Australia), 2-[4-(2-Hydroxyethyl)]-1-piperazine ethane sulfonic acid buffer 0.02 mol/L (HEPES, Trace Biosciences, Sydney, Australia), sodium bicarbonate 1.5 g/L, penicillin/streptomycin 50 mg/L, 2-mercaptoethanol 5×10^{-5} mol/L and L-glutamine 1 mg/L to a concentration of 65536 μ mol/L (micromolar) and then filtered through a 0.22 μ m filter (Sartorius, Hannover, Germany). Final concentrations used were quadrupling dilutions of 16384 to 0.0625 μ mol/L.

CsA

For the transplant survival study CsA (Neoral, Novartis Pharmaceutical, Australia) was diluted in olive oil and administered by gavage at doses of 0.5, 2, 3, 4 mg/kg per day to DA rats. For the study on its blood levels CsA was delivered by gavage at the appropriate dose once daily from day 0 to day 9. Venous blood (0.3 mL) was then collected from the tail veins of DA rats using a 1 mL syringe with a 25 gauge needle two hours after gavage of CsA (C₂ level). Samples were then processed at Hunter New England Area Pathology Services (John Hunter Hospital Newcastle, NSW, Australia) using a homogeneous enzyme immunoassay (EMIT 2000, Dade Behring-Syva, Deerfield, Illinois, United States) performed on a Cobas Mira (Roche, Basel, Switzerland). For the MLR CsA was diluted in RPMI medium to 40.96 μ mol/L, filtered through a 0.22 μ m filter and used in quadrupling dilutions of 10.24 to 0.00015625 μ mol/L.

MLR assay

Responder cells were isolated at 4 °C from pooled, all DA available lymph nodes; stimulator cells were isolated at 4 °C from PVG spleens and both were prepared as previously described^[6]. Final cell concentrations for use in the MLR were 2×10^6 /mL responders and 2×10^6 /mL stimulators. The stimulators were irradiated with 3000 rad (radiation absorbed dose) using a linear accelerator (Varian, Palo Alto, California, United States) before use in the MLR.

For the MLR 2×10^5 responder cells were co-cultured with 2×10^5 PVG stimulator cells for 72 h. All assays for given doses of CAST or CsA were done in triplicate. During incubation cells were exposed to final concentrations of CAST in quadrupling dilutions of 16384 to 0.0625 μ mol/L or final concentrations of CsA in quadrupling dilutions of 10.24 to 0.00015625 μ mol/L or a combination of both drugs. The cultures were pulsed with H³ - thymidine (Amersham, United Kingdom) at 1.0 μ Ci/well for 18 h and then harvested on to nitrocellulose filters using a Filter Mate Cell Harvester (Packard Instrument Company,

Meriden, United States) then counted on a microplate scintillation counter (Packard Instrument Company, Meriden, United States). The mean count per minute (cpm) \pm SD was the function used to express the results.

Cardiac transplant survival

The survival curves for heterotopic cardiac transplants were established for CAST by dose and for CsA by dose separately: Groups received CAST at 50, 100, 150, 200 or 300 mg/kg per day over 7 d; other groups received CsA at 0.5, 2, 3 or 4 mg/kg per day over 7 d. For the interaction studies the groups were: CsA 0.5 mg/kg plus CAST 100 mg/kg, CsA 0.5 mg/kg plus CAST 200 mg/kg, CsA 2 mg/kg plus CAST 50 mg/kg, CsA 2 mg/kg plus CAST 100 mg/kg, CsA 2 mg/kg plus CAST 200 mg/kg, CsA 3 mg/kg plus CAST 50 mg/kg or CsA 3 mg/kg plus CAST 100 mg/kg. The control group consisted of allografts with neither CAST nor CsA. Previous work has established that the osmotic pump with 0.9% saline does not prolong allograft survival^[6]. Permanent prolongation was defined as 100 d survival.

Blood levels of CsA in the presence of castanospermine

The study consisted of 9 groups: CsA 2, 3 or 4 mg/kg each in combination with CAST 0 (saline), 100 or 200 mg/kg. C₂ levels (ug/L) were then measured on day 3, 6 (both on pump) and 9 (off pump).

MLR

The T cell responses in the MLR relating the proliferation and dose were used to determine the IC₅₀s for CsA and CAST separately. To study the interaction between the two drugs the range of doses selected for CsA or CAST was the IC₅₀ for either drug plus the two dose concentrations that were immediately greater or smaller. A series of MLRs for CsA each with a different CAST dose was then done.

Transplant data

In this study "time to death" was chosen as the outcome measure. The survival time of transplants was truncated at 100 d and therefore the survival times beyond 100 d are unknown. Survival analysis techniques, which model these censored observations, have been used. Specifically, accelerated failure time models that assume survival times follow a Weibull distribution were used^[15].

The extent to which dose of CAST can impact on the association between dose of CsA and survival can only be estimated where the marginal effect of either drug does not reach its maximum. Therefore we only examined whether the dose of CAST was an effect modifier of the association between CsA and survival for the dose ranges of CAST by CsA of (0 to 200) mg/kg by (0 to 2) mg/kg and separately (0 to 100) mg/kg by (0 to 3) mg/kg. Hazard ratios (HR)

were used to describe the effect of CsA and CAST, and their interaction, on survival. The HR for these data can be interpreted as the relative risk of death at a given follow-up time associated with each one-unit increase in the treatment. With an interaction term in the model, the HR associated with the main effect of one of the treatments is only applicable when the other treatment is held at zero; this is true because the interaction term allows the HR of one treatment to depend on the level of the other treatment. The HR associated with the interaction term is the additional effect of having the two treatments above the individual effects of the two treatments.

MLR data

The effect of treatment with CsA and CAST on lymphocyte count was explored using linear regression within a linear mixed model framework. The outcome measure in the regression models was the natural logarithm (log) of the lymphocyte count and the main predictors of interest were dose of CsA and CAST. Experimental number was included as the adjusting unit to adjust for any variation that may have occurred in experimental conditions. The likelihood ratio statistic was used to compare the models with and without the interaction term of CsA by CAST. The data indicate that the relationship between CsA and lymphocyte count or between CAST and lymphocyte count is not monotonic with a small increase in the lymphocyte count observed at very low doses. Therefore it was not appropriate to assume that the dose response relationship is linear and so dose of CsA and dose of CAST were included in the model as categorical variables. Therefore no assumption is made about the relationship of dose and the natural log of lymphocyte count.

Statistical analysis

In this study synergy is defined as a positive interaction between CsA and CAST which means that their combined effects are greater than the sum of their individual effects. The definition of statistical interaction is logically equivalent to the definition of effect-measure modification and is usually described as "departure from additivity of effects on the chosen outcome scale"^[16]. This definition implies that the presence or absence of statistical interaction between two factors depends on the scale chosen to measure the effect.

RESULTS

Interaction between castanospermine and CsA in rat cardiac transplantation

The numbers of transplants that survived to 100 d and the mean transplant survival times are listed in Tables 1 and 2 and Figure 1. Castanospermine

Table 1 Effect of cyclosporin A or castanospermine or both upon cardiac allograft survival

No. of subjects and (number alive at 100 d) for each dose group of cyclosporin A by castanospermine ^{1,2}		Castanospermine dose ^{3,4}					
Cyclosporin A dose ⁴	0.0	14 (0)	7 (0)	7 (0)	7 (1)	6 (1)	6 (4)
	0.5	7 (0)		7 (2)		6 (4)	
	2.0	10 (0)	7 (0)	11 (7)		6 (5)	
	3.0	6 (0)	6 (1)	6 (6)			
	4.0	6 (5)					

¹PVG donor into DA recipient; ²The syngeneic control, DA into DA, was 4 (4); ³Survival times are truncated at 100 d; ⁴Drug doses are given in mg/kg per day body weight.

Table 2 Effect of cyclosporin A or castanospermine or both upon cardiac allograft survival

Mean survival for each dose group of cyclosporin A by castanospermine ^{1,2}		Castanospermine dose ^{3,4}					
Cyclosporin A dose ⁴	0.0	7.5	9.7	13.1	31.7	45	75.7
	0.5	7.4		38.9		73.8	
	2.0	8.4	13.2	75.5		99.3	
	3.0	10.7	30.7	100			
	4.0	85.2					

¹PVG donor into DA recipient; ²The mean survival of the syngeneic control (DA into DA) was 100 d; ³Survival times are truncated at 100 d; ⁴Drug doses are given in mg/kg per day body weight.

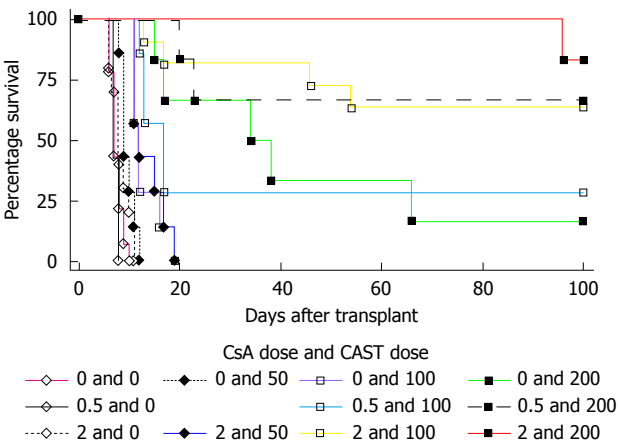


Figure 1 Cardiac graft survivals in rats treated with a range of doses of castanospermine only, a range of doses of cyclosporin A only or a combination of both. The doses of CAST and CsA are given in mg/kg per day. When the two drugs are used together the survival is greater than the sum of the two drugs alone ($P < 0.001$ when dose of CsA and dose of CAST are treated as continuous variables): Compare CsA 2 mg/kg alone plus CAST 100 mg/kg alone with the combination of CsA 2 mg/kg and CAST 100 mg/kg. CAST: Castanospermine; CsA: Cyclosporin A.

clearly prolonged transplant survival times in a dose dependent manner even at relatively low doses. Cyclosporin A also prolonged transplant survival times in a dose dependent manner particularly at doses

Table 3 Analysis of the interaction between cyclosporin A and castanospermine upon cardiac allograft survival

Output from the accelerated failure time model with weibull distribution for cyclosporin A doses of 0 to 2 mg/kg per day and castanospermine doses of 0 to 200 mg/kg per day			
Variable	HR	95%CI	P value
Cyclosporin A dose	0.958	0.668-1.374	0.817
Castanospermine dose	0.982	0.976-0.988	< 0.001
Interaction	0.986	0.981-0.992	< 0.001

Table 4 Analysis of the interaction between cyclosporin A and castanospermine upon cardiac allograft survival

Output from the accelerated failure time model with weibull distribution for cyclosporin A doses of 0 to 3 mg/kg per day and castanospermine doses of 0 to 100 mg/kg per day			
Variable	HR	95%CI	P value
Cyclosporin A dose	0.852	0.662-1.0954	0.211
Castanospermine dose	0.978	0.968-0.987	< 0.001
Interaction	0.986	0.981-0.992	< 0.001

above 2 mg/kg. The results of statistical analyses of the interactions between the two drugs are listed in Tables 3 and 4. Using accelerated failure time models the effect of dose of CsA on the association between CAST and survival was analysed in the dose ranges of CsA by CAST of (0 to 2) mg/kg by (0 to 200) mg/kg and (0 to 3) mg/kg by (0 to 100) mg/kg. There was a statistically significant interaction between CsA and CAST in both dose ranges (both $P < 0.001$). In the dose ranges of CsA by CAST of (0 to 2) mg/kg by (0 to 200) mg/kg, the HR associated with CsA was 0.958, with CAST was 0.982 and with the interaction term was 0.986. This means the addition of one mg/kg of CsA together with one mg/kg of CAST reduced the risk of death by 7.2% at each point in the follow-up period, which is captured by the combined HR of 0.928 ($0.958 \times 0.982 \times 0.986$).

The effect of castanospermine upon the blood level of CsA

This was studied to determine whether the synergistic interaction between CAST and CsA *in vivo* was simply due to an increased blood level of CsA in the presence of CAST. The results of CsA levels in the presence of CAST are listed in Table 5 and upon cessation of CAST in Table 6. The addition of CAST did not significantly increase the CsA levels on day 3 or day 6 for all CsA doses studied. Furthermore, at day 3 the CsA levels were similar for all doses of CAST but at day 6 the CsA levels tended to decrease with increasing doses of CAST. This difference in the trend of the CsA levels between day 6 and day 3 was statistically significant at each dose of CsA (CsA 2 mg/kg $P = 0.02$; CsA 3 mg/kg $P = 0.04$; CsA 4 mg/kg $P = 0.001$). Cessation of CAST by removal of the pump did not significantly

Table 5 Effect of the dose of castanospermine delivered by a pump on the blood level of cyclosporin A

Blood level of CsA ¹					
CsA dose ²	d	Castanospermine dose ²	No.	Mean	SD
2 ^{3,4}	3	0	5	189.2	73.34
	3	100	5	299.8	53.53
	3	200	5	313.0	131.56
	6	0	5	477.0	78.97
	6	100	5	326.6	110.48
	6	200	5	280.2	126.69
3 ^{3,5}	3	0	5	520.6	177.18
	3	100	5	450.2	218.76
	3	200	4	506.5	271.96
	6	0	5	1061.80	256.22
	6	100	5	784.80	107.83
	6	200	4	439.75	160.51
4 ^{3,6}	3	0	5	711.80	184.61
	3	100	5	601.40	121.33
	3	200	5	1031.60	287.18
	6	0	5	1110.20	252.20
	6	100	5	1152.20	127.67
	6	200	5	556.20	192.41

¹CsA levels are given in $\mu\text{mol/L}$; ²CsA and CAST doses are given in mg/kg per day body weight; ³No significant increase in CsA level for no CAST *vs* CAST at day 3 or day 6; ⁴For each CsA dose the difference in trend of day 6 values compared with day 3 was significant: CsA 2 mg/kg per day $P = 0.02$; ⁵CsA 3 mg/kg per day $P = 0.04$; ⁶CsA 4 mg/kg per day $P = 0.001$. CAST: Castanospermine; CsA: Cyclosporin A.

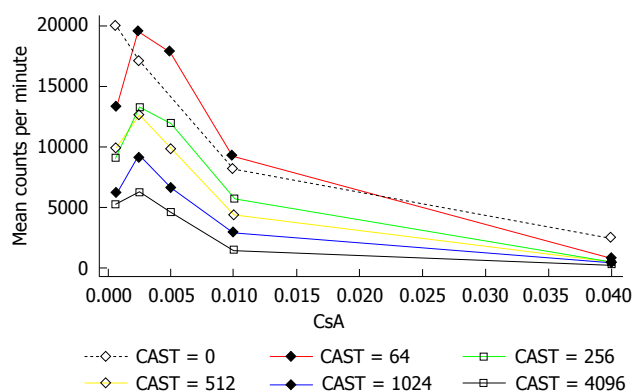


Figure 2 Mean number of lymphocytes for increasing doses of cyclosporin A by dose of castanospermine. The doses of CAST and CsA are given in $\mu\text{mol/L}$. There is a reduction in lymphocyte count for increasing doses of CsA or increasing doses of CAST. The absolute reduction in lymphocytes for a given dose of CsA decreases with decreasing doses of CAST. CAST: Castanospermine; CsA: Cyclosporin A.

decrease the CsA level: On the contrary, when using CsA at 2 mg/kg cessation of CAST significantly increased the CsA level ($P = 0.002$).

The interaction between castanospermine and CsA in the MLR

The interaction between CAST and CsA in the MLR is represented in Figures 2 and 3. There is a reduction in the number of lymphocytes with increasing doses of CsA for all dose levels of CAST and the absolute reduction in lymphocytes for a given dose of CsA

Table 6 Effect of removal of the pump delivering castanospermine on blood level of cyclosporin A

Blood level of CsA ¹					
CsA dose ²	On pump	Castanospermine dose ²	No.	Mean	SD
2 ³	Yes	0	10	333.10	167.84
		100	10	313.20	83.06
		200	10	296.60	122.98
	No	0	5	513.20	170.76
		100	5	560.00	254.00
		200	5	355.40	105.29
3 ⁴	Yes	0	10	791.20	352.83
		100	10	617.50	239.87
		200	8	473.13	209.79
	No	0	5	849.40	455.77
		100	5	671.20	421.57
		200	4	824.50	153.44
4 ⁴	Yes	0	10	911.00	295.81
		100	10	876.80	313.14
		200	10	793.90	340.42
	No	0	5	968.80	429.26
		100	5	1188.60	453.13
		200	5	589.40	290.93

¹CsA levels are given in $\mu\text{mol/L}$; ²CsA and CAST doses are given in mg/kg per day body weight; ³Off pump significantly increased compared with on pump ($P = 0.02$); ⁴No significant difference between on pump *vs* off pump values. CAST: Castanospermine; CsA: Cyclosporin A.

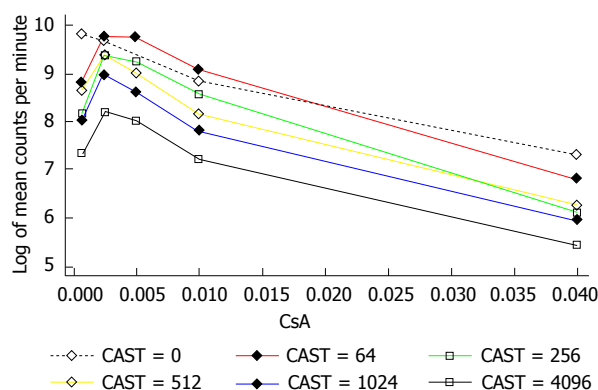


Figure 3 Natural logarithm of the mean number of lymphocytes for increasing doses of cyclosporin A by dose of castanospermine. The doses of CAST and CsA are given in $\mu\text{mol/L}$. There is a dose dependent reduction in the logarithm of the lymphocyte count for CsA alone ($P < 0.001$) or for CAST alone ($P < 0.001$). But when the reduction is analysed there is not a synergistic interaction ($P = 0.89$). CAST: Castanospermine; CsA: Cyclosporin A.

decreases with decreasing doses of CAST (Figure 2). A more appropriate scale to assess this biological interaction, however, is the natural logarithm (log) of lymphocytes given that proliferation is likely to occur due to a doubling of the current number. The results contained in Figure 3 show there is a reduction in the natural log of the number of lymphocytes with increasing doses of CsA which is similar for all doses of CAST ($P < 0.001$). This implies that the percentage reduction in the number of lymphocytes with increasing dose of CsA is constant for all doses of CAST. Further, there was no statistically significant interaction between CsA and CAST ($P = 0.89$).

DISCUSSION

The major findings in this study are that CAST and CsA interacted synergistically in the prolongation of rat cardiac allograft survival but did not interact synergistically in the MLR despite showing additive dose dependent inhibition with CsA. Further, the blood level of CsA was not increased by the addition of CAST. By contrast it was increased when CAST was ceased while using CsA at 2 mg/kg but not at the other 2 doses of CsA.

In clinical practice the nephrotoxicity of CsA is a major unsolved problem. Cyclosporin A causes interstitial fibrosis, tubular atrophy (IFTA) and arteriolar hyalinosis and therefore can contribute to graft failure^[17]. There is controversy, however, about the extent that CsA nephrotoxicity alone causes graft failure; some argue that it is the major cause^[17] while others consider it minor causing 0.7% of graft losses^[18]. The use of a second agent acting in synergism with CsA provides a method of managing the nephrotoxicity because it allows dose reduction in CsA (and thus toxicity) without compromising graft survival. Reduction in the dose of CsA can be expected to alleviate nephrotoxicity given the inverse relationship between CsA dose and IFTA^[19]. Our study shows that because CAST interacts synergistically with CsA and is relatively nontoxic^[6] it holds promise of reducing the toxicity of CsA when combined with it. But there are many remaining points of assessment before castanospermine can be considered for the clinic. Other studies have also shown synergistic interactions between CsA and dexamethasone and between CsA and rapamycin which have allowed safe reduction in CsA dose. A second method of managing CsA nephrotoxicity is the use of a specific antagonist: For instance, darusentan alleviates CsA nephrotoxicity in rats by blocking the type A endothelin receptor^[20] but to date there is no antagonist in clinical use.

Three explanations for the synergistic interaction between CAST and CsA were examined in our studies. First it is not due to simple inhibition of the hepatic metabolism of CsA because CAST did not increase the CsA level (Table 5). By contrast CAST reduced the blood level of CsA at one of the three doses studied (Table 6). Our hypothesis for these findings is that CAST may impair the mechanism used for the absorption of CsA in the small bowel known to depend upon a glycoprotein transporter. This mechanism may be competitively inhibited at low doses of CsA by CAST but at higher doses of CsA the inhibition is less effective. Second, although CAST inhibits the MLR by inhibiting signal transduction from the IL-2 receptor^[21] it did not act synergistically with CsA in the MLR (Figures 2 and 3). It did however reduce the MLR with CsA in an additive dose dependent manner. This finding implies that CAST may act at sites other than the T cell which proliferates in the MLR. Third, our previous immunohistochemistry studies in rats treated with CAST revealed clusters of mononuclear cells (MNCs)

about the basement membrane of venules while sparing the interstitial infiltrate in cardiac allografts^[6]; these findings are consistent with the observations of Willenborg *et al*^[8] in rats with experimental autoimmune encephalomyelitis treated with CAST.

We therefore propose that CAST may impair the passage of MNCs through this basement membrane of the venules. The evidence for this proposal is the following. The basement membrane contains heparan sulphate proteoglycan perlecan which acts as a barrier to cell entry^[10]. It can be broken down by heparanase which is present in MNCs and endothelial cells^[11]. Castanospermine has been shown to inhibit heparanase and sulfatase in endothelial cells^[13], to inhibit heparanase within intragraft alloreactive cells^[22] and to inhibit lysis of extracellular matrix which also contains HSPG^[13]. Furthermore, in a murine model of autoimmune insulinitis inhibition of heparanase conserved the basement membrane of islet clusters which contained heparan sulphate^[11]. Hence an explanation for the synergistic interaction of CAST and CsA may be the reduction in heparanase production from alloreactive cells by CAST thus strengthening the impermeability of the vascular basement membrane. To our knowledge this site is not affected by CsA.

The strengths of our study are that it definitively establishes for the first time that CAST and CsA act synergistically in prolonging rat allograft survival and, second, the explanation cannot be found in its effect on T cell proliferation nor the metabolism of CsA. The weakness of our study is that this work is in inbred rats only and therefore work in higher animal models is required before one can reasonably hope for amelioration of the adverse effects of CsA by dose reduction.

Although we conclude that CAST and CsA interact synergistically in this model further study of its effect on heparanase and heparan sulphate concentrations in organ allograft transplantation is necessary. *In vivo* and *in vitro* migration studies are also needed to challenge the proposal that the basement membrane is a key site of action of CAST.

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COMMENTS

Background

The field of organ transplantation which is a major medical advance still has some fundamental problems to solve. One of these is the adverse effects of immunosuppressive drugs that are necessary for the duration of the transplant for the vast majority of recipients. It is an exception for recipients to become

tolerant to their transplants implying that their immune responses have accepted the foreign transplants. Now, the major adverse effects of immunosuppressive agents are cancer and infection although nephrotoxicity, diabetes mellitus and osteoporosis are also common. One approach to managing adverse effects is the use of another immunosuppressive agent which acts synergistically with the first agent. Thus reduction in dose of the first agent can be done without inducing rejection. Because dose is reduced its toxicity may also be reduced provided the second agent is relatively non-toxic. In this study the authors have used this strategy when analysing the immunosuppressive ability of castanospermine a biological derived from the Moreton Bay Chestnut tree.

Research frontiers

The authors aimed to study the interaction between castanospermine and cyclosporin A (CsA) which is a common maintenance immunosuppressive agent in organ transplantation. The major adverse effect of CsA is nephrotoxicity which is dose dependent. So first the study of the interaction needs to be done in an animal model transplant system.

Innovations and breakthroughs

They study establishes the positive interaction between castanospermine and CsA and therefore justifies studying the mechanism of its immunosuppressive effect. They have found that the synergism is unlikely to be due to inhibition of T-cell proliferation nor interference in the metabolism of CsA. They have other evidence referenced here suggesting that castanospermine may act by inhibiting migration of cells through the basement of the transplant. Impairment of heparanase in T cells seems to be the key.

Applications

Although clinical use of castanospermine or a derivative is the long term aim of this work further study of its mechanism and toxicity profile are needed first.

Terminology

There are several key components of the allograft rejection response. One of these is the T cell that secretes IL-2 a cytokine that causes T cell proliferation. Cyclosporin A interferes with the production of IL-2 and is a strong immunosuppressant. Another is the B cell that presents antigen to the T cell and also enables antibody production from plasma cells. Rituximab monoclonal antibody inhibits B cell production. Castanospermine acts differently focussing upon migration of cells into the transplant.

Peer-review

The authors have reviewed and answered the peer reviewers' comments. They liked the idea of developing an immunosuppressive agent that was synergistic with CsA in organ transplantation. They understood that it could have clinical benefit but that other studies in outbred animals about adverse effects and immunosuppressive ability of castanospermine are needed first. They also encouraged further study of the reasons behind synergism and in particular how castanospermine can inhibit cell migration.

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Retrospective Study

New Nodule-Newer Etiology

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Author contributions: Mehta AC participated in the design of the study, data collection, statistical analysis, interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Mehta AC was the guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article; Wang J and Abuqayyas S participated in the data collection, interpretation of the results and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Abuqayyas S performed the statistical analysis; Garcha P, Lane CR, Tsuang W and Budev M participated in the interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Akindipe O participated in the conception of the study, interpretation of the results and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted.

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Abstract

AIM: To evaluate frequency and temporal relationship between pulmonary nodules (PNs) and transbronchial biopsy (TBBx) among lung transplant recipients (LTR).

METHODS: We retrospectively reviewed 100 records of LTR who underwent flexible bronchoscopy (FB) with TBBx, looking for the appearance of peripheral pulmonary nodule (PPN). If these patients had chest radiographs within 50 d of FB, they were included in the study. Data was compared with 30 procedures performed among non-transplant patients. Information on patient's demographics, antirejection medications, anticoagulation, indication and type of lung transplantation, timing of the FB and the appearance and disappearance of the nodules and its characteristics were gathered.

RESULTS: Nineteen new PN were found in 13 procedures performed on LTR and none among non-transplant patients. Nodules were detected between 4-47 d from the procedure and disappeared within 84 d after appearance without intervention.

CONCLUSION: FB in LTR is associated with development of new, transient PPN at the site of TBBx

in 13% of procedures. We hypothesize that these nodules are related to local hematoma and impaired lymphatic drainage. Close observation is a reasonable management approach.

Key words: Peripheral pulmonary nodule; Flexible bronchoscopy; Transbronchial biopsy; Lung transplant

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Core tip: Transbronchial biopsy (TBBx) is routinely performed in lung transplant recipients (LTR). The development of pulmonary nodules (PNs) in this population is common. We investigated LTR who developed PNs post TBBx to determine the temporal relationship between the procedure and the timing of appearance and disappearance of these nodules. Our conclusion is that TBBx in LTR is associated with development of transient nodules at the site of TBBx in 13% of procedures. We hypothesize that these nodules are related to local hematoma and impaired lymphatic drainage. Close observation is a reasonable management approach.

Mehta AC, Wang J, Abuqayyas S, Garcha P, Lane CR, Tsuang W, Budev M, Akindipe O. New Nodule-Newer Etiology. *World J Transplant* 2016; 6(1): 215-219 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/215.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.215>

INTRODUCTION

Lung transplantation (LTx) is a well-accepted treatment modality for end stage pulmonary diseases such as interstitial lung disease (ILD), cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and pulmonary artery hypertension (PAH). Since the mid-80s more than 51000 patients have undergone lung transplantation (www.ISHLT.org/). Flexible bronchoscopy (FB) is routinely performed in this population based on clinical grounds and/or as a surveillance to rule out subclinical rejection. LTx is being performed at our institution for over 25 years and over 1500 procedures have been performed. For the last five years we have performed an average of 900 bronchoscopies per year on this group of patients.

Peripheral pulmonary nodule (PPN) is a common clinical challenge for the pulmonologist given that it presents with a wide range of differential diagnosis. When present in the LTR, these nodules represent even a greater challenge due to the possibilities of opportunistic infection, post-transplant lymphoproliferative disorder (PTLD) and other malignancies^[1].

Prompt evaluation and appropriate treatment for the PPN are essential in this high-risk population.

Recently we have noticed transient appearance of PPNs in lung transplant recipients (LTR) who underwent FB with a transbronchial biopsy (TBBx). These nodules prompted diagnostic workup in some individuals but were eventually thought to be related to the procedure. The following study was carried out to evaluate the relationship between FB with TBBx and the new PPN in this group focusing on the nodule's characteristics and the temporal relationship with the procedure.

MATERIALS AND METHODS

Study group

We retrospectively reviewed 100 bronchoscopy records of LTR who underwent FB with TBBx between January 2013 and March 2014 at our institution. If either a chest X-ray or a computed tomography (CT) was performed within 50 d of the procedure on these patients they were considered for the study. Patients with preexisting lung nodule of known or unknown etiology prior to the FB were excluded from the study.

Pulmonary nodule

PPN was defined as a focal pulmonary lesion or opacity, round or oval in shape, which measured less than 3 cm in diameter and appeared within 50 d after the bronchoscopy.

Data collection

Data collection included patient demographics, antirejection and anticoagulation medication used, indication and type of lung transplantation (single vs bilateral), timing of the FB in relation to the transplantation, site of the TBBx, bronchoscopy complications, histological findings and microbiological culture results, number of the nodules, site, shape, size and presence or absence of cavitation. Once a nodule was detected all available post-bronchoscopy radiographic studies were reviewed to judge the outcome of the nodule and/or the day of disappearance. The day of appearance and disappearance of the nodule was also tabulated. The patient's clinical status was noted and was correlated with the appearance and disappearance of the nodules from the available medical records.

Control group

A control group was created by reviewing bronchoscopy records of non-transplant patients who underwent FB with TBBx during the same period and had a chest radiograph performed within 50 d of the procedure. Similar data as in the LTR was collected from these patients if they were found to have a PPN.

Flexible bronchoscopy

A surveillance bronchoscopy is routinely performed at our institution among the LTR at 3, 6 and 12 wk, and 6, 9 and 12 mo following the LTx. If rejection is detected,

Table 1 Demographics of lung transplant recipients with pulmonary nodules

Patient	Sex	Age	Indication for LTX	Type of LTX	Anticoagulation
1	M	71	IPF/UIP	Right	Warfarin
2	F	42	COPD	Right	
3	F	60	CB	Bil	
4	F	54	PVOD	Bil	LMWH
5	M	62	COPD	Bil	
6	M	69	IPF	Left	
7	M	29	PVOD	Bil	
8	F	50	ILD/MCTD/PSS	Bil	
9	M	32	ILD/PSS with PHTN	Bil	
10	M	31	CF	Bil	

LTX: Lung transplantation; IPF: Idiopathic pulmonary fibrosis; COPD: Chronic obstructive pulmonary disease; CB: Constrictive bronchiolitis; PVOD: Pulmonary veno-occlusive disease; ILD: Interstitial lung disease; MCTD: Mixed connective tissue disease; PSS: Progressive systemic sclerosis; PHTN: Pulmonary hypertension; CF: Cystic fibrosis; LMWH: Low molecular weight heparin.

a follow-up bronchoscopy is performed 3 wk following the completion of appropriate treatment. A clinical bronchoscopy is performed on an as needed basis. All bronchoscopies are performed under conscious sedation and fluoroscopic guidance. A bronchoalveolar lavage (BAL) is obtained from a non-dependent portion of the lung in all patients to stain and/or culture for opportunistic infections.

Transbronchial biopsy

For the surveillance procedure, our common practice is to obtain a total of 6 pieces of tissue in a single lung transplant (SLTx) recipient and 8 pieces of tissue in recipients of bilateral transplant (BLTx). All the biopsies are obtained from either a single segment or two separate segments of the dependent lobe of the lung at the discretion of the bronchoscopist. All tissue specimens are processed for histological examination in an usual fashion. When antibody mediated rejection (AMR) is suspected, biopsies are sent for C3d and C4d immunofluorescent staining.

The Institutional Review Board of the Cleveland Clinic, Cleveland, Ohio, approved the study. Due to the retrospective nature of the study, there was no need to obtain patient consent.

RESULTS

In the LTR group, we found 19 new nodules after 13 procedures performed on 10 LTR patients (Tables 1 and 2). All nodules were found at the same site of the TBBx (Figures 1 and 2). Nine of these nodules were rounded (47%) and 10 were oval in shape (53%). Fourteen nodules were solid (74%) and 5 were cavitory in nature (26%) (Figure 3). Nodule size (greatest diameter) ranged between 0.4 to 3 cm with a mean of 1.4 cm. Nodules were detected within 4

Table 2 Characteristics of the pulmonary nodules

FB	DOA	DOD	n	Size (cm)	Shape	Nature	Location
1	21	71	1	1.1	Round	Solid	RML
2	17	84	1	2.3	Round	Solid	RLL
3	16	12	2	1.2, 2.2	Round oval	Solid	RLL
4	13	60	2	1.1, 3	Round oval	Solid	RLL
5	27	25	2	1 × 0.7, 0.5 × 0.4	Oval	Solid	LUL, LLL
6	14	33	1	1 × 1.1	Oval	Solid	RML
7	4	9	1	1.5 × 2.5	Oval	Cavitory	LUL
8	21	33	1	1 × 1.1	Oval	Solid	LUL
9	10	19	1	2.2	Round	Solid	LLL
10	8	53	1	1.4 × 1.1	Oval	Cavitory	LUL
11	4	37	4	2, 2, 2, 1.2	Round	Cavitory Solid	LUL, LLL 3
12	28	48	1	0.4	Round	Cavitory	LLL
13	47	35	1	0.7	Round	Cavitory	RLL

FB: Flexible bronchoscopy; DOA: Day of appearance; DOD: Day of disappearance; RML: Right middle lobe; RLL: Right lower lobe; LUL: Left upper lobe; LLL: Left lower lobe.

to 47 d (mean 25 d) after the FB with TBBx and they disappeared within 9 to 84 d (mean: 38.3).

The male to female ratio was (1.5:1), age ranged between 29 to 71 years with a mean of 39.3 years. In these patients, LTx was performed for different indications, IPF in two patients, COPD in two patients, constrictive bronchiolitis in one patient, CF in one patient, pulmonary veno-occlusive disease in two patients, interstitial lung disease due to progressive systemic sclerosis in one patient and mixed connective tissue disease in one patient. Seven of these patients had BLTx (70%) and 3 SLTx (30%). Eight of them were on antirejection medication, Tacrolimus. Two patients were on chronic anticoagulation with either warfarin or low molecular weight heparin (LMWH) in which the therapy was appropriately stopped prior to the procedure. Two patients were on aspirin. Complications reported included minimal bleeding of less than 40 mL in seven procedures, one procedure had more than 40 mL blood loss.

In five patients, no acute or chronic rejection was found. Mild acute vascular rejection was found in two patients, mild acute rejection in three patients, chronic airway rejection in one and in one more patient scattered giant cells were found on the biopsy.

Other associated radiographic findings that were reported included blunting of the right costophrenic angle in one patient, mosaic attenuation and scattered ground glass opacities in another patient.

In all 13 procedures, the results of BAL were negative for viral, bacterial, mycobacterial and fungal infections.

In the control group, there were 30 patients. The indications for the FB with TBBx included (many of them did have confirmed diagnosis): Sarcoidosis, cryptogenic organizing pneumonia (COP), ILD, MCTD, bronchiolitis, asthma and COPD. No new nodules were

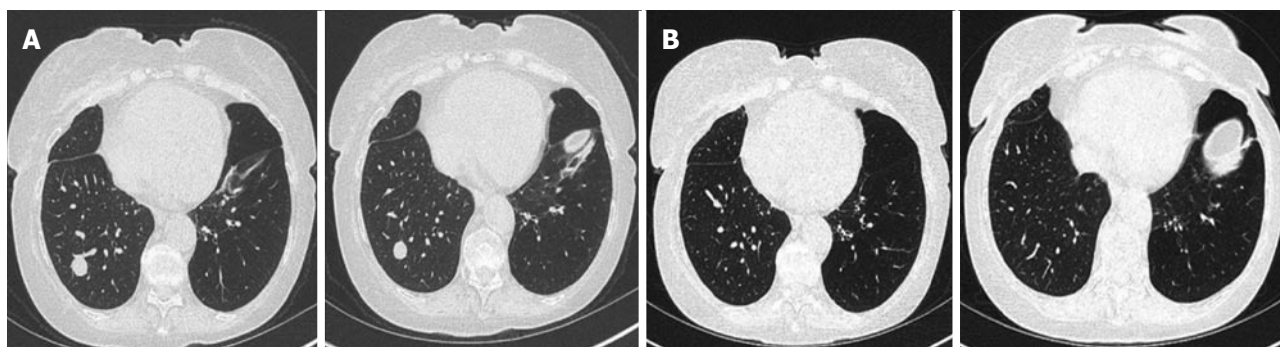


Figure 1 Computed tomography of chest. A: Day 40. Note a well circumscribed, round pulmonary nodule involving the right lower lobe. Transbronchial biopsy was obtained from this site 40 d earlier; B: Day 90. Note the total resolution of the right lower lobe nodule.

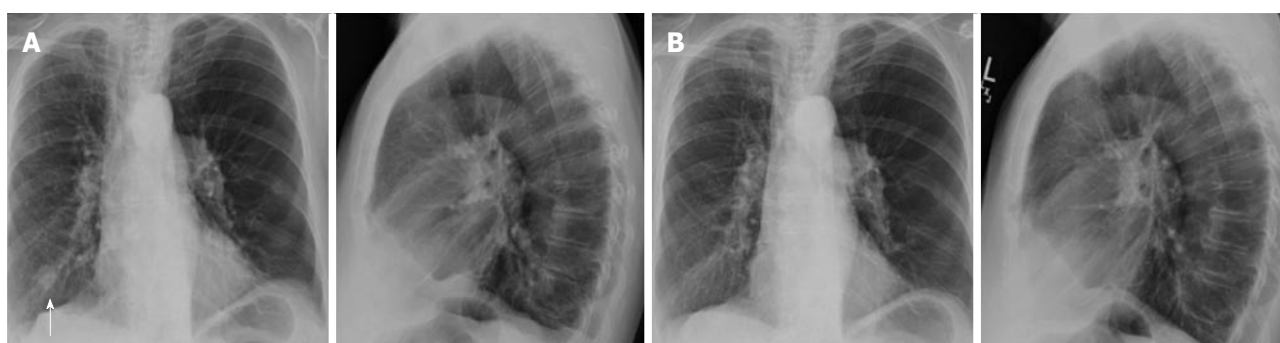


Figure 2 Posteroanterior and lateral views of the chest. A: Day 40. Note a well circumscribed, round pulmonary nodule involving the RLL, 2 cm in diameter. Transbronchial biopsy was obtained from this site 40 d earlier; B: Day 90. Note the total resolution of the RLL nodule. RLL: Right lower lobe.

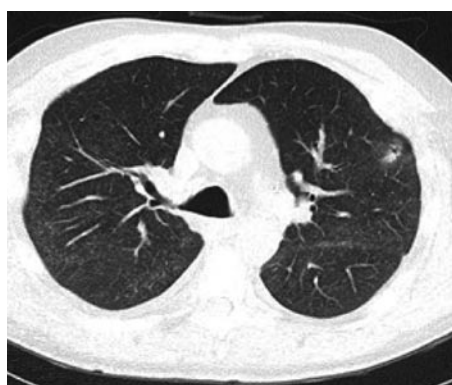


Figure 3 Computed tomography of chest revealing a cavitating lung nodule involving lingual. A transbronchial biopsy was obtained from the site 21 d earlier.

detected in this group of patients.

DISCUSSION

Part of the success of lung transplant is attributed to the flexible bronchoscopy. Most patients either undergo surveillance or require a clinical bronchoscopy with TBBx to rule out rejection, infection or malignancy. Even though there is no proven benefit of surveillance bronchoscopy over clinically indicated procedures, the former has been accepted as a common practice for

early detection of subclinical rejection^[1-3].

It is a conservative estimate that over 200000 pulmonary nodules will be detected in year 2014 in the United States, outside the lung cancer screening program^[4].

PPNs are a common radiographic finding, and are still considered a clinical dilemma. The PPN among LTR is of added significance as it involves differential diagnosis such as PTLN (39%), Invasive Pulmonary Aspergillosis (IAP) (37%) and other opportunistic infections^[5-8].

Our study revealed that LTRs are also at risk of developing PPN nodule following a TBBx. This finding is rarely reported in the literature^[9-11].

This finding is unique to the transplant population as it was not detected in our control group. These nodules can develop in 13% of the procedures performed on LTR. The location suggests that they developed directly as a result of the TBBx and are most likely due to a local hematoma and impaired pulmonary lymphatic drainage in the LTR^[12]. We speculate that size of the nodule may depend upon the number of samples obtained from a single location.

The nodules could be single, multiple, solid, round, oval solid or cavitating. They seem to be associated with neither infection nor rejection and not related to the type of transplantation. They could appear as early as 4 d after the FB and may take up to 86 d to resolve.

Given the fact that they resolve spontaneously, their diagnosis and management require only a good temporal relationship and a close follow-up.

As compared to the early 80s a larger number of lung transplants are being performed today including in patients with selected co-morbidities. Besides, today we rely on chest CT scans more than on plain chest X-rays. These may be the reasons behind the delayed recognition of these iatrogenic pulmonary nodules.

The weakness of our study is that we could recruit very few patients in our control group as rarely non-transplant recipients underwent radiographic studies following the bronchoscopy. We sincerely doubt that this would have affected our observations as TBBxs have been performed in non-transplant recipients for over 40 years and no PPN have been reported in this group.

All physicians involved in caring for LTRs should be cognizant of this newer iatrogenic etiology of a PPN. The awareness will avoid unnecessary, expensive work up in this unique group of patients.

COMMENTS

Background

Peripheral pulmonary nodule (PPN) is a common clinical challenge. This entity is even more challenging when detected in lung transplant recipients (LTR). Flexible bronchoscopy (FB) is routinely performed following lung transplantation. The authors incidentally noted development of new PPN in LTR following a FB with a transbronchial biopsy (TBBx). This finding has a potential to initiate unnecessary diagnostic work-up. Purpose of the study was to evaluate frequency and the temporal relationship between the nodule and the TBBx among the LTR, with an intention to avoid unwarranted testing.

Research frontiers

Lung nodules are commonly found in LTR. Previous reports have focused on infection, malignancy and rejection as potential causes. The study revealed that LTRs are also at risk of developing PPN nodule following a TBBx. The authors aim to raise the awareness of such nodules with a goal to avoid unwarranted testing.

Innovations and breakthroughs

In this study, the occurrence of PPN following TBBx in LTR was 13% compared to 0% in non LTR. The focus of our study, in comparison to others, was to investigate these temporary nodules (size, time of appearance and disappearance, shape and consistency).

Applications

All physicians involved in caring for LTRs should be cognizant of this newer iatrogenic etiology of a PPN. The awareness will avoid unnecessary, expensive work up in this unique group of patients.

Terminology

FB with TBBx: Flexible bronchoscopy with the application of transbronchial biopsy, is a commonly used method for routine surveillance as well as clinically indicated procedures in LTR.

Peer-review

This is a well organized manuscript. The authors incidentally noted development of new PPN in LTR following a FB with a TBBx. This finding has a potential to initiate unnecessary diagnostic work-up.

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Retrospective Study

Incidence and risk factors for early renal dysfunction after liver transplantation

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Abstract

AIM: To determine renal dysfunction post liver transplantation, its incidence and risk factors in patients from a Belgian University Hospital.

METHODS: Orthotopic liver transplantations performed from January 2006 until September 2012 were retrospectively reviewed ($n = 187$). Patients with no renal replacement therapy (RRT) before transplantation were classified into four groups according to their highest creatinine plasma level during the first postoperative week. The first group had a peak creatinine level below 12 mg/L, the second group between 12 and 20 mg/L, the third group between 20 and 35 mg/L, and the fourth above 35 mg/L. In addition, patients who needed RRT during the first week after transplantation were also classified into the fourth group. Perioperative parameters were recorded as risk factors, namely age, sex, body

mass index (BMI), length of preoperative hospital stay, prior bacterial infection within one month, preoperative ascites, preoperative treatment with β -blocker, angiotensin-converting enzyme inhibitor or non steroidal anti-inflammatory drugs, preoperative creatinine and bilirubin levels, donor status (cardiac death or brain death), postoperative lactate level, need for intraoperative vasopressive drugs, surgical revision, mechanical ventilation for more than 24 h, postoperative bilirubin and transaminase peak levels, postoperative hemoglobin level, amount of perioperative blood transfusions and type of immunosuppression. Univariate and multivariate analysis were performed using logistic ordinal regression method. Post hoc analysis of the hemostatic agent used was also done.

RESULTS: There were 78 patients in group 1 (41.7%), 46 in group 2 (24.6%), 38 in group 3 (20.3%) and 25 in group 4 (13.4%). Twenty patients required RRT: 13 (7%) during the first week after transplantation. Using univariate analysis, the severity of renal dysfunction was correlated with presence of ascites and prior bacterial infection, preoperative bilirubin, urea and creatinine level, need for surgical revision, use of vasopressor, postoperative mechanical ventilation, postoperative bilirubin and urea, aspartate aminotransferase (ASAT), and hemoglobin levels and the need for transfusion. The multivariate analysis showed that BMI (OR = 1.1, $P = 0.004$), preoperative creatinine level (OR = 11.1, $P < 0.0001$), use of vasopressor (OR = 3.31, $P = 0.0002$), maximal postoperative bilirubin level (OR = 1.44, $P = 0.044$) and minimal postoperative hemoglobin level (OR = 0.059, $P = 0.0005$) were independent predictors of early post-liver transplantation renal dysfunction. Neither donor status nor ASAT levels had significant impact on early postoperative renal dysfunction in multivariate analysis. Absence of renal dysfunction (group 1) was also predicted by the intraoperative hemostatic agent used, independently of the extent of bleeding and of the preoperative creatinine level.

CONCLUSION: More than half of receivers experienced some degree of early renal dysfunction after liver transplantation. Main predictors were preoperative renal dysfunction, postoperative anemia and vasopressor requirement.

Key words: Liver transplantation; Acute kidney injury incidence; Perioperative complications; Acute kidney injury risk factors; Creatinine/blood; Severity renal failure

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Core tip: One hundred and eighty-seven liver transplantations performed between 2006 and 2012 were retrospectively analyzed. Patients were classified into four groups according to their highest creatinine plasma level during the first postoperative week relying on sequential organ failure assessment renal classification.

Perioperative parameters were recorded as risk factors. Univariate and multivariate analysis were performed. Fifty-eight percent of recipients experienced some degree of early postoperative renal dysfunction. The multivariate analysis showed that body mass index, preoperative creatinine level, use of vasopressor, hemostatic drug, postoperative bilirubin peak level and postoperative hemoglobin minimum level but not the donor status (cardiac dead or brain dead donor) were independent predictors of post-transplantation early renal dysfunction.

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INTRODUCTION

Renal failure is one of the main complications after orthotopic liver transplantation (OLT), with severe impact on early and long-term outcomes^[1]. Renal function could even predict patients' survival before and after liver transplantation^[2,3]. The prevalence of acute kidney injury (AKI) after OLT varies from 12% to 70% according to AKI definition^[4-7]. Its pathogenesis is multifactorial and includes functional pre-renal hyperazotemia and acute tubular necrosis or apoptosis^[4,8]. Highlighting AKI risk factors associated with OLT may help to reduce the prevalence of early renal dysfunction (and improve global outcome) *via* the development of therapeutic strategies aiming at reducing these risks.

Extensive research has suggested that many preoperative factors may favour the occurrence of AKI after OLT such as preoperative kidney dysfunction and hepatorenal syndrome (HRS), pre-OLT low serum albumin level, hypovolemia, ascites, concomitant chronic diseases leading to kidney injury (diabetes mellitus, hypertension), hepatitis C (which is associated with multiple glomerular diseases including membranous glomerulonephritis, mixed essential cryoglobulinemia and membranoproliferative glomerulonephritis^[9,10]), Child-Pugh score and Meld score^[10-14], all with conflicting evidence. During surgery, kidneys have to deal with further insults such as hypovolemia, inferior vena cava clamping and its associated increased pressure at the kidney level, hemorrhage and anemia, hemodynamic instability, blood transfusion, extended surgical procedure and some particular surgical techniques^[9,15,16].

Moreover, it is reported that renal function relies on the liver graft quality. Renal prognosis is deemed to be worse with organs issued from cardiac death donors^[17].

Postoperative additional factors such as radiological contrast media, sepsis and immunosuppressive drugs

(calcineurin inhibitors) promote renal failure^[9,18].

The primary goal of our single center retrospective study was to estimate the incidence and severity of early postoperative renal dysfunction in OLT recipients and to highlight the perioperative AKI risk factors and their significance. The role of donation after circulatory death (DCD) was particularly looked into.

MATERIALS AND METHODS

Data were collected from a consecutive series of patients who underwent OLT at the University Hospital of Liege (Belgium) from January 2006 until September 2012. This analysis was limited to this time frame to avoid selection bias due to new recommendations in the handling of transplanted patients. We analyzed OLT patients developing acute renal failure (ARF) in the early postoperative course up to and including postoperative day 7 (primary outcome).

Data collection was based on a prospective clinical research database taking into account hospitalization data (preoperative hospital stay and infection occurrence), baseline demographic characteristics [age, gender, body mass index (BMI) and co-morbid conditions], preoperative clinical and biological data (urea, creatinine and bilirubin levels), perioperative septic status, ascites, previous treatment with β -adrenoreceptor blockers, angiotensin-converting enzyme inhibitors (ACEI) and non-steroidal anti-inflammatory drugs (NSAIDs). We did not exclude patients with HRS pre-OLT from the study but we excluded patients who required preoperative renal replacement therapy (RRT).

A single surgical team, all members of which were specifically trained in OLT, performed all procedures. Intraoperative collected variables included liver graft source (cardiac dead or brain dead donor), need for surgical revision, need for transfusion [type of blood product administered: Red cells concentrate (RCC), fresh frozen plasma (FFP) or platelet] and need for vasoactive drugs. Furthermore, we secondarily analysed the impact of the hemostatic agent used (aprotinin until October 2007, tranexamic acid later on - the only significant modification to protocol during the study period).

Post operative data during the first week were collected: Need for transfusion (amount and type of blood product on days 0, 1 and 7), postoperative day 1 lactate peak level, minimum hemoglobin level, need for vasopressors, time to extubation, bilirubin peak level, aminotransferases peak levels, urea and creatinine peak levels, need for postoperative RRT and immunosuppressive drugs (tacrolimus, cyclosporine A or other immunosuppressive drug). The local triple immunosuppressive regimen consisted of a calcineurin inhibitor (cyclosporine or tacrolimus), an antiproliferative drug and a corticosteroid. Whole

blood levels of calcineurin inhibitor were measured by chemiluminescence microparticle immunoassay (Architect[®] from Abbott).

We separated patients into four groups according to their renal function (relying on sequential organ failure assessment score stratification), based on the highest creatinine plasma level during the first postoperative week. The first group had a creatinine level below 12 mg/L, the second group between 12 and 20 mg/L, the third group between 20 and 35 mg/L, and the fourth above 35 mg/L. Patients who needed RRT during the first week after transplantation were also classified in the fourth group.

Statistical analysis

Statistical analysis was performed by the University's biomedical statisticians.

Univariate analysis was performed to identify variables associated with primary outcome as potential confounders. The results are presented as mean and standard deviation for normally distributed variables or median (interquartile range) for non-normally distributed variables. Several variables underwent a logarithmic transformation in order to standardize their distributions. Normality was checked by Shapiro-Wilk's test.

RRT: Comparisons between RRT and categorized variables were made by a χ^2 test whereas comparisons with continuous variables were made using logistic regression.

Comparisons between the 4 groups of renal dysfunction with categorized variables were made by a χ^2 test whereas comparisons with continuous variables were made using ANOVA or the Kruskal-Wallis' non-parametric test according to normality of variables. Ordinal logistic regression was performed in order to take the groups' order into account and hence renal dysfunction severity (group 4 "more severe" than group 3 "more severe" than group 2 "more severe" than group 1).

The results are considered as significant with an uncertainty level of 5% ($P < 0.05$). Statistical analyses were carried out using software SAS version 9.3.

Multivariate model

Variables included in the model are variables with a P -value lower than 0.10 in univariate analysis.

RESULTS

There were 78 patients in group 1 (41.7%), 46 in group 2 (24.6%), 38 in group 3 (20.3%) and 25 in group 4 (13.4%). Twenty patients required RRT: 13 (7%) during the first week after transplantation (group 4). There were 7 (3.7%) early deaths within 28 d after transplantation (Table 1).

Considering the 4 aforementioned groups, severity of renal dysfunction was correlated in univariate analysis

Table 1 Univariate analysis for severity of post orthotopic liver transplantation acute kidney injury

Variable	Whole group (<i>n</i> = 187)	Group 1 (<i>n</i> = 78)	Group 2 (<i>n</i> = 76)	Group 3 (<i>n</i> = 38)	Group 4 (<i>n</i> = 25)	<i>P</i> value between groups
Preoperative						
Age (yr)	56 ± 10	54 ± 10	56 ± 10	58 ± 9	57 ± 12	0.055
Sex (male)	147 (79)	61 (78)	32 (70)	33 (87)	21 (84)	0.410
BMI (kg/m ²)	26 ± 4.5	25 ± 4	26 ± 5	26 ± 5	26 ± 5.0	0.055
Hospital stay (d)	3 ± 8	2.2 ± 4.8	4.2 ± 12.9	2.7 ± 7.9	6.4 ± 9.6	0.150
Bilirubin (mg/L)	25 (12-66)	17.4 (8.7-44.8)	23.2 (13.1-60.6)	32.3 (15.8-64.9)	56.3 (23.1-115.0)	< 0.0001
Creatinine (mg/L)	9.5 (7.4-12.3)	7 (6.6-9.3)	10.4 (8.0-12.7)	11.5 (9.3-14.5)	13.4 (6.6-16.0)	< 0.0001
Urea (g/L)	0.47 ± 0.35	0.34 (0.20-0.42)	0.40 (0.30-0.59)	0.42 (0.33-0.68)	0.64 (0.38-0.92)	< 0.0001
Ascites	138 (73)	50 (64)	37 (80)	30 (79)	21 (84)	0.015
β blockers	67 (37)	24 (31)	18 (39)	17 (46)	8 (33)	0.400
ACEI	18 (10)	8 (11)	4 (9)	4 (11)	2 (8)	0.770
NSAIDs	5 (3)	1 (1)	2 (4)	1 (3)	1 (4)	0.480
Prior bacterial infection	62 (33)	16 (20)	18 (39.1)	13 (34.2)	15 (60)	0.0007
Intraoperative						
DCD	63 (34)	25 (32)	17 (37)	12 (32)	9 (36)	0.790
Infection	50 (27)	17 (22)	12 (26)	13 (34)	8 (32)	0.140
Vasopressors	86 (46)	18 (23)	25 (54)	25 (66)	18 (72)	< 0.0001
Surgical revision	45 (24)	12 (15)	12 (26)	11 (29)	10 (40)	0.0087
Transfusion	169 (90)	66 (85)	44 (96)	37 (97)	22 (88)	0.060
Postoperative						
Lactates D1 (mg/L)	434 ± 230	394 (270-509)	375 (279-484)	428 (283-527)	435 (334-711)	0.200
Minimum hemoglobin (g/dL)	8.0 (7.0-9.2)	8.9 (7.8-10.3)	7.7 (6.7-8.5)	7.55 (6.8-8.5)	6.7 (6.5-8.0)	< 0.0001
Bilirubin peak (mg/L)	40 (23-77.6)	37 (18-77)	32 (24-82)	51 (37-73)	60 (33-128)	0.006
ASAT (UI/L)	733 (372-1248)	554 (333-966)	804 (472-1988)	875 (399-1300)	822 (505-2458)	0.001
ALAT (UI/L)	617 (380-1068)	569 (332-941)	698 (399-1085)	546 (397-1113)	695 (407-1133)	0.260
Urea (g/L)	0.88 (0.6-1.3)	0.57 (0.46-0.69)	0.97 (0.80-1.14)	1.38 (1.21-1.64)	1.87 (1.52-2.18)	< 0.0001
Mechanical ventilation > 24 h	56 (30)	18 (23)	9 (20)	16 (42)	13 (52)	0.0026
Mechanical ventilation days	1 (1-2)	1 (1-1)	1 (1-1)	1 (1-2)	2 (1-2)	0.0014
RRT	20 (11)	4 (5)	2 (4)	1 (3)	13 (52)	< 0.0001
ICU stay (d)	3 (2-5)	2 (2-4)	3 (2-4)	5 (4-7)	6 (4-13)	0.0046
Tacrolimus	177 (95)	77 (99)	43 (94)	35 (92)	22 (92)	0.089
Cyclosporin	21 (11)	5 (6)	7 (15)	6 (16)	3 (13)	0.170
Additional immunosuppressant	185 (98)	77 (99)	46 (100)	38 (100)	24 (96)	0.550
Transfusion RCC D0 (U)	1 (0-3)	0 (0-2)	2 (0-4)	2 (1-5)	2 (1-5)	0.0007
Transfusion RCC D1 (U)	0 (0-1)	0 (0-0)	0 (0-1.5)	0 (0-2)	0 (0-4)	< 0.0001
Transfusion RCC D7 (U)	2 (0-5)	0 (0-3)	3 (1-6)	4 (2-7)	4 (2-12)	< 0.0001
Transfusion FFP D0 (U)	4 (2-6)	3 (0-6)	4 (2-7)	6 (3-9)	6 (3-8)	0.0031
Transfusion FFP D1 (U)	0 (0-2)	0 (0-0)	0 (0-2)	2 (0-3)	2 (0-4)	< 0.0001
Transfusion FFP D7 (U)	6 (2-10)	4 (1-6)	6 (2.5-10)	8 (4-11)	8 (6-15)	< 0.0001
Transfusion platelets D0 (CUP)	1 (0-1)	0 (0-1)	1 (0-1)	1 (0-1)	1 (1-2)	0.0008
Transfusion platelets D1 (CUP)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0.0022
Transfusion platelets D7 (CUP)	1 (0-2)	0 (0-1)	1 (0-2.5)	1 (0-2)	2 (1-4)	< 0.0001

BMI: Body mass index; ACEI: Angiotensin-converting enzyme inhibitors; NSAIDs: Non-steroidal anti-inflammatory drugs; DCD: Donation after circulatory death; ASAT: Aspartate aminotransferase; ALAT: Alanine amino transferase; RRT: Renal replacement therapy; ICU: Intensive care unit.

with patient BMI, ascites, prior bacterial infection, preoperative bilirubin, urea and creatinine levels, surgical revision, intraoperative vasopressor requirement, postoperative mechanical ventilation, postoperative urea, bilirubin, aspartate amino transferase (ASAT) peak levels and minimum hemoglobin levels, intensive care unit (ICU) length of stay and transfusion of each type of products (RCC, FFP and platelet cups).

Results are presented as mean ± SD if normal distribution, median (P25-P75) if non normal continuous variable, *n* (%) if categorical variable.

Using multivariate analysis, the ordinal multiple logistic regression analysis identified 5 independent predictors of increased postoperative creatinine peak

level among our whole OLT population, namely BMI, preoperative creatinine level, use of vasopressor, postoperative bilirubin peak level and minimum postoperative hemoglobin level. It is to be noted that neither the donor status (cardiac death or brain death) nor transaminase levels were independent risk factor for AKI (Table 2).

Post hoc analysis of renal data into two chronological groups according to the hemostatic agent used showed that the occurrence of AKI (group 2, 3 and 4 together) was higher with tranexamic acid than with aprotinin, even when adjusting for preoperative creatinine (OR = 2.23, 95%CI: 1.13-4.41, *P* = 0.021) and regardless of the extent of bleeding (Table 3).

Table 2 Multivariate analysis for increased post orthotopic liver transplantation serum creatinine level

	OR	95%CI	P value
BMI (kg/m ²)	1.10	1.03-1.18	0.0044
Preoperative increased creatinine (ln - mg/L)	11.07	5.28-23.23	< 0.0001
Vasopressors use	3.31	1.75-6.29	0.0002
Postoperative minimum Hemoglobin (ln - g/dL)	0.06	0.01-0.29	0.0005
Postoperative bilirubin peak (ln - mg/L)	1.44	1.01-2.05	0.044

BMI: Body mass index; ln: Natural logarithm.

DISCUSSION

AKI remains a common disorder after OLT, despite advances in surgical technique, anesthesia, post-operative care and immunosuppressive therapy. We found 58% of OLT recipients to have some degree of renal dysfunction highlighted by an increase in serum creatinine level during the first postoperative week. The rate of AKI varies among studies. Cabezueto *et al*^[4] and Rymarz *et al*^[19] observed an AKI prevalence of around 30% over the first week after surgery, while Junge *et al*^[10] found only 12% patients developing AKI during the first week after OLT. The incidence of post-transplantation acute renal dysfunction is related to an increased mortality rate^[20,21].

RRT requirement

When focusing on AKI severity, RRT requirement concerned 20 on 187 of our patients (11%), 13 (7%) of them within the first postoperative week. Likewise, in Faenza's study^[22], 8% of OLT patients experienced ARF requiring RRT during the postoperative period. They found that ARF requiring RRT conferred an excessive risk of in-hospital mortality ($n = 8$, 40%). This increased risk cannot be explained solely by a more pronounced severity of illness and provides evidence that ARF is a specific, independent risk factor for a poor prognosis^[22]. According to the literature, 3% to 20% of RRT-naïve patients who undergo OLT ultimately require postoperative RRT^[23] with an associate increase in mortality rate^[13,24].

Our results identified five parameters independently associated with a postoperative increased serum creatinine level.

Preoperative renal impairment

Some degree of preoperative renal impairment was a main factor highlighted by our study, as shown by others^[4,10,12,19], especially since biological markers are delayed and reflect advanced renal damages^[25]. Intrinsic chronic kidney disease predisposes patients with end-stage liver failure to acute renal dysfunction^[26]. Furthermore, hemodynamic preoperative factors promote the risk of ARF in cirrhotic patients: Kidney

Table 3 Post hoc multivariate analysis highlighting the effect of anti-hemorrhagic treatment strategy on acute kidney injury occurrence

Risk not being into the 1 th group in multivariate analysis	OR	95%CI	P value
Antihemorrhagic treatment period	3.36	1.44-7.85	0.005
Preoperative increased creatinine (ln - mg/L)	1.36	1.20-1.54	< 0.0001
Bleeding (100 mL)	1.03	1.01-1.06	0.011

ln: Natural logarithm.

hypoperfusion is due to intravascular hypovolemia associated with parietal edema, hypoalbuminemia and hormone-induced vasodilatation of splanchnic circulation^[26,27]. Renin angiotensin aldosterone axis is also disturbed. Edema of renal parenchyma itself can also play a role in this phenomenon^[28].

A link between acute liver failure (ALF) and ARF is described in the literature. Seventy percent of patients with ALF developed AKI, and 30% received RRT. Patients with severe ARF had higher international normalized ratio values, more severe encephalopathy and shock than patients without renal dysfunction^[29].

Vasopressor requirements

Like other authors, we observed an adverse role of vasoconstrictor therapy during surgery^[13]. Nevertheless, maybe vasopressor requirement rather than vaso-pressor use is responsible for renal impairment. With cirrhosis, systemic arterial vasodilation is observed. Indeed, portal hypertension is associated with a release of vasodilatory substances (NO, prostacyclins). Moreover, vasodilation opens arteriovenous shunts. As a result, a hyperkinetic syndrome with an increase in the cardiac flow and a fall of the systemic blood pressure is observed in cirrhotic patients. During surgery, significant hemodynamic disturbances occur following liver mobilizations (dislocation), in addition to hepatomegaly in some cases, inducing a venous return decrease. Massive blood losses can occur especially in presence of adhesions. Inferior cava vein clamping reduces once more venous return (up to 60%) and decreases cardiac flow (about 40% to 60%). Clamp withdrawal increases transient severe hypotension.

A surgical revision is needed when significant bleeding persists after correction of biological coagulation parameters, leading to anemia, hypotension, tissue hypoperfusion and cellular oxygen deprivation. These situations are associated with greater hemodynamic instability leading to renal hypoperfusion.

Sepsis-associated vasodilation further increases these circulatory derangements. Sepsis-related AKI doesn't seem to be related to renal global hypoperfusion but rather to renal hyperemia with an intra-renal blood flow redistribution. The exact pathophysiology of sepsis-induced AKI is still not clear and seems multifaceted, with components of inflammatory injury,

ischemia-reperfusion (I-R) injury, endothelial cell dysfunction, coagulation disturbance and apoptosis^[30]. Moreover, recent findings suggest that pathophysiologic mechanisms of sepsis-induced AKI are different from non-septic AKI^[31].

It is reported that vasoplegia-induced hypotension is correlated with progressive AKI during severe sepsis, relying on the Finnaki study's results^[32]. On the other hand, generous fluid infusion and fluid overload in septic patients are also associated with progressive AKI^[33,34].

Anemia and transfusion requirements

We found a significant impact of both postoperative anemia and transfusions on the incidence of early AKI. ARF severity was correlated to all transfused blood products in univariate analysis.

Data issued from literature are somewhat inconsistent regarding the effect of anemia and transfusion on renal function.

Villanueva *et al.*^[35] did not find any significant repercussion on the occurrence of acute kidney injury of different transfusion strategies with hemoglobin thresholds of 7 g/dL and 9 g/dL in 921 patients with upper gastro intestinal bleeding.

On the other hand, AKI is thought to happen when a combination of insults inducing renal hypoxia, inflammation and oxidative stress occurs in vulnerable patients^[36,37]. Kidneys are known to be highly vulnerable to hypoxic injury in the setting of reduced oxygen delivery because of anemia^[38,39]. Decreased renal oxygen delivery is due to hypotension, hemodilution and impaired renal blood flow.

On one hand, several studies have highlighted the harmful effect of the need for transfusion on renal function of liver recipient patients^[11]. As a matter of fact, transfused erythrocytes may favour kidney injury because of the functional and structural alterations that they undergo during storage^[40]. These include depletion of adenosine triphosphate and 2,3-diphosphoglycerate, loss of ability to generate nitric oxide, increased adhesiveness to vascular endothelium, release of pro-coagulant phospholipids, accumulation of pro-inflammatory molecules as well as free hemoglobin and iron^[40,41]. Furthermore, erythrocytes undergo progressive structural changes during storage that lead a considerable proportion (up to 30%) of them to be rapidly removed from the circulation by macrophages^[42], which may then release some of scavenged hemoglobin-iron complexes into circulation^[43,44]. As a result, stored erythrocytes may, at least for a few hours after they are transfused, paradoxically weaken tissue oxygen delivery, enhance the inflammatory cascade, and worsen tissue oxidative stress^[39,40,45,46]. Furthermore, a significant need for intraoperative transfusion of all type of blood products in previously non anaemic patient can be a reflection of either a more severe preoperative liver dysfunction

with severe coagulation impairment, or a prolonged intervention with surgical difficulties and hemodynamic alterations. In contrast with what precedes, some authors even recommend an increased intraoperative vasopressor use aiming at reducing transfusion requirement. It is reported that norepinephrine can improve outcome and reduce mechanical ventilation duration without effect on renal function when comparing a restrictive fluid strategy and a liberal fluid strategy called placebo during OLT surgery^[47].

Obviously, a particular attention must be paid for hemostasis and coagulation optimization.

Finally, there is a theoretical anti ischemic preconditioning effect of aprotinin, selective cyclooxygenase-2 inhibitors and oral anti-diabetics (sulfonylurea, glitazones) which inhibit potassium channels^[48]. Aprotinin is not used anymore and has been replaced by tranexamic acid to limit blood losses. The unique major modification in intraoperative management of liver transplant recipients in our center is the discontinued use of aprotinin in October 2007 (it was pulled out from international market given the concern that aprotinin increased risk of complication and death in the intraoperative period). Paradoxically, when stratifying renal data in two groups according to the antihemorrhagic agent used, we observed that the occurrence of renal failure was higher with tranexamic acid than with aprotinin, even when adjusted for preoperative creatinine level. This effect was not in relation with an increased intraoperative bleeding.

Hyperbilirubinemia

Because of donors' paucity, sub optimal transplants coming from living donors, split or domino procedures and cardiac death donors often result in early hyperbilirubinemia, which is deemed to be due to suboptimal graft^[49]. Hyperbilirubinemia is due to miscellaneous etiologies such as small for size syndrome and aged living donor, acute cellular rejection, graft preservation injury, surgical complications, sepsis or drug toxicity^[50] with a higher prevalence in the context of living donors in the literature. Serum bilirubin level is a useful predictor of short-term (< 1 year) graft poor outcome^[51].

Early postoperative hyperbilirubinemia can be considered as a sign of liver impairment from different causes (*i.e.*, surgical complications, infection or acute graft rejection) but it may in itself also potentiate other insults such as kidney failure^[52]. When early hyperbilirubinemia is not an isolated phenomenon but presents with hepatocellular failure, *i.e.*, persistent coma, coagulopathy and elevated serum transaminase level, it is encompassed in the diagnosis of "primary non function" (or less severe early allograft dysfunction). In this particular situation, the patient also needs to be on prolonged mechanical ventilation and requires iterative transfusions. A rapid new liver transplantation is mandatory under these circumstances. Primary

non function is described as more frequent after “uncontrolled DCD donors” (*i.e.*, with a prolonged warm ischemia) and believed to be the consequence of severe I-R injury in relation with warm injury^[53]. Delayed bilirubin increasing is often due to biliary complications (bile leakage and bile duct stricture).

I-R

Besides aforementioned hemodynamic phenomena, liver I-R injury occurs after liver transplantation and circulatory shock, leading to significant morbidity and mortality. There is substantial evidence towards hepatic I-R injury resulting in an intense inflammatory response initiated by oxidative stress in the liver parenchyma during reperfusion. Hepatic I-R injury is associated with a systemic inflammatory response syndrome through a combination of immunologic, toxic and inflammatory factors (cytokines release), which can cause AKI through hemodynamic mechanisms and direct tubular cell death^[30,54-57].

Nevertheless, unlike previous studies^[17,58,59], we did not find any significant relationship between DCD and renal dysfunction. In 2012, Leithead *et al.*^[58] published the results of a single-center study conducted on 88 consecutive DCD liver transplant recipients. During the immediate postoperative period, DCD liver transplantation was associated with an increased incidence of AKI compared with donation after brain death (DBD). Interestingly, increased perioperative peak ASAT, a surrogate marker of hepatic ischemia reperfusion injury, was the only significant predictor of renal dysfunction after DCD transplantation. Organs recovered from a DCD have some degree of oxygen deprivation during the time after the heart stops beating, which is called warm ischemia. One of the explanations of the lower impact of DCD on renal function in our data, in comparison with Leithead's studies, may be related to the differences in the legislation between the two countries. In the United Kingdom, discontinuation of therapy for DCD is carried out in the ICU, in the same condition than withdrawal of active treatment for a patient who is not a potential donor, *e.g.*, in the presence of the family. Organ donation may not be possible if the dying process is prolonged and may result in an unacceptable warm ischemic time^[60]. Moreover, warm ischemia increases graft susceptibility to damages induced by cold injury.

The Belgian legislation authorizes treatment withdrawal (in the context of the DCD) within the operating theatre, which reduces considerably warm ischemia duration. Two minutes are awaited after circulatory arrest before establishing death followed by a 5-min “no touch” phase before skin incision^[61]. This enables the warm ischemia time to be as short as possible.

Another sensitive ethical issue in DCD concerns organ preservation measures to protect organ viability

until transplantation^[62]. A tool to reduce I-R impact lies in preconditioning operations. Preconditioning consists of an improvement of the tolerance to ischemia (for 1 to 2 h) by brief episodes of flow occlusion or pharmacological means^[63-65].

Preconditioning by halogenated anesthetics is related to several cellular mechanisms partially elucidated, such as the ATP dependant potassium channel opening (preserving mitochondrial function) and mitochondrial permeability transition pore closure [reducing the amount of radical oxygen species (ROS)]^[66-69]. These phenomena correspond to the early phase of the cellular protection; its duration is limited to 1-2 h. Preconditioning technique is possible only for a surgery where ischemia is programmed. Sevoflurane has also a protective effect on renal function (cystatine C) after coronary bypass surgery according to a double blinded multicenter study^[67]. Pharmacological preconditioning by volatile anesthetics may protect non-cardiac organs against I-R^[68,69].

Leithead *et al.*^[17] also showed an association between cold ischemic time (CIT) and perioperative AKI.

These findings strongly suggest that a sustained CIT is a causative factor for poor outcome (of the transplanted organ but also global) after DCD liver transplantation^[70]. Cold ischemia duration corresponds to the time elapsed between infusion of preservation fluid and the moment when the graft is perfused in the receiver. Shorter the time, better the results of transplantation. Beyond 13 h of cold ischemia on a whole liver, the risk of primary non-function becomes important. In addition to its non-specific effects, cold ischemia enhances graft immunogenicity and host allo-responsiveness. The ischemic injury, a localized process of cellular metabolic disturbances, results from glycogen consumption, lack of oxygen supply and adenosine triphosphate (ATP) depletion^[71]. Reperfusion abruptly reintroduces large amount of oxygen in the previously deprived cells. The mitochondrial respiratory chain, functionally damaged by ischemia, cannot accurately use this excess of oxygen. The reactivation of the ionic pumps rapidly corrects the acidosis, but at the cost of a sodium and calcium overload, potentially very harmful for the cell. Instead of synthesizing ATP, mitochondrion produces free ROS. It leads, by lipidic peroxidation, to cellular membranes damages (including mitochondrial membrane), but also to an indirect inflammation activation by leucocytes recruitment and by stimulating cytokines production, especially tumor necrosis factor- α (TNF- α) and interleukin-1 beta^[72-74]. Cytokines are mainly produced in the liver by the Kupffer cells^[75] but also by the extra-hepatic macrophages^[76]. TNF- α propagates the inflammatory response^[77]. Cytokines induce a local and general inflammatory syndrome followed by tissue edema. At reperfusion, body is flooded by degradation substances, such as lytic enzymes (ASAT, lactate

dehydrogenase), lactates, potassium, H⁺ ions... which can induce severe metabolic acidosis, renal failure, ARDS, heart failure or even multiple organ failure^[78]. A similar situation is observed with the harmful remote effects of mesenteric I-R, where released mediators are involved in multi organ failure occurrence^[79]. I-R phenomenon may clarify the stronger association we found between ASAT and AKI than between alanine amino transferase (ALAT) and AKI, even if ALAT is more liver specific than ASAT.

Eurotransplant is responsible for allocation of donor organs in Belgium. A match list is generated by a computer algorithm that takes into account all medical and ethical criteria. Another potential explanation of the difference between Leithead's report and our data perhaps relies on the policy of preferential allocation by Eurotransplant of an organ coming from a DCD to the donor's transplantation center (to reduce cold ischemic duration in those organs which have already experienced warm ischemia).

The recipient selection is also important since organs coming from a DCD are selectively reserved to uncomplicated cases to ensure short cold ischemic time (by avoiding cases with extensive history of abdominal surgery or portal-vein thrombosis)^[53].

Likewise in our study, a recent meta-analysis focusing on post OLT complications also failed to detect a significant difference in complication rates (including renal failure) in the subgroup of cardiac death donors^[80].

Immunosuppressive drugs

Unexpectedly, we did not found any significant impact of immunosuppressive drugs on early AKI. Nevertheless, nephrotoxicity associated with calcineurin inhibitors (CNI), *e.g.*, cyclosporine and tacrolimus, is common and occurs either acutely or after chronic use. Acute injury is believed to be dose and concentration-dependent. However, it may be observed in patients with therapeutic blood concentrations. CNI-induced AKI is believed mainly to come from afferent glomerular arteriolar vasoconstriction, reduced renal blood flow and ultrafiltration coefficient and, as a result, decreased glomerular filtration rate. This may be attributable to an increased production of vasoconstrictive factors (such as thromboxane A₂ and endothelin) together with a decrease in renal vasodilatory prostaglandins and inhibition of nitric oxide^[9,18,81-84]. CNI-associated AKI may develop early in therapy. It can occur within a few days after the initiation of either cyclosporine or tacrolimus. Early CNI-induced AKI generally improves once the cyclosporine or tacrolimus dose is reduced or discontinued. In contrast, late CNI-induced chronic renal failure is associated with interstitial nephritis and is usually irreversible^[18-82].

In our institution, usual immunosuppressive therapy is based on low dose tacrolimus (serum target of 5-8 ng/mL), mycophenolic acid and steroids. It

corresponds to the renal sparing immunosuppression regime in other studies^[17,58,59,85], where renal sparing immunosuppression could significantly reduce early kidney dysfunction in comparison with their standard immunosuppressant treatment with CNI (serum tacrolimus target of 8-10 ng/mL), azathioprine and decreasing dose of steroids.

Limitations

Serum creatinine is the most established, simple, and inexpensive estimation of renal function. It is the primary method of detection of all forms of renal failure. Usually, monitoring renal function mostly relies on the results of the serum creatinine level and the estimated glomerular filtration rate calculated with the use of Levey's modification of diet in renal disease and Cockcroft-Gault formulas with an additional monitoring of diuresis. Relying on the risk injury failure loss and end-stage renal disease (RIFLE) classification introduced in 2002, modified as AKI network (AKIN) classification since 2005, the AKI term currently integrates a wide range of renal dysfunctions, starting with a very early and slight renal dysfunction with minimal changes in the serum creatinine level (stage 1, risk), through moderate changes (stage 2, injury), to an advanced renal failure (stage 3, failure).

One limitation of the study is the lack of use of the RIFLE, AKIN or more recent Kidney Disease Improving Global Outcomes criteria to define the degree of acute kidney injury. Moreover, as well in our study than in all the AKI definitions mentioned above, the use of serum creatinine (sCr) as renal dysfunction marker is also questionable in the context of liver failure.

Even if sCr remains the most practical biomarker and the most commonly used for renal function evaluation, it presents many weaknesses in clinical practice since it is influenced by body weight, muscle mass, race, age, gender, protein intake and muscle metabolism. Body weight and muscle mass probably explain why BMI is an independent significant factor of postoperative increased creatinine level. In the particular case of a cirrhotic patient, it is also affected by a decreased formation of creatinine from muscles (due to muscle waste)^[86], a decreased hepatic capacity to produce creatinine, an increased renal tubular secretion of creatinine^[87], a low dietary protein intake to avoid hyperammonemia^[7], an impairment of creatinine dosage with bilirubin high level^[88] and an increased volume of distribution responsible for dilution of sCr. As a consequence, measurements of sCr in patients with cirrhosis overestimate glomerular filtration rate (GFR) or kidney function. Even more, creatinine is not an early reflection of GFR variations (substantial rises in serum creatinine are often not witnessed until 48-72 h after the initial kidney insult^[89,90]) and rapid deterioration of renal function can be underestimated in the first days. In addition, significant renal disease can exist with minimal or

no change in creatinine because of renal reserve or enhanced tubular secretion of creatinine^[91,92]. On the other hand, slight modifications of serum creatinine level can be due to variation of body water content, corresponding to a false positive elevation. Although a decreased urinary output is the second criteria used in all those scores, it is admitted that use of urinary output in patients with cirrhosis and ascites is inadequate since these patients suffer from sodium retention and often present oliguria, even if they have a relatively preserved GFR^[93]. Moreover urinary output is frequently artificially enhanced by use of diuretics.

A “troponin-like” biomarker of AKI that is easily measured, unchanged by other biological variables, and capable of both early detection and risk stratification would considerably help for the diagnosis of AKI. It has been proposed that new biomarkers of renal function may be added to the diagnosis of AKI^[94]. Nevertheless, recent studies focusing on critical patients have shown disappointing conclusions regarding the impact of routine use of neutrophil gelatinase-associated lipocalin (NGAL) analysis^[95-97].

Anyway, by using serum creatinine evolution for 7 d after transplantation, we estimated that a perioperative event would be emphasized by an increase in creatinine level, even with a 48 h delay in comparison with other biomarkers such as NGAL^[98]. The aim of this study was not here to detect a renal damage as quickly as possible but to highlight all the perioperative factors which may affect kidney function.

On the other hand, we only excluded from our analyses patients with previous renal failure requiring RRT (but not patients with moderate renal dysfunction). Even if it is easily conceivable that a kidney with less reserve will be more prone to functional deterioration compared to a healthy kidney, our study design reflects more real life situation in ICU management of AKI post OLT, taking into account that patients without previous oliguria or elevated serum creatinine could indeed have lost a substantial number of nephrons.

In conclusion, our study demonstrated that AKI after liver transplantation is a common complication since more than half of liver transplanted patients experienced some degree of early renal dysfunction after transplantation. BMI, hyperbilirubinemia, preoperative renal dysfunction, perioperative circulatory instability requiring the use of vasopressor and postoperative anemia are independent predictors of AKI occurrence.

Despite the reputable poor quality of the graft in DCD, neither comparison between DCD and DBD, nor ASAT level were associated with post-OLT AKI by multivariate analysis.

Besides targeting improvement of graft quality, a particular attention must be paid to avoid preoperative additive kidney damages, to optimize intraoperative hemodynamics and to consider treatment in order to reduce transfusion requirements.

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COMMENTS

Background

Acute renal dysfunction is a frequent complication in the perioperative period of liver transplantation, with an impact on renal and vital outcomes in some cases. Moreover, acute renal failure has multifactorial etiologies with possible complex interactions.

Research frontiers

Since acute renal failure is frequent and may result from multiple etiologies with additional extra renal confounding factors and, moreover, is delayed from its cause, there is no unique treatment to prevent or resolve renal dysfunction. Highlighting significant risk factors of renal dysfunction should allow focusing on these parameters and reducing their impact in the future.

Innovations and breakthroughs

The authors found a high prevalence of perioperative renal dysfunction after liver transplantation. Previous studies evaluated the late renal impact after liver transplantation and prolonged immunosuppressive treatment, but few of them focused on the perioperative period to highlight renal repercussions at that time-limited but crucial period. Among studies focusing on renal function during early postoperative period, organs from donation after cardiac death (DCD) seemed to be associated with more renal dysfunction than with liver from brain dead donors. The authors did not find the same association. It seems extremely important since donor shortage will lead to an increasing proportion of transplantations from DCD rather than from donation after brain death.

Applications

The authors observed that preoperative renal dysfunction, body mass index, vasopressor, postoperative low hemoglobin and high bilirubin levels were independent risk factors for developing renal dysfunction. While it seems difficult to act on BMI or on previous renal function, optimizing hemodynamics and coagulopathy management appears useful.

Terminology

Acute renal dysfunction is defined as a sudden reduction in renal filtration ability, induced by one or more harmful phenomena. It leads to serum ions imbalance, blood accumulation of waste substances, fluid retention and metabolic acidosis. Acute renal failure can be fatal and requires intensive treatment. Nevertheless, it may be a reversible condition. Early postoperative period is defined in this study as the first week following liver transplantation. When focusing on renal function, since usual (bio)markers of renal failure are delayed, this period reflects hemodynamic and metabolic changes encountered just before, during and immediately after surgical intervention (early surgical complications). Donation after cardiac/circulatory death and donation after brain death: Donation after cardiac/circulatory death is a donor in refractory cardiac arrest or suffering from devastating and irreversible organ injury (usually brain injury) and awaiting cardiac arrest, but who does not meet formal brain death criteria. In these later cases, it is decided to withdraw care. When the

patient's heart stops beating, the organs are harvested in the operating room. Organs from a cardiac dead donor have some degree of oxygen deprivation during warm ischemia, *i.e.*, the time after the heart stops beating. Donation after brain death occurs when a person has a disastrous and irreversible brain injury, which causes total cessation of all brain function (including upper brain structure and brain stem). Brain death is not a coma nor a vegetative state but a real dead condition where cardio respiratory function is sustained by artificial devices (*e.g.*, mechanical ventilation).

Peer-review

The manuscript is a single center retrospective study that aims at estimating the incidence and severity of early postoperative renal dysfunction in orthotopic liver transplantation recipients and at highlighting the perioperative acute kidney injury risk factors and their significance, with particular attention to the role of DCD. The manuscript is well-written and deserves publication, as it carries a useful message to the clinicians involved in transplantation.

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Retrospective Cohort Study

Total pancreatectomy and islet autotransplantation: A decade nationwide analysis

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Abstract

AIM: To investigate outcomes and predictors of in-hospital morbidity and mortality after total pancreatectomy (TP) and islet autotransplantation.

METHODS: The nationwide inpatient sample (NIS) database was used to identify patients who underwent TP and islet autotransplantation (IAT) between 2002-2012 in the United States. Variables of interest were inherent variables of NIS database which included demographic data (age, sex, and race), comorbidities (such as diabetes mellitus, hypertension, and deficiency anemia), and admission type (elective *vs* non-elective). The primary endpoints were mortality and postoperative complications according to the ICD-9 diagnosis codes which were reported as the second to 25th diagnosis of patients in the database. Risk adjusted analysis was performed to investigate morbidity predictors. Multivariate regression analysis was used to identify predictors of in-hospital morbidity.

RESULTS: We evaluated a total of 923 patients who underwent IAT after pancreatectomy during 2002-2012. Among them, there were 754 patients who had TP + IAT. The most common indication of

surgery was chronic pancreatitis (86%) followed by acute pancreatitis (12%). The number of patients undergoing TP + IAT annually significantly increased during the 11 years of study from 53 cases in 2002 to 155 cases in 2012. Overall mortality and morbidity of patients were 0% and 57.8 %, respectively. Post-surgical hypoinsulinemia was reported in 42.3% of patients, indicating that 57.7% of patients were insulin independent during hospitalization. Predictors of in-hospital morbidity were obesity [adjusted odds ratio (AOR): 3.02, $P = 0.01$], fluid and electrolyte disorders (AOR: 2.71, $P < 0.01$), alcohol abuse (AOR: 2.63, $P < 0.01$), and weight loss (AOR: 2.43, $P < 0.01$).

CONCLUSION: TP + IAT is a safe procedure with no mortality, acceptable morbidity, and achieved high rate of early insulin independence. Obesity is the most significant predictor of in-hospital morbidity.

Key words: Total pancreatectomy; Pancreatectomy; Islet auto transplantation; Chronic pancreatitis; Insulin independency

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Core tip: Total pancreatectomy (TP) is the last resort to control the severe pain in patients with chronic pancreatitis due to the morbidity of the operation and the frequent severe resultant diabetes. Islet autotransplantation (IAT) following TP is reported, by well experienced groups, to be an effective therapy to prevent post-surgical diabetes. However, there is limited nationwide data analysis during the last few decades. The objective of this study was to investigate outcomes and predictors of in-hospital morbidity and mortality after TP + IAT.

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INTRODUCTION

Chronic pancreatitis (CP) is a progressive inflammation of the pancreas resulting in irreversible damage of the pancreas structure and function. CP has a broad spectrum of symptoms ranging from steatorrhea and malabsorption to diabetes and abdominal pain^[1]. Managing the symptoms is critical in order to provide optimum treatment. Any uncontrolled symptoms may hinder the treatment approach, affecting a patient's quality of life and activity. Diabetes and malabsorption can be managed by insulin and oral pancreatic enzyme

supplements respectively; however, the primary challenge is pain management^[2]. Although multiple factors and mechanisms have been hypothesized and investigated, the pathogenesis of the pain in CP remains unknown^[3]. Therapeutic options for the pain are limited but include extensive surgery, less invasive endoscopic procedures, and medical management. Although an aggressive approach, partial or total pancreatectomy (TP) is on occasion, the only therapeutic option that can provide complete relief in patients with severe pain that could not be alleviated by other treatments^[4-7].

Although the utilization of pancreatectomy in patients with CP show positive results in managing pain, there are various unsolicited complications associated with the procedure. Exocrine insufficiency and surgical diabetes have been identified as the most significant complications. Islet autotransplantation (IAT) combined with total or partial pancreatectomy can be effective in preventing or minimizing surgical diabetes^[8-11]. The surgical technique includes TP and pylorus- and distal-sparing duodenectomy with orthotopic reconstruction by means of duodenostomy and choledochoduodenostomy. During TP, the blood supply to the pancreas should be preserved as long as possible to lessen the effects of warm ischemia on the islets. To do so, never separate the distal pancreas from the splenic vessels. If the splenic vessels are ligated in the hilum, the spleen may survive on its collateral vessels, but usually it has to be taken^[9].

The utilization of IAT following the surgical procedure of TP was introduced by Sutherland *et al.*^[12] in 1977. Since then, several centers have followed this dual procedure in patients with CP^[13-17]. After pancreas excision, the duodenum and spleen (if attached) were removed on the back table. Purified enzyme blend (collagenase) was injected to the pancreatic main duct to separate islet from pancreatic tissue using modified Ricordi method. Then, digested pancreatic tissue with islets were infused into liver through the portal vein^[10]. Because this dual procedure is surgically quite different from simple TP, the morbidity rate and related risks differ. Therefore, the morbidity rate for this procedure will be higher than simple TP procedure^[7,18,19].

Despite the higher morbidity rate, several studies have reported that TP + IAT procedure produces significant pain relief, reduced narcotic dependency, and decreased life-threatening hypoglycemic episodes. These benefits support the primary goal of utilizing this treatment^[7,20-22].

In the last few decades, no nationwide retrospective analysis of the trends and short term outcomes of TP + IAP have been reported. To our knowledge, this is the first research study to use nationwide inpatient sample (NIS) database to report the most common indications, short term outcomes, and predictors of in-hospital morbidities of patients who underwent combined TP and IAT in the United States.

Table 1 Demographics and clinical characteristics of patients who underwent total pancreatectomy and islet autotransplantation

Variables		TP and islet auto-transplantation (sample size = 754)
Age	Mean \pm SD (yr)	39 \pm 13
	Median (yr)	41
Sex	Female	513 (68%)
Race	White	260/295 (88%) ¹
	Black or African American	20/295 (6.7%) ¹
	Hispanic	5/295 (1.6%) ¹
	Asian	5/295 (1.6%) ¹
	Other	5/295 (1.6%) ¹
Comorbidity	Diabetes mellitus	202 (26.8%)
	Hypertension	188 (25%)
	Deficiency anemia	153 (20.4%)
	Chronic pulmonary disease	98 (13.1%)
	Drug abuse	88 (11.7%)
	Coagulopathy	63 (8.3%)
	Alcohol abuse	44 (5.9%)
	Obesity	25 (3.3%)
	Weight loss	22 (29.13%)
	Elective	660 (87.7%)
Admission type	Non-elective	93 (12.3%)
	Chronic pancreatitis	648 (86%)
Patient diagnosis/indication of surgery	Acute pancreatitis	90 (12%)
	Other diagnosis	15 (2.1%)
Other factors	Preoperative fluid and electrolyte disorders	216 (28.7%)

¹Race data are available only for 295 patients from nationwide inpatient sample database.

MATERIALS AND METHODS

Patients and database

A retrospective analysis of the NIS database from 2002 through 2012 was performed for this study. NIS is the largest inpatient care database in the United States maintained by the Agency for Healthcare Research. It is an annually compiled database containing information on more than 8 million hospital admissions each year, which represents 20% of all United States hospital discharges to calculate population estimates^[23]. The informed consent was obtained from individual patients within the individual hospital's patient consent forms by NIS. This study evaluated patients who underwent IAT and TP according to the International Classification of Diseases, 9th Revision, clinical modifications (ICD-9-CM) procedure codes of 52.84 and 52.6 during 2002-2012. We extracted all the patients who had undergone IAT from database, then we selected patients who also had TP. Patients' diagnoses of surgery were extracted using ICD-9-CM diagnosis codes of 577.1 for CP and 577.0 for acute pancreatitis (AP).

Variables of interest were inherent variables of NIS database which included demographic data (age, sex, and race), comorbidities (such as diabetes mellitus, hypertension, and deficiency anemia), and admission

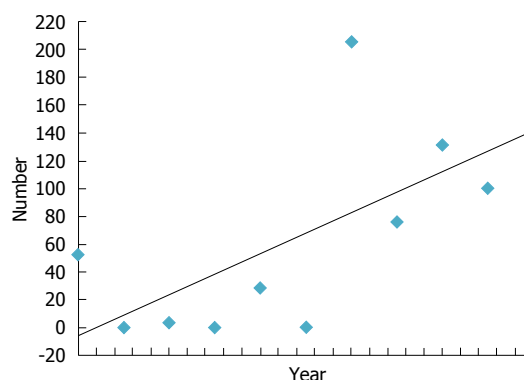


Figure 1 Number of total pancreatectomy and islet autotransplantation cases by year in United States from 2002-2012.

type (elective vs non-elective). The primary endpoints were mortality and postoperative complications according to the ICD-9 diagnosis codes which were reported as the second to 25th diagnosis of patients in the database. Risk adjusted analysis was performed to investigate morbidity predictors.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, Version 22 (SPSS Inc., Chicago, IL). The main analysis was multivariate analysis using logistic regression. The associations of morbidity with the variable of interest were examined using a multivariable logistic regression model. We included all the potential confounder variables in the model as covariates which were all variables of the study. The estimated adjusted odds ratio (AOR) with a 95%CI was calculated. The level of significance was set at $P < 0.05$.

RESULTS

Patient characteristics

We identified 923 patients who underwent IAT during 2002-2012. Among them, there were 754 patients who had TP and IAT. The mean and median patient age were 39 \pm 13 and 41 years old respectively; the majority of the patients were Caucasian (88%) and female (68.3%). Overall, 87.7% of patients were operated electively. The most common comorbidity was diabetes mellitus (26.8%) followed by hypertension (25%). Also, 20.4% of patients had anemia prior the operation. The most common indication of TP was CP (86%) followed by acute pancreatitis (12%). The mean hospitalization length of patients was sixteen days. Demographics and clinical characteristics of patients are shown in Table 1.

There was a steady increase in number of patients who underwent TP + IAT during 2002-2012 (Figure 1). The number of patients increased from 53 in 2002 to 155 cases in 2012. Also, the number of procedures was significantly higher during 2008-2012 compared to 2002-2007 (667 vs 87, $P < 0.01$). The overall

Table 2 Postoperative complications of patients who underwent total pancreatectomy and islet autotransplantation

Complications	Rate
Mortality	0 (0%)
Overall morbidity	435 (57.8%)
Post surgical hypoinsulinemia	318 (42.3%)
Acute renal failure	90 (12%)
Wound infection	63 (8.4%)
Pneumonia	56 (7.4%)
Hemorrhagic complications	50 (6.6%)
Peritoneal abscess	34 (4.5%)
Thrombosis of portal vein	25 (3.3%)
Acute myocardial infarction	15 (2%)
Wound disruption	14 (1.9%)
Acute respiratory failure	10 (1.3%)
Thromboembolic complications	10 (1.3%)
Deep vein thrombosis	¹
Biliary stricture	1

¹Too small to report.

mortality and morbidity of patients who underwent TP + IAT was 0% and 57.8% respectively (Table 2).

Predictors of morbidity

Post-surgical hypoinsulinemia was reported in 42.3% of patients, indicating 57.7% of patients were insulin independent during hospitalization. Also, 8.4% of patients had wound infections (Table 2).

Risk adjusted analysis of factors associated with morbidity of patients is reported in Table 3. Patients with obesity (AOR: 3.02, $P < 0.01$), preoperative fluid and electrolyte disorders (AOR: 2.71, $P < 0.01$), alcohol abuse (AOR: 2.63, $P < 0.01$), and weight loss (AOR: 2.43, $P = 0.03$) had significantly higher morbidity risk.

DISCUSSION

CP is associated with severe pain that may cause serious effects on a patient's quality of life and activity. TP has been established as the last resort for patients with refractory chronic pain. However, many studies have shown significant improvements in patient quality of life, as well as reduction of narcotic use^[24-26]. The combination of TP + IAT allows removal of the entire diseased gland while minimizing the risk of surgical diabetes. Post-operative narcotic use, insulin use, and standardized pain assessments have been reported by several groups, however the data on risks and morbidities of TP + IAT were limited to single-institution series. In addition, a large scale analysis of nationwide patients has not yet been reported^[7,20,21,25].

This study focuses on morbidity rates and short-term outcomes of the patients during hospitalization. The data showed an overall morbidity of 57.8%, which is consistent with data reported in existing literature that have shown morbidity in 58%-69% of patients^[7,21,24]. Despite a high morbidity, the mortality rate was 0% in patients with TP + IAT when

Table 3 Risk adjusted analysis of morbidity predictors of patients who underwent total pancreatectomy and islet autotransplantation (multivariate analysis)

Variables		Adjusted odds ratio	95%CI	P value
Age	Age	1.01	1.01-1.02	0.82
Sex	Female	1.95	1.30-2.94	< 0.01
Comorbidity	Diabetes mellitus	1.06	0.68-1.63	0.78
	Hypertension	0.70	0.45-1.08	0.11
	Deficiency anemia	0.85	0.57-1.27	0.43
	Chronic pulmonary disease	0.56	0.34-0.91	0.19
	Drug abuse	0.55	0.33-0.93	0.27
Other factors	Coagulopathy	1.24	0.63-2.44	0.52
	Alcohol abuse	2.63	1.23-5.63	0.01
	Obesity	3.02	1.00-9.11	0.049
	Weight loss	2.43	1.64-3.60	< 0.01
	Preoperative fluid and electrolyte disorders	2.71	1.79-4.09	< 0.01

compared to other studies where the rate indicated 0%-3.5%^[7,22,27]. The zero mortality rate can be explained by the fact that NIS database exclusively contains patient information only while they are hospitalized. Therefore, the data for mid-term and long-term complications are not available in the NIS. Among comorbid conditions, we found obesity to have the strongest association with morbidity of patients who underwent TP + IAT. Obesity alone is a significant risk factor for many surgical complications such as wound infection, blood loss, and a longer operation time^[28]. On the other hand, many clinical studies have shown that obesity may contribute to recovering more viable islets from pancreas isolation and achieving better metabolic control when compared to lean patients who undergo TP + IAT^[29,30]. The data suggested that physicians should objectively evaluate both negative and positive effects of obesity before surgical therapy. In addition, we found fluid and electrolyte disorders as a second morbidity predictor, which indicated that the pre-operative care and reversing fluid and electrolyte status is critical to minimizing potential post-surgical morbidities.

Patients become insulin dependent after TP due to the lack of beta-cells. IAT is an effective treatment preventing surgical diabetes after TP in patient with CP. Recently, Sutherland *et al*^[22] showed that there was a 30% insulin-independence rate in a single-center study after long-term follow-up^[16]. Furthermore, other clinical studies have shown comparative insulin-free rates during the last decade^[15,21,27]. In this study, the data clearly indicates that IAT can achieve more than a 50% insulin-free rate if using combination of TP + IAT. However, the limitation of this study was that we were only able to analyze the short-term outcomes during the hospitalization.

TP + IATs were performed mainly in a limited amount of medical facilities due to the highly required equipped facilities and well experienced isolation team.

However, the total number of patients who underwent TP + IAT in the United States has been continuously increasing during the last decade. Considering the outcomes of no mortality, acceptable morbidity, and islet graft function during the hospitalization, this procedure may be applicable for more centers nationwide.

The main limitation of the study was that it is retrospective which makes any definitive conclusion difficult. The number of transplanted patients was limited in this study; therefore, the power of the study was too low to run multivariate analysis. Also, 61.4% of the race variable's data was missing. NIS does not provide information regarding long term outcomes and follow up information of patients; therefore, there is no available data for quality of life measurement and narcotic independency status. Despite these limitations, this study is one of the first studies reporting and analyzing outcomes of patients who underwent TP and IAT with a nationwide database.

Between 2002-2012, the overall number of patients who underwent TP + IAT has been increasing.

The most common indication of the procedure was CP followed by AP. This study showed that TP + IAT is a safe and feasible procedure with no mortality and with acceptable morbidity rates, and that insulin independence can be achieved. Obesity and fluid and electrolyte disorders are the most significant predictors of in-hospital morbidity.

COMMENTS

Background

Chronic pancreatitis (CP) has a broad spectrum of symptoms and signs that interferes with patient's daily performance and quality of life. Exocrine insufficiency and severe pain are the significant manifestations that require proper management. The standard treatments include medical, endoscopic, and surgical intervention. Total pancreatectomy (TP) is the last resort treatment for pain management in CP patients. TP is related with high rate of morbidity and complications. Post surgical hypoinsulinemia is one of the important TP complications, which needs a proper intervention. Islet autotransplantation (IAT) following TP helps to decrease hypoinsulinemia episodes.

Research frontiers

This study is the first TP + IAT nationwide analysis. The authors think that TP + IAT must have a nationwide application to provide the best care for patients. The findings of this study support the fact that utilizing IAT after TP may help patients to experience less hypoinsulinemia episodes. For evaluating the pain control measures, studies with long term follow up is needed.

Innovations and breakthroughs

TP + IATs has been performed mainly in a limited amount of medical facilities due to the highly required equipped facilities and well experienced isolation team. But, this is the first nationwide analysis, which evaluates in-hospital mortality and morbidity. Considering the outcomes of no mortality, acceptable morbidity, and islet graft function during the hospitalization, this study suggests that TP + IAT may be applicable for more centers nationwide.

Applications

Patients with advanced stage CP who suffer from pain will benefit from IAT. Patients with IAT after pancreas removal can achieve insulin independence status and less pain. These benefits help patients to have better life quality and

performance in their daily life.

Terminology

CP is progressive inflammatory changes that happens in pancreas gland leads to physiological and structural damage. This process result in exocrine and endocrine malfunction. IAT is a procedure to isolate pancreatic islet cells and transplant these cells into the patient's body. The transplanted islet cells have physiologic function to secrete insulin, which prevents hypoinsulinemia episodes.

Peer-review

Very good result of TP and IAT in patients of chronic pancreatitis on a large series of retrospective study.

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Retrospective Cohort Study

Single vs dual (*en bloc*) kidney transplants from donors ≤ 5 years of age: A single center experience

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Abstract

AIM: To compare outcomes between single and dual *en bloc* (EB) kidney transplants (KT) from small pediatric donors.

METHODS: Monocentric nonprospective review of KT from pediatric donors ≤ 5 years of age. Dual EB KT was defined as keeping both donor kidneys attached to

the inferior vena cava and aorta, which were then used as venous and arterial conduits for the subsequent transplant into a single recipient. Donor age was less useful than either donor weight or kidney size in decision-making for kidney utilization as kidneys from donors < 8 kg or kidneys < 6 cm in length were not transplanted. Post-transplant management strategies were standardized in all patients.

RESULTS: From 2002-2015, 59 KT were performed including 34 dual EB and 25 single KT. Mean age of donors (17 mo *vs* 38 mo, $P < 0.001$), mean weight (11.0 kg *vs* 17.4 kg, $P = 0.046$) and male donors (50% *vs* 84%, $P = 0.01$) were lower in the dual EB compared to the single KT group, respectively. Mean cold ischemia time (21 h), kidney donor profile index (KDPI; 73% *vs* 62%) and levels of serum creatinine (SCr, 0.37 mg/dL *vs* 0.49 mg/dL, all $P = \text{NS}$) were comparable in the dual EB and single KT groups, respectively. Actuarial graft and patient survival rates at 5-years follow-up were comparable. There was one case of thrombosis resulting in graft loss in each group. Delayed graft function incidence (12% dual EB *vs* 20% single KT, $P = \text{NS}$) was slightly lower in dual EB KT recipients. Initial duration of hospital stay (mean 5.4 d *vs* 5.6 d) and the one-year incidences of acute rejection (6% *vs* 16%), operative complications (3% *vs* 4%), and major infection were comparable in the dual EB and single KT groups, respectively (all $P = \text{NS}$). Mean 12 mo SCr and abbreviated MDRD levels were 1.17 mg/dL *vs* 1.35 mg/dL and 72.5 mL/min per 1.73 m² *vs* 60.5 mL/min per 1.73 m² (both $P = \text{NS}$) in the dual EB and single KT groups, respectively.

CONCLUSION: By transplanting kidneys from young pediatric donors into adult recipients, one can effectively expand the limited donor pool and achieve excellent medium-term outcomes.

Key words: Donor age; Donor weight; *En bloc* kidney transplant; Kidney donor profile index; Single kidney transplant; Small pediatric donor

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Core tip: We evaluated outcomes in 59 kidney transplants (KT) from young pediatric donors ≤ 5 years of age including 34 dual *en bloc* (EB) and 25 single KT. Mean donor age and weight were significantly lower in the dual EB compared to the single KT group. Actuarial graft and patient survival rates at 5-years follow-up were comparable as were other outcomes. With appropriate recipient selection, excellent mid-term results can be attained by transplanting kidneys from small pediatric donors into adult recipients, which effectively expands the limited donor pool. Kidney donor profile index is predictive of survival for single KT but is not accurate for predicting dual EB KT outcomes from young pediatric donors.

Al-Shraideh Y, Farooq U, El-Hennawy H, Farney AC, Palanisamy A, Rogers J, Orlando G, Khan M, Reeves-Daniel A, Doares W, Kaczmarek S, Gautreaux MD, Iskandar SS, Hairston G, Brim E, Mangus M, Stratta RJ. Single *vs* dual (*en bloc*) kidney transplants from donors ≤ 5 years of age: A single center experience. *World J Transplant* 2016; 6(1): 239-248 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/239.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.239>

INTRODUCTION

The burgeoning crisis between organ demand and supply, particularly in kidney transplantation (KT), has fueled initiatives to safely and successfully expand the limited donor pool. Since 2002, the kidney waiting list has doubled from 50000 to > 100000 candidates and waiting times have increased from a median of 3 to > 5 years^[1]. At present, nearly 30% of patients waiting on the kidney list have been on dialysis for at least 6 years^[1]. For patients awaiting KT, only 48% will ever actually receive a KT^[1,2]. Since 2004, the total number of KT [both from living and deceased donors (DD)] performed each year in the United States has remained static and ranges has between 16000 and 17000^[1]. In the last decade, the total number of kidney DDs has slowly increased from 6325 to 7547 annually commensurate with a decrease in living donors. Among these DDs, the annual number ≤ 5 years of age range from 200 to 300, which accounts for approximately 4% of kidney DDs^[3]. The prolonged waiting times for KT and associated longer periods on dialysis have been associated with significant morbidity and mortality^[4].

Dual *en bloc* (EB) KT was first described by Carrel^[5] in 1908 in a xenograft model. Transplantation of dual EB pediatric DD kidneys into an adult was first reported in 1972^[6]. Historically, transplantation of small, pediatric, DD kidneys into adults was reported to be technically challenging and associated with vascular and urinary complications, acute rejection, delayed graft function (DGF), and the development of hyperfiltration injury^[7-11]. For these reasons, many transplant centers were reluctant or refrained completely from utilizing kidneys from small pediatric donors because they were considered "marginal"^[12-14]. However, several studies in the new millennium have demonstrated that excellent outcomes could be achieved with dual EB KT secondary to improvements in donor management, organ recovery and preservation techniques, antibody identification and crossmatch methodology, recipient selection and management, surgical techniques and immunosuppression^[15-20].

Consequently, dual EB KT has become more widely accepted and has been extended to include both donation after cardiocirculatory death (DCD) donors and infant donors < 5 kg body weight^[21]. However, the lower limits of acceptable age or body

weight for single KT are currently unknown and many pediatric kidneys from donors either < 5 years or < 20 kg are transplanted dual EB rather than split into two recipients. Because dual EB KT halves the number of potential transplant recipients, in the past decade there has been growing interest in single KT from small pediatric donors^[22-26]. Whereas dual EB KT maximizes graft function, single KT maximizes resource availability^[27-29]. A few comparative studies of single vs dual EB KTs from pediatric donors into adult recipients have been published both from registry and monocentric analyses^[30-33]. The aim of this study was to report our monocentric retrospective data spanning 12.5 years with dual EB vs single KT from small pediatric donors \leq 5 years of age in patients receiving standardized management algorithms.

MATERIALS AND METHODS

Study design

We conducted a retrospective chart review of all DD KTs performed from small pediatric donors \leq 5 years of age at our center from 7/02-1/15 with a mean follow-up of 56 mo. During this 12.5 year study period, a total of 59 DD KTs met the entry criteria and were categorized into dual EB and single KT groups for purposes of comparison.

Definitions

Dual EB KT was defined as keeping both donor kidneys attached to the aorta and inferior vena cava, which were then used as arterial and venous conduits for the subsequent transplant into a single recipient. DGF was defined as the need for dialysis for any reason in the first week post-transplant. Renal allograft loss was defined as death with a functioning graft (DWFG), allograft nephrectomy, return to dialysis, kidney re-transplantation, or return to the pretransplant serum creatinine (Scr) level in a preemptively transplanted patient.

Donor evaluation and selection

In order to estimate the donor creatinine clearance (CrCl), the Cockcroft-Gault calculation was used. We relied mainly on the donor body weight and actual kidney size and anatomy to determine whether or not to use the kidneys either for dual EB, single KT or not at all. In our dual EB KT experience, the youngest donor age was 5 mo (7.7 kg body weight) and the lowest donor weight was 6.8 kg (7 mo of age). Donor age was less useful than either donor weight or kidney size in our decision-making for kidney utilization as we usually refused kidneys from donors < 8 kg or kidneys < 6 cm in length. In our single KT experience, the youngest donor was 15 mo of age and lowest donor weight was 13.0 kg. However, similar to our lower limits of donor acceptability for dual EB KT, size of the vessels (aorta and inferior vena cava for dual EB, renal

artery and vein for single KT) was the ultimate factor that determined whether kidneys could be separated and safely transplanted into two recipients. In contrast to our adult DD KT experience, machine preservation of small pediatric donor kidneys was rarely performed.

Recipient selection

Whenever possible, based on allocation criteria, we attempted to select patients < 60 years of age for small pediatric donor kidneys. We specifically avoided selecting pediatric recipients < 12 years of age. Early in our experience, we transplanted 2 teenagers (ages 13 and 15 years), both of whom suffered early graft loss [one thrombosis secondary to recurrent fulminant focal segmental glomerulosclerosis (FSGS), one severe rejection secondary to noncompliance]. Consequently, we subsequently decided to consider the pediatric age group (who already receive priority towards young adult donors) as an exclusion criterion to KT from small pediatric donors at our center.

Similar to donor assessment, body weight was more useful in adult recipient selection than age. We attempted to select recipients < 180-200 lbs. in weight in order to avoid large mismatches between kidney and recipient size. In addition, we selected low immunological risk patients including primary transplants with a 0% panel reactive antibody (PRA) level, matching for human leukocyte antigens (HLA), and compatible B and T cell flow cytometry crossmatches in accordance with guidelines promulgated by the United Network for Organ Sharing (UNOS)^[34,35]. Reasons for selecting low immunological risk patients included concerns regarding the success of treating early acute rejection in the setting of limited nephron mass (prior to kidney growth) coupled with the hazards of performing biopsies on small pediatric donor kidneys.

All KTs from small pediatric donors were performed with informed consent from the recipient, acknowledging that there might be higher risks of DGF and technical complications unique to transplanting these types of kidneys. Other considerations in appropriate recipient selection included favorable vascular anatomy (no severe concentric iliac atherosclerosis), adequate bladder capacitance and function (to accommodate 2 ureteral anastomoses), no chronic anti-coagulation (warfarin or clopidogrel) or history of thrombophilia, adequate cardiac function and reserve (ejection fraction > 40%-50%, no atrial fibrillation or significant valvular disease), absence of either significant pulmonary or systemic hypertension, no orthostasis or history of hypotension, no prior pelvic/retroperitoneal surgery or irradiation, and absence of high risk for recurrent kidney disease.

Surgical techniques

Donor kidneys were recovered dual EB with aorta, inferior vena cava and bilateral ureters in continuity; no attempt was made to perform any dissection

Table 1 Donor, transplant and recipient characteristics

Mean \pm SD	Dual <i>en bloc</i> KT n = 34	Single KT n = 25	P value
Donor age (yr)	1.4 \pm 0.8	3.3 \pm 1.2	< 0.001
Donor gender: Male	17 (50%)	21 (84%)	0.01
Donor: African American	13 (38%)	7 (28%)	NS
Donor weight (kg)	11.0 \pm 2.6	17.4 \pm 3.1	0.046
Import organ (non-local)	17 (50%)	14 (56%)	NS
Calculated CrCl (mL/min)	99 \pm 50	111 \pm 60	NS
Pre-retrieval SCr (mg/dL)	0.37 \pm 0.26	0.49 \pm 0.24	NS
DCD donors	6 (17.6%)	3 (12%)	NS
Cause of death: Trauma	19 (56%)	11 (44%)	NS
Cold ischemia time (h)	21.0 \pm 7.8	20.9 \pm 6.4	NS
KDPI (%)	73.2 \pm 9.1	62.2 \pm 10.4	NS
HLA-mismatch	4.2 \pm 1.4	4.2 \pm 1.4	NS
0-Antigen mismatch	0	1 (4%)	NS
0% PRA	30 (88%)	24 (96%)	NS
PRA > 40%	2 (5.9%)	1 (4%)	NS
CMV donor+/recipient-	5 (14.7%)	2 (8%)	NS
Retransplant	1 (3%)	3 (12%)	NS
Recipient age (yr)	38.0 \pm 12.1	45.7 \pm 16.1	0.040
Recipient gender: Male	21 (62%)	13 (52%)	NS
Recipient: African American	17 (50%)	12 (48%)	NS
Recipient weight (kg)	72.2 \pm 14.7	75.2 \pm 12.0	NS
Recipient with diabetes	6 (17.6%)	6 (24%)	NS
Preemptive transplant	4 (11.8%)	5 (20%)	NS
Duration of dialysis	41.2 \pm 27.2	43.5 \pm 32.6	NS
Pretransplant (mo)			
Waiting time (mo)	25.2 \pm 13.6	25.4 \pm 27.2	NS

CrCl: Creatinine clearance; KT: Kidney transplantation; SCr: Serum creatinine; DCD: Donation after cardiac death; KDPI: Kidney Donor Profile Index; HLA: Human leukocyte antigen; PRA: Panel reactive antibody; NS: Not significant.

along the aorta, vena cava or renal hila in the donor. Back bench preparation of the dual EB specimen included oversewing the supra-renal vena cava and aorta with careful, meticulous dissection of the infra-renal vena cava and aorta with individual ligation of lumbar and mesenteric branches. Minimal dissection was performed in the renal hila in order to preserve any accessory vessels. Perinephric fat was left on the kidneys and suture fixation of the upper poles antero-medially was performed to maintain correct graft orientation. The dual EB allograft was transplanted extraperitoneally with end-to-side anastomoses between the distal donor vena cava and the right external iliac vein and between the distal donor aorta and the right external iliac artery. Separate parallel extravesical ureteroneocystostomies over two small (3.5–4 French) indwelling stents were performed to the dome of the bladder, attempting to make the ureters as short as possible. Single pediatric donor kidneys were transplanted in a fashion similar to standard adult KT using an extraperitoneal approach, the distal external iliac vessels as targets, and generous vena caval and aortic cuffs or patches around the orifices of the renal vein and artery, respectively. Ureteroneocystostomy was performed in an extravesical fashion over a single indwelling double-J ureteral stent (5–6 French), again attempting to make the ureter as short as

possible without tension. Both EB and single pediatric allografts were affixed either to the lateral pelvic wall or retroperitoneum using perinephric fat or capsule as needed in order to avoid torsion.

Immunosuppression and post-transplant management

Nearly all DD KT patients received either rabbit antithymocyte globulin or alemtuzumab induction as previously reported^[34–36]. Daily immunosuppression maintenance therapy included mycophenolate mofetil, tacrolimus, and either early corticosteroid withdrawal or rapid tapering as previously reported^[36]. Ultrasound-guided percutaneous kidney biopsies were performed to evaluate renal allograft dysfunction and to diagnosis and grade acute rejection. However, because of small kidney size and the theoretical risk for a higher complication rate, we did not perform surveillance kidney biopsies in these patients. All patients received surgical site, anti-fungal, anti-viral, and anti-Pneumocystis prophylaxes as previously published^[34–36]. Most patients received aspirin as prophylaxis but anti-coagulation agents were not specifically administered. Infections were categorized as major if the patient required hospitalization for either diagnosis or treatment. SCr levels were used to determine renal allograft function. In addition, the abbreviated modification of diet in renal disease (MDRD) formula was used to determine glomerular filtration rate (GFR)^[37].

Statistical analysis

Both retrospective and prospective data were analyzed and confirmed by medical record review with approval from the Wake Forest University Health Science Institutional Review Board. Statistical review of the study was performed by a biomedical statistician. Actual graft and patient survival rates were reported, and actuarial and death-censored graft survival rates were also established using Kaplan-Meier methodology with comparisons using the log-rank test. A two-tailed *P* value of < 0.05 was considered significant.

RESULTS

From 2002–2015, we performed 59 KTs from young pediatric donors \leq 5 years of age including 34 dual EB and 25 single KTs. The majority of dual EB KTs (23/34 = 68%) were performed since 2010 whereas the majority of single KTs (16/25 = 64%) were performed prior to 2010. Mean age of donors (17 mo vs 38 mo, *P* < 0.001), mean weight (11.0 kg vs 17.4 kg, *P* = 0.046) and male donors (50% vs 84%, *P* = 0.01) were lower in the dual EB compared to the single KT group, respectively (Table 1). All but 4 of the dual EB KT donors were \leq 2 years of age whereas all but 6 of the single KT donors were \geq 3 years of age. Organ import (52%), DCD donors (15%), mean cold ischemia (21 h) and terminal SCr levels (0.37 mg/dL vs 0.49 mg/dL, all *P* = NS) were comparable

Table 2 Results

Mean \pm SD	Dual <i>en bloc</i> KT <i>n</i> = 34	Single KT <i>n</i> = 25	<i>P</i> value
Patient survival	32 (94.1%)	20 (80%)	0.12
Graft survival	31 (91.2%)	17 (68%)	0.04
Follow-up (mo)	52 \pm 38	74 \pm 41	NS
Death-censored graft survival	31/33 (93.9%)	17/21 (81%)	0.19
DWFG	1 (3%)	4 (16%)	0.15
Months to DWFG	15	54 \pm 6.5	NS
Delayed graft function	4 (11.8%)	5 (20%)	NS
# Days to SCr < 3.0 mg/dL	4.7 \pm 4.5	8.9 \pm 7.2	NS
Initial length of stay (d)	5.4 \pm 2.9	5.6 \pm 3.4	NS
Acute rejection in 1 st year	2 (5.9%)	4 (16%)	NS
Surgical complications	1 (2.9%)	1 (4%)	NS
12 mo SCr (mg/dL)	1.17 \pm 0.3	1.35 \pm 0.3	NS
12 mo GFR (mL/min per 1.73 m ²)	72.5 \pm 18.4	60.5 \pm 18.1	NS
4 yr SCr (mg/dL)	1.0 \pm 0.4	1.17 \pm 0.4	NS
4 yr GFR (mL/min per 1.73 m ²)	81 \pm 21.9	64.4 \pm 18.1	NS

KT: Kidney transplantation; SCr: Serum creatinine; DWFG: Death with a functioning graft; GFR: Glomerular filtration rate; NS: Not significant.

in the dual EB and single KT groups, respectively. The longest cold ischemia times were 45 h for a dual EB and 35 h for a single KT. Only one donor (in the dual EB group) had evidence for acute kidney injury with a terminal SCr level > 1.0 mg/dL. In the single KT group, both kidneys from the same donor were transplanted at our center in 6 cases (12 KT). Mean kidney donor profile index (KDPI) was 73% for dual EB vs 62% for single KT donors ($P = \text{NS}$).

Other than mean recipient age (38 dual EB vs 46 years single KT, $P = 0.04$), there were no differences in recipient variables between groups (Table 1). Nearly 50% of recipients were African American. With a mean 52 mo follow-up in dual EB compared to 74 mo follow-up in single KT recipients, actual graft (91% vs 68%, $P = 0.04$) and patient (94% vs 80%, $P = 0.12$) survival rates were slightly higher in dual EB compared to single KT recipients, respectively (Table 2). Death-censored kidney graft survival rates were 93.9% and 81% ($P = 0.19$), respectively. Actuarial patient and graft survival rates are shown in Figures 1 and 2 ($P = \text{NS}$). Survival rates were similar up to 4 years follow-up in the each group after which time graft survival decreased in the single KT group. There was no influence of recipient gender or ethnicity on outcomes.

As previously mentioned, patients #3 and #4 in our dual EB KT experience were both teenagers who developed early graft failure (at 5 mo secondary to noncompliance and at 2 d secondary to thrombosis related to fulminant recurrence of FSGS, respectively). Patient #3 subsequently died 5 years later secondary to a hemorrhagic stroke (in the absence of retransplantation because of a high PRA level); the only other death (and graft loss) in the dual EB KT group was a 28 years old male who experienced

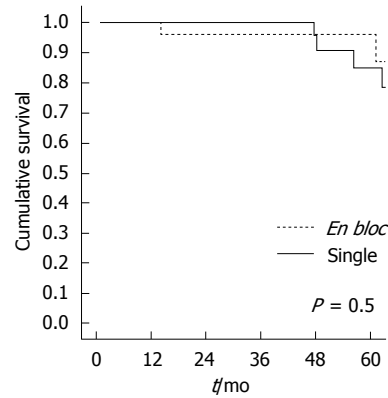


Figure 1 Actuarial patient survival rates among recipients of dual *en bloc* vs single kidney transplantation from young pediatric donors.

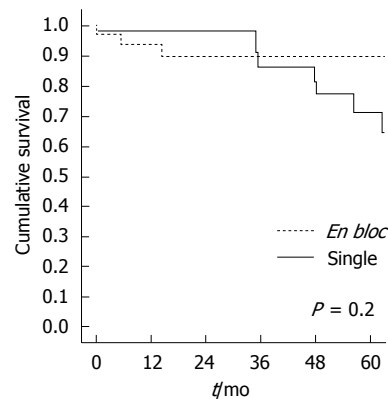


Figure 2 Actuarial graft survival rates among recipients of dual *en bloc* vs single kidney transplantation from young pediatric donors.

DWFG at 15 mo post-transplant; the cause of death was unknown. However, one patient developed a near 50% lower pole infarction of one kidney secondary to a missed accessory renal artery that was managed expectantly without sequela. Another patient developed a partial lower pole infarction of the left kidney secondary to a missed accessory renal artery that was also successfully managed expectantly. A third patient developed a lower pole infarct of the right kidney secondary to a missed accessory renal artery and underwent allograft nephrectomy of the left kidney on post-operative day #1 because of venous thrombosis. Fortunately this latter patient has acceptable renal function from the remaining right kidney and no evidence of a ureteral complication with limited follow-up. One recipient developed dual ureteral strictures at 15 mo following dual EB KT secondary to acute cellular and antibody-mediated rejection related to medication noncompliance. The strictures were initially managed with percutaneous nephrostomies followed by placement of chronic internalized ureteral stents that are changed at frequent intervals.

In the single KT group, there were 5 deaths (4 DWFGs) occurring at a mean of 70 mo post-KT; none occurred until 4 years or more post-KT. Causes of

death include 2 strokes, 2 pneumonias/respiratory failure, and one unknown. There were 8 graft losses including 4 DWFGs, 2 secondary to acute and chronic rejection, 1 chronic allograft nephropathy and one early thrombosis. There were no urological or other surgical complications in either group.

During this same period in time, we performed 758 standard criteria donor (SCD) KT (excluding young pediatric donors) in 722 recipients with an age mean of 50.4 years. With 63 mo mean follow-up, actual patient and graft survival rates in SCD KT recipients were 83.9% [$P = 0.15$ compared to dual EB (94%), $P = NS$ compared to single KT (80%)] and 70.4% [$P = 0.006$ compared to dual EB (91%), $P = NS$ compared to single KT (68%)], respectively. The kidney graft survival rate (censored for death) following SCD KT was 79.6% [$P = 0.04$ compared to dual EB (93.9%), $P = NS$ compared to single KT (81%)]. From 2008-2015, we performed 180 living donor KT in 179 patients with an age mean of 47.4 years. With a 40 mo mean follow-up, actual patient and graft survival rates were 92.7% [$P = NS$ compared to dual EB (94%), $P = 0.05$ compared to single KT (80%)] and 88.9% [$P = NS$ compared to dual EB (91%), $P = 0.01$ compared to single KT (68%)], respectively. The kidney graft survival rate (censored for death) following living donor KT was 93.6% [$P = NS$ compared to dual EB (93.9%), $P = 0.065$ compared to single KT (81%)].

The DGF rate (12% dual EB vs 20% single KT, $P = NS$) was slightly lower in dual EB KT recipients. Duration of hospitalization (mean 5.4 d vs 5.6 d) and the one-year incidences of acute rejection (6% vs 16%), operative complications (3% vs 4%), and major infection were comparable in the dual EB and single KT groups, respectively (all $P = NS$). Mean 12 mo SCr and aMDRD levels were 1.17 mg/dL vs 1.35 mg/dL and 72.5 mL/min per 1.73 m² vs 60.5 mL/min per 1.73 m² (both $P = NS$) in the dual EB and single KT groups, respectively. At 4 years follow-up, the corresponding values were 1.0 mg/dL vs 1.17 mg/dL and 81 mL/min per 1.73 m² vs 64.4 mL/min per 1.73 m² in the dual EB and single KT groups, respectively.

DISCUSSION

Historically, kidneys from donors at the extremes of age have been considered as marginal organs for KT because of concerns regarding technical complications and long-term functional outcomes^[38]. Most of the recent expansion in organ donation has occurred at the older extreme of age^[1]. However, unlike kidneys from older donors, kidneys transplanted from pediatric donors into adult recipients have the capacity to grow to a normal adult renal size within a few months of KT and represent an under-utilized resource^[39]. Both conversion and utilization rates are lower with younger DD age^[3,31,33]. Small pediatric donor KT is gaining wider acceptance but is still regarded as controversial by some and is not universally accepted. The total

number of nephrons in each kidney (estimated at a mean of approximately 1.0 million) is attained by 36 wk of gestation; subsequent renal "growth" occurs by hypertrophy rather than increases in nephron number^[40,41]. Excellent outcomes with pediatric dual EB KT have been published from recent reports, which in theory reduces concerns regarding functional outcomes and graft longevity because of the potential for growth coupled with the increased nephron mass associated with transplantation of both kidneys^[20,31-33]. However, there exists a persistent unwillingness to separate small pediatric donor kidneys for KT into two recipients, and no consensus exists as to when single KT can be safely and successfully performed^[42-46].

Previous studies have suggested that pediatric dual EB KT should be performed for donors < 10 kg whereas "splitting" kidneys for use in two recipients is appropriate when the donor is > 20 kg in size^[20,24,26]. However, donors weighing between 10-20 kg represent a "gray area" in achieving the proper balance between utilization and outcomes^[31,33]. In a large retrospective UNOS registry analysis of donors < 10 years of age from 1995-2007, Kayler *et al*^[24] reported that kidneys from donors with a 15-19, 10-14, and < 10 kg body weight were used for dual EB KT in 40%, 65%, and 86% of adult recipients, respectively^[24]. In a subsequent UNOS registry analysis of donors < 10 years of age spanning 1987-2007, Sureshkumar *et al*^[25] reported that kidneys from donors with a 10-13, 13-15, 15-20, and > 20 kg body weight were used for dual EB KT in 63%, 49%, 24%, and 4% of adult recipients, respectively. In addition, they noted that although pediatric dual EB kidneys functioned "better" than single kidneys for all pediatric donor weight groups studied, "acceptable" graft outcomes could be achieved with single KT from donors > 10 kg because the graft failure risk declined above this donor size.

In 2011, Laurence *et al*^[26] constructed a decision analysis model based on existing literature in order to predict outcomes (expressed as life years) for waitlist patients according to whether they underwent dual EB or single KT from a pediatric donor. At all ages of recipients studied, the combined projected life years of both recipients of solitary KT exceeded the projected life years of a dual EB KT. However, for recipients of kidneys from donors < 10 kg, there was an estimated net loss of life years following solitary KT irrespective of recipient age group.

Other studies have reported that outcomes following dual EB KT are comparable to those achieved following living donor KT whereas outcomes following single KT from pediatric donors are comparable to those achieved following SCD KT and superior to those achieved following ECD KT^[27,43,45,46]. In our experience, we likewise found that dual EB KT outcomes were comparable to concurrent living donor KT and superior to SCD KT at our center whereas outcomes following single KT from pediatric donors were inferior to living donor KT and similar to those achieved following SCD

KT. Although these findings may be explained in part by variations in recipient age, differences persisted even when we censored for DWFG.

We conducted a retrospective review spanning 12.5 years of our clinical experience in KT from small pediatric donors (defined as ≤ 5 years of age) and compared outcomes between recipients of dual EB vs single KTs. The majority of dual EB KTs (69%) were performed since 2010 whereas the majority of single KTs (64%) were performed prior to 2010. In our dual EB KT experience, the youngest donor age was 5 mo (7.7 kg body weight) and the lowest donor weight was 6.8 kg (7 mo of age). Donor age was less useful than either donor weight or kidney size in our decision-making for kidney utilization as we usually refused kidneys from donors < 8 kg or kidneys < 6 cm in length. Over time, we have become more comfortable with performing dual EB KTs from smaller pediatric donors; 14 of the 34 dual EB donors were < 10 kg body weight and 50% were age ≤ 12 mo. In our single KT experience, the youngest donor was 15 mo of age and lowest donor weight was 13.0 kg. However, similar to our lower limits of donor acceptability for dual EB KT, size of the vessels (inferior vena cava and aorta for dual EB, renal vein and artery for single KT) and ureters were the ultimate factors that determined whether kidneys could be separated and safely transplanted into two recipients.

Recipient selection is paramount to success in KT from small pediatric donors. Similar to donor assessment, we found that body weight was more useful in adult recipient selection than age. We attempted to select recipients < 180 - 200 lbs in weight in order to avoid large mismatches between kidney and recipient size in an attempt to minimize the risk of hyperfiltration injury^[47-50]. However, we specifically excluded pediatric recipients from consideration after a negative experience with dual EB KT in 2 teenagers who developed early graft loss. Some authors have reported that the risk of graft failure may be higher when transplanting kidneys from small pediatric donors into pediatric recipients^[20,24,28,32,43]. The primary reason to avoid transplanting small pediatric donor kidneys into pediatric recipients (in the absence of a primary renal disease with a high recurrence rate) is to avoid anastomosing small donor vessels to small recipient vessels in relatively hypotensive (compared to adults) patients, which may result in early technical failure. At present, 90% of all pediatric DD kidneys are transplanted into adult recipients, 37% of whom are aged 50 years and older^[41]. However, recent studies are beginning to question the prohibition of pediatric recipients from receiving pediatric donor kidneys as improving results are being reported and size-matching between donors and recipients seems logical from a functional and growth perspective^[21,29].

We have observed that small pediatric donors are assigned relatively high scores in the new KDPI (overall mean 69% in our experience) because of the

negative cumulative impact of reduced donor height, weight, and age in the calculation. The UNOS KDPI is derived from the kidney donor risk index that explicitly incorporates 10 donor factors (such as donor age, hypertension, diabetes, ethnicity, height, weight, cause of death, SCr, hepatitis C status, and whether the donation occurred after cardiocirculatory death) to rank order the relative quality of kidneys into a continuous score as defined by an aggregate population relative risk^[51,52]. However, many of the KDPI variables do not "fit" for small pediatric donors, particularly in the setting of dual EB KT. For example, the mean KDPI in our single KT experience was 62%, which translates roughly to an expected graft survival rate at 5 years follow-up of 69%. Our observed graft survival rate at 5 years follow-up in this group was 70%. Conversely, the mean KDPI in the dual EB KT group was 73%, which translates roughly to an expected graft survival rate at 5 years follow-up of 66%. However, our observed graft survival rate at 5 years follow-up in this group was 90%. Consequently, one might contend that the KDPI is not applicable in this setting and a new predictive algorithm may be needed not only for dual EB KT in particular but perhaps dual KT in general.

Other important aspects of recipient selection included informed consent and selecting low immunological risk patients (primary transplants with a low PRA level, HLA-matching, negative T and B cell flow crossmatches) so as to avoid the need to either biopsy or treat for acute rejection. Additional recipient "contraindications" to either dual EB or single KT from small pediatric donors included severe pulmonary or systemic hypertension, orthostasis or severe hypotension, low ejection fraction, severe iliac vascular disease, presence of an abnormal urinary bladder (either anatomically or functionally), high risk for recurrent kidney disease, history of thrombophilia or need for anti-coagulation.

Based on this experience, we found that excellent mid-term outcomes can be attained from young pediatric donors; our protocol at present is to perform dual EB KT from donors < 15 kg and single KT from donors ≥ 15 kg. Limitations of our study design include its retrospective nature and relatively small number of KTs in each group whereas strengths include intermediate-term follow-up and standardized management algorithms pertaining to donor and recipient selection, surgical technique, immunosuppression and post-transplant management. It is well established that small pediatric donor kidneys increase in size and have excellent function in adult recipients provided that technical complications or acute rejection do not occur^[8,39,53]. Pediatric donor kidneys appear to have an excess capacity for hypertrophy, which translates into an absolute increase in GFR over time^[39,43,46,49,54]. Because pediatric dual EB kidneys have double the nephron mass compared to single KT, studies have shown that these recipients may attain renal function that is similar to or even

better than functional outcomes achieved following living donor KT^[43,45,49]. In our experience, renal function improved in both groups from 1 to 4 years following KT but the improvement observed in the dual EB KT group was more notable.

Fortunately, we did not note in our study an increase in technical complications associated with the utilization of small pediatric donor kidneys. There was one thrombosis resulting in early graft loss in each group and no early ureteral complications mandating any re-operation or intervention. A study of UNOS data demonstrated a 5% thrombosis risk among donors between 12 and 17 years of age compared to a 10% rate of vascular thrombosis using donors < 5 years of age^[15]. This study also showed inferior outcomes with single grafts from donors > 15 kg compared to using dual EB kidneys from donors < 5 years of age. Other risk factors for inferior outcomes in this study included retransplants, those with a body mass index > 24 kg/m², black recipients, and prolonged ischemia time^[15]. Some studies have demonstrated that small donor kidneys may have a higher risk of late graft failure if transplanted into large recipients^[48,50,55]. Consequently, the relative sizes of the recipient and donor need to be considered. When the donor weight is greater than 14 kg and the individual renal allografts measure greater than 6 cm in length, then separation of EB pairs can be contemplated. Other series have shown that kidneys from donors 1-3 year of age and/or weighing 9-15 kg can be successfully transplanted EB and those from donors > 3 years of age and/or weighing > 15 kg can be successfully transplanted as single grafts^[13,30]. Our experience mirrors and supports these previous recommendations. Moreover, we would like to underscore the fact that in the new Kidney Allocation System, the KDPI for small pediatric donor kidneys does not accurately represent the outcomes that can be achieved with dual EB KT.

COMMENTS

Background

The burgeoning crisis between organ supply and demand, particularly in kidney transplantation, has fueled initiatives to safely and successfully expand the limited donor pool. Historically, transplantation of small pediatric donor kidneys into adult recipients was reported to be technically challenging and associated with an increased risk of vascular and urinary complications, acute rejection, delayed graft function, and the development of hyperfiltration injury. For these reasons, many transplant centers are reluctant to transplant kidneys from small pediatric donors, which results in lower conversion and utilization rates among young donors.

Research frontiers

Most of the recent expansion in organ donation has occurred at the older extreme of age. However, unlike kidneys from older donors, kidneys transplanted from small pediatric donors into adult recipients have the capacity to grow to a normal adult renal size and represent an under-utilized resource. Transplantation of kidneys from small pediatric donors is gaining wider acceptance but is still regarded as controversial by some and is not universally accepted. Moreover, criteria for using these kidneys either as single or dual *en bloc* (EB) transplants are evolving. Previous studies have suggested that

pediatric dual EB kidney transplants (KT) should be performed for donors < 10 kg whereas "splitting" kidneys for use in two recipients is appropriate when the donor is > 20 kg in size. However, donors weighing between 10-20 kg represent a "gray area" in achieving the proper balance between utilization and outcomes.

Innovations and breakthroughs

The authors conducted a retrospective review spanning 12.5 years of the authors' clinical experience in kidney transplantation from small pediatric donors (defined as ≤ 5 years of age) and compared outcomes between recipients of dual EB vs single KT. In the authors' dual EB KT experience, the youngest donor age was 5 mo (7.7 kg body weight) and the lowest donor weight was 6.8 kg (7 mo of age). Over time, the authors have become more comfortable with performing dual EB KT from smaller pediatric donors; 14 of the 34 dual EB donors were < 10 kg body weight and 50% were age ≤ 12 mo. In the authors' single KT experience, the youngest donor was 15 mo of age and lowest donor weight was 13.0 kg. Recipient selection is paramount to success as we attempted to avoid large mismatches between kidney and recipient size. However, the authors specifically excluded pediatric recipients from consideration. The authors established that dual EB outcomes were comparable to concurrent living donor kidney and superior to standard criteria adult deceased donor KT whereas outcomes following single kidneys from small pediatric donors were inferior to concurrent living donor kidney and similar to those achieved following standard criteria adult deceased donor KT at the center.

Applications

Based on this experience, the authors verified that excellent intermediate-term outcomes can be achieved from young pediatric donors; the authors' current policy is to perform dual EB KT from donors < 15 kg and single KT from donors ≥ 15 kg. Moreover, the authors have observed that small pediatric donors are assigned relatively high scores in the new Kidney Donor Profile Index (KDPI) because of the negative cumulative impact of reduced donor height, weight, and age in the calculation. In the new Kidney Allocation System, however, the KDPI for small pediatric donor kidneys does not accurately predict outcomes that can be achieved with dual EB KT, suggesting that a new predictive algorithm may be needed in this setting.

Terminology

Dual EB KT are performed by keeping both donor kidneys attached to the aorta and inferior vena cava, which are then used as arterial and venous conduits for the subsequent transplant of both kidneys as a single unit into one recipient. The KDPI is derived from the kidney donor risk index that explicitly incorporates 10 donor factors (such as donor age, hypertension, diabetes, ethnicity, height, weight, cause of death, serum creatinine, hepatitis C status, and whether the donation occurred after cardiocirculatory death) to rank order the relative quality of kidneys into a continuous score as defined by an aggregate population relative risk.

Peer-review

This manuscript of Yousef Al-Shraideh *et al.* exhaustively described a current issue, directly related to the ever-existing problem of acute organ shortage, namely the optimum use of small paediatric donors.

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Recurrence of lymphangioleiomyomatosis: Nine years after a bilateral lung transplantation

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Abstract

Lymphangioleiomyomatosis (LAM) is a rare, slowly progressive lethal lung disease primarily afflicting young women. LAM is characterized by proliferation of abnormal smooth muscle cells that target the lungs, causing cystic destruction and eventual respiratory failure leading to death. Recent ten year mortality due to end stage LAM has been reported to be approximately 10%-20%, but may vary. The decline in lung function in LAM is gradual, occurring at a rate of about 3% to 15% per year but can vary from patient to patient. But recently therapy with mammalian target of rapamycin (mTOR) inhibitors such as sirolimus has shown promising results in the stabilization of lung function and reduction of chylous effusions in LAM. Lung transplantation is a viable option for patients who continue to have decline in lung function despite mTOR therapy. Unique issues that may occur post-transplant in a recipient with LAM include development of chylous effusion and a risk of recurrence. We describe a case of LAM recurrence in a bilateral lung transplant recipient who developed histological findings of LAM nine years after transplantation.

Key words: Lymphangioleiomyomatosis; Mammalian target of rapamycin inhibitors; Lung transplantation; Sirolimus; Lung rejection

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Core tip: Lymphangioleiomyomatosis (LAM) is a rare, slowly progressive lethal lung disease characterized by proliferation of abnormal smooth muscle cells that target the lungs, causing cystic destruction and eventual respiratory failure and death. Mammalian target of rapamycin (mTOR) inhibitors such as sirolimus have shown promise in stabilization of lung function. Lung transplantation is a viable option when lung function continues to decline despite use of mTOR inhibitors. However, recurrence of LAM in transplanted

lung has been reported. We describe a case of LAM recurrence in a bilateral lung transplant recipient nine years after transplantation, our therapeutic approach once recurrence was documented with review of the literature.

Zaki KS, Aryan Z, Mehta AC, Akindipe O, Budev M. Recurrence of lymphangioleiomyomatosis: Nine years after a bilateral lung transplantation. *World J Transplant* 2016; 6(1): 249-254 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i1/249.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i1.249>

INTRODUCTION

Lymphangioleiomyomatosis (LAM) is a rare, progressive, cystic lung disease of young women characterized by abnormal proliferation of smooth muscle like LAM cells causing pulmonary tissue destruction and cystic changes^[1]. LAM is commonly sporadic (S-LAM) however 30%-40% of cases are related with tuberous sclerosis complex (TSC-LAM) carrying mutations in TSC1 or TSC2 genes^[1,2]. Interestingly, TSC2 mutation has also been reported in sporadic type which is indicative of genetic basis for LAM^[1]. Patients with LAM can have several clinical findings including dyspnea on exertion, thoracic lymphadenopathy, recurrent pneumothorax, chylothorax and chylous ascites as well as angiomyolipomas and lymphangiomyomas^[3]. Histologically, LAM is characterized by infiltration of abnormal spindle shaped smooth muscle cells called LAM cells. They express common melanoma related antigens (HMB-45, gp-100, MART-1) and smooth muscle antigens (S100) which are useful in histological identification^[3]. Regardless of association with TSC, LAM cells have bi-allelic inactivation of TSC which is a tumor suppressor gene leading to activation of mammalian target of rapamycin (mTOR) pathway and uncontrolled proliferation and metastasis of LAM cells. Because of existence of genetic aberration in smooth muscle cell in organs other than the lungs and their ability to metastasize, recurrence of LAM after lung transplantation has been reported even in the absence of angiomyolipomas. Generally the lung function decline is extremely slow and may take up to 1-2 decades before LAM patients developed respiratory failure. Early hormonal treatment was thought to be beneficial but Oprescu *et al*^[4] in 2013 showed that such therapy doesn't improve the outcome. mTOR therapy with sirolimus has showed to stabilize lung function and improve quality of life. In patients that have exhausted all medical therapies, lung transplantation may be the only option. The recurrence of LAM following lung transplantation is rare and only nine cases have been reported in the literature^[1,5-10]. The largest LAM database from Europe demonstrated only single digit recurrence rate of LAM after transplantation (6%-7%)^[10,11]. Due to the rarity of LAM and low rate

of recurrence following lung transplantation, there is a paucity in our current knowledge regarding the treatment and rate of its progression. Although looking at the LAM registry in general, out of the nine patients who underwent transplantation the most common cause of death was respiratory failure (44%) followed by infection but no documentation was noted regarding recurrence as a cause of death^[4]. Here, we present the tenth case of recurrence of LAM following bilateral lung transplantation (BLT) and describe our therapeutic approach once the recurrence was demonstrated.

CASE REPORT

A 66-year-old African-American woman underwent sequential BLT for LAM in 1999. Her initial diagnosis of LAM was established at age 51 years when she was found to have cystic changes involving the lungs and histo-pathologic findings of abnormal proliferation of LAM cells on biopsy. The lung was the only organ involved with no evidence of angiomyolipomas before and after the transplant. Her early post-lung transplantation regimen included prednisone, tacrolimus, mycophenolate mofetil along with trimethoprim-sulfamethoxazole for pneumocystis jiroveci and acyclovir for viral prophylaxis. She underwent left upper lobe lobectomy for pseudomonas abscess in 2000 with no decline in her lung function or findings of chronic lung allograft dysfunction. Eight years later, she developed right upper lobe mass and nodules along with declining lung function and underwent BAL with transbronchial biopsy (TBBX). Her BAL demonstrated *Aspergillus Ustis*, *Pseudomonas* and *Mycobacterium avium-intracellulare* infection, which was treated with voriconazole, inhaled amphotericin-B, ciprofloxacin, azithromycin and ethambutol. There was no evidence of acute or chronic rejection at that time. Her symptoms improved with returning of FEV1 back to her baseline. Follow up bronchoscopy and TBBX in December 2008 revealed presence of bundles of smooth muscle cells with sparse atypical spindle/LAM cells without evidence of acute or chronic rejection or infection. Even though the immunohistochemical studies for HMB-45 were negative likely due to scant number of LAM cells, in the absence of other findings clinical diagnosis of LAM recurrence was made. She did well during the following years with stable lung function and her immunosuppression remained the same. In March 2011, she developed dyspnea on exertion despite stable lung functions which led to a bronchoscopy with TBBX which showed similar findings of LAM cells without rejection or infection. She was placed on sirolimus which was discontinued after six months of therapy due to the need for an urgent surgery. In December 2013, one year later she noticed worsening of dyspnea with gradual decline in FEV1 from 1.36 to 1.0 L (Table 1). On chest X-ray right upper lobe interstitial and nodular changes were

Table 1 Serial pulmonary functions in a lung transplant recipient for lymphangioleiomyomatosis

	PreTx-1999	PostTx-2000	2009	2011	2013	2014
FVC	0.81 (27%)	1.70 (57%)	2.06 (71%)	1.90 (80%)	1.83 (79%)	1.76 (77%)
FEV1	0.26 (10%)	1.39 (56%)	1.36 (59%)	1.33 (71%)	1.12 (62%)	1.0 (56%)
FEV1/FVC	32.1 (39%)	81.6 (100%)	65.7 (83%)	69.9 (89%)	61.2 (78%)	57.1 (73%)

FVC: Forced vital capacity; FEV: Forced expiratory volume.



Figure 1 Chest X-ray postero-anterior view at 15 years. Note right upper zone nodular and interstitial opacities.

noticed (Figure 1). A computed tomography (CT) of the chest showed right upper lobe nodules with bilateral interstitial thickening and scattered ground glass opacities which were unchanged from 2008 (Figure 2). A flexible bronchoscopy with BAL and TBBX again showed sparse LAM cells (Figure 3) negative for HMB-45 with no evidence of infection and acute or chronic rejection suggesting LAM recurrence as likely cause of her symptoms and findings on CT.

In an effort to stabilize lung function, tacrolimus was switched to sirolimus monotherapy resulting in brief stabilization of lung function. She subsequently developed respiratory failure due to HINI viral infection and mycoplasma pneumonia a few months later. However, despite therapy for the viral and mycoplasma infections her lung functions continued to deteriorate with a decline in her functional status, this was thought to be due to chronic lung allograft dysfunction of bronchiolitis obliterans type. She was not considered for re-transplantation due to her deconditioned state and age. She ultimately entered hospice care and died of complications likely due to chronic rejection along with LAM recurrence.

DISCUSSION

LAM is a rare disease with prevalence of 2 per 1 million of the population^[3]. It almost exclusively affects young women. With respect to the rarity of LAM and limited knowledge on treatment and prognosis of these patients, here we presented a fifteen year follow up post-bilateral lung transplant of a patient with LAM recurrence. It

is evident from the literature that LAM could recur as early as within two years after the lung transplantation. Although the recurrence of LAM is rare, the post-transplant survival of these patients when compared to all other indications of transplant is better^[11]. But the number of patients that have undergone transplantation for LAM as the primary indication is very small and predications regarding this disease and survival post-transplant should be tempered.

To date lung transplantation represents one of the most effective and acceptable therapeutic option for LAM patients with respiratory failure. Both single and BLTs have been performed (Table 2). The estimated five year post lung transplant survival among LAM patients is between 60%-70%. The recurrence is rare, and the rate between 3.7%-7% has been reported in the largest European and United States studies^[10,11]. It is likely that recurrence rate could be higher in long term survivors as early recurrence may be asymptomatic. These studies demonstrated that respiratory failure, BOS and infectious complications are the most common causes of death in the later period post-transplant similar to other cases of transplant. The LAM recurrence is rare and doesn't compromise long term survival. As in our patient LAM recurrence diagnosis was made after nine years post-transplant and remained asymptomatic for at least four more years.

Due to the limited knowledge regarding specific treatment of LAM, the goal remains relief of symptoms and management of complications. In 2011 MILES study showed promising results of sirolimus in LAM patients with stabilization of lung function with improvement in quality of life and functional performance^[12]. In Europe, the dose of rapamycin varies individually from 0.5 mg every other day, to 2 mg daily while in MILES study the dose was adjusted by keeping serum levels between 5-15 µg/dL^[10,12]. As LAM recurrence post lung transplant is mostly asymptomatic it is unclear when to start mTOR inhibitors. It is less likely that a large, randomized trial in this group of patients post-transplant can be carried out due to the rare nature of this disease; however our clinical acumen supports the notion that in lung transplant recipients with LAM, sirolimus should be considered as a primary anti-rejection medication either as mono or as dual therapy with a calcineurin inhibitors (CNI). Theoretically, therapy with mTOR inhibitors is likely to delay the progression or

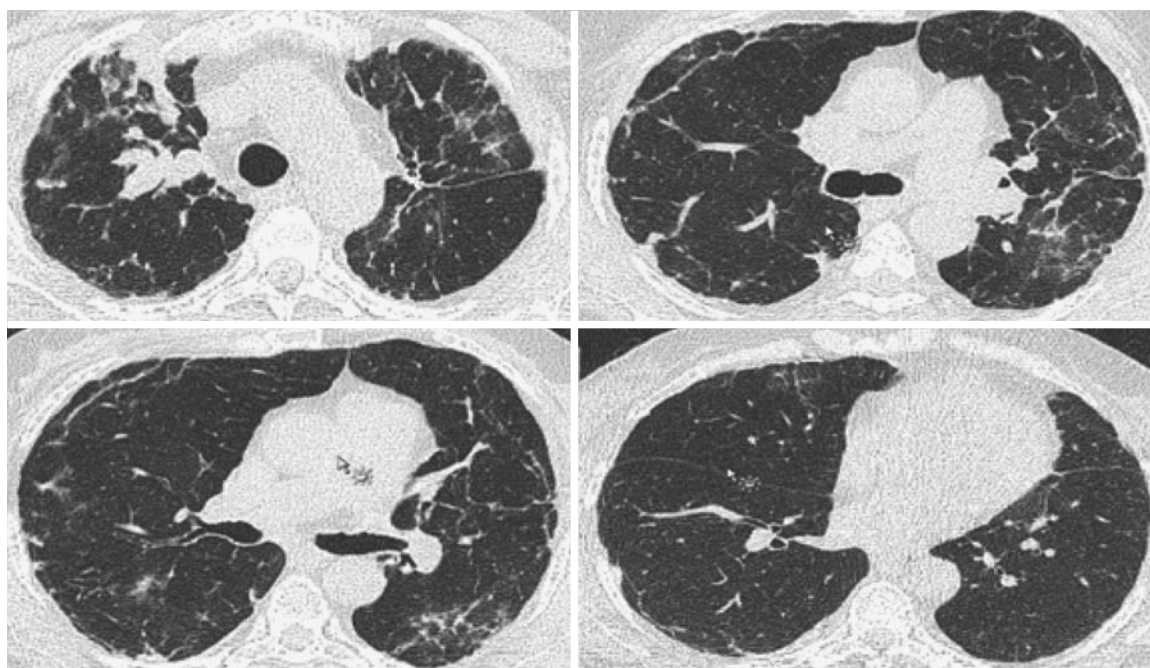


Figure 2 Computed tomography of the chest. RUL nodules with bilateral interstitial thickening and scattered ground glass opacities.

Table 2 Summary of cases with recurrence of lymphangioleiomyomatosis following lung transplantation

Ref.	No. of patients	Type of transplant	Age at transplantation (yr)	Donor	Post-transplant immunosuppressive drugs	Post-transplant complications	Outcomes
O'Brien <i>et al</i> ^[5]	1	Single right	NA	NA	NA	NA	NA
Bittmann <i>et al</i> ^[8,9]	1	Single right	34	Male Cadaveric	NA	Pneumothorax	Survival 2 yr COD: pneumothorax and hypoxemia
Karbowniczek <i>et al</i> ^[11]	1	Single right	42	Male cadaveric	Cyclosporine, Azathioprine, Prednisone	Chylous pleural effusion	Survival 2 yr COD: Aspergillus pneumonia, Recurrence of LAM was confirmed on autopsy
Chen <i>et al</i> ^[7]	1	Bilateral Living-donor lobar	23	Mother and sister	NA	Massive chylous pleural effusion and ascites	Not known, but she was diagnosed with recurrence of LAM in left lung 2 yr after transplantation due to characteristics cystic changes and pathological confirmation
Sugimoto <i>et al</i> ^[6]	1	Bilateral Living-donor lobar	23	Brother	Tacrolimus, Prednisone	Un-eventful course	Dyspnea and pleural effusion following 5 yr post-transplant, sirolimus 1-2 mg/d helped resolve pleural effusion and improved lung function and symptoms
Benden <i>et al</i> ^[10]	4	NA	NA	NA	Cyclosporine, Tacrolimus, Prednisone, Azathioprine	Surgical complications, respiratory tract infections, pneumothorax, pulmonary embolism	Not specified for recurrence of LAM, 5 yr survival was estimated to be 34%

NA: Not available; COD: Cause of death; LAM: Lymphangioleiomyomatosis.

recurrence of LAM. However, there are no randomized trials to support the recommendation due to the rarity of the disease and its presentations. It is advisable to place the patients on lifelong mTOR inhibitors following the lung transplantation to delay the recurrence of LAM in the allograft. Intolerance or complications of mTOR

inhibitors may limit their use in some patients, who may then require re-transplantation.

Our case highlights the possibility of LAM recurrence following BLT. Though rare, it remains asymptomatic and doesn't seem to affect long term survival. The most common cause of death remains respiratory failure,

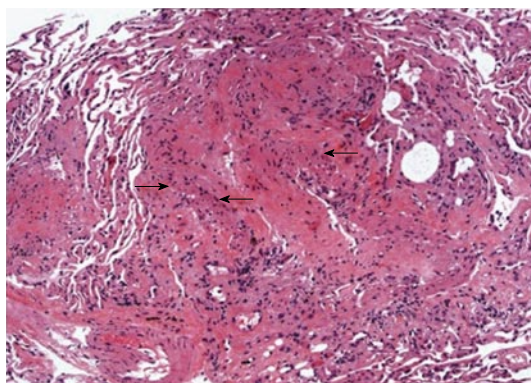


Figure 3 Histopathological examination of the transbronchial biopsy revealing spindle shaped lymphangioleiomyomatosis (arrows) cells suggestive of recurrence.

development of BOS and infectious complications. Sirolimus should be considered as a primary anti-rejection medication either as monotherapy or as dual therapy with a CNI in this patient population but timing of initiation remains under debate.

COMMENTS

Case characteristics

A 66 year of women post bilateral lung transplantation for lymphangioleiomyomatosis (LAM) presented with dyspnea on exertions 9 years post transplantation.

Clinical diagnosis

Her clinical examination remained unremarkable and didn't change since prior visits.

Differential diagnosis

Acute cellular rejection, chronic rejection, obliterative bronchiolitis syndrome, opportunistic infection, recurrence of LAM.

Laboratory diagnosis

All laboratory work up was within normal limits.

Imaging diagnosis

Chest X-ray showed chronic right upper lobe interstitial and nodular changes. CT of the chest showed right upper lobe nodules with bilateral interstitial thickening and scattered ground glass opacities which were unchanged from prior studies.

Pathological diagnosis

Histopathological examination of the transbronchial biopsy revealing spindle shaped LAM cells without evidence of infection or rejection, suggestive of LAM recurrence.

Treatment

Calcineurin inhibitor immunosuppressive therapy was switched to sirolimus monotherapy but had to be stopped due to surgery. Later again restarted resulted in brief stabilization of lung function. However the patient developed complications of infection and rejection which proved to be fatal.

Related reports

Lung transplantation represents one of the most effective and acceptable therapeutic option for LAM patients with respiratory failure. The recurrence is rare and mostly remains asymptomatic. Sirolimus has shown to stabilized lung function in patients with LAM. However, post transplantation its role is not clear.

Term explanation

Bronchitis obliterans syndrome is a form of chronic lung allograft dysfunction that commonly presents with obstructive ventilatory defect and decline in forced expiratory volume in 1 s post lung transplantation.

Experiences and lessons

LAM is a rare disease and its recurrence post lung transplantation is even rarer. Sirolimus therapy slows the progression of disease in patient with LAM. This clinical acumen supports the notion that in lung transplant recipients with LAM, sirolimus should be considered as a primary anti-rejection medication either as monotherapy or as dual therapy with a calcineurin inhibitors. Intolerance or complications of mammalian target of rapamycin inhibitors may limit their use in some patients, who may then require re-transplantation.

Peer-review

It is a very rare phenomenon.

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Exocrine drainage in vascularized pancreas transplantation in the new millennium

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Abstract

The history of vascularized pancreas transplantation largely parallels developments in immunosuppression and technical refinements in transplant surgery. From the late-1980s to 1995, most pancreas transplants were whole organ pancreatic grafts with insulin delivery to the iliac vein and diversion of the pancreatic ductal secretions to the urinary bladder (systemic-bladder technique). The advent of bladder drainage revolutionized the safety and improved the success of pancreas transplantation. However, starting in 1995, a seismic change occurred from bladder to bowel exocrine drainage coincident with improvements in immunosuppression, preservation techniques, diagnostic monitoring, general medical care, and the success and frequency of enteric conversion. In the new millennium, pancreas transplants are performed predominantly as pancreatico-duodenal grafts with enteric diversion of the pancreatic ductal secretions coupled with iliac vein provision of insulin (systemic-enteric technique) although the systemic-bladder technique endures as a preferred alternative in selected cases. In the early 1990s, a novel technique of venous drainage into the superior mesenteric vein combined with bowel exocrine diversion (portal-enteric technique) was designed and subsequently refined over the next ≥ 20 years to recreate the natural physiology of the pancreas with first-pass hepatic processing of insulin. Enteric drainage usually refers to jejunal or ileal diversion of the exocrine secretions either with a primary enteric anastomosis or with an additional Roux limb. The portal-enteric technique has spawned a number of newer and revisited techniques of enteric exocrine drainage including duodenal or gastric diversion. Reports in the literature suggest no differences in pancreas transplant outcomes irrespective of type of either venous or exocrine diversion. The purpose of this review is to examine the

literature on exocrine drainage in the new millennium (the purported “enteric drainage” era) with special attention to technical variations and nuances in vascularized pancreas transplantation that have been proposed and studied in this time period.

Key words: Pancreas transplantation; Portal-enteric drainage; Simultaneous pancreas-kidney transplant; Systemic-bladder drainage; Enteric conversion; Solitary pancreas transplant; Systemic-enteric drainage

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Core tip: The history of vascularized pancreas transplantation largely parallels advances in surgical techniques. Prior to 1995, most pancreas transplants were performed with delivery of insulin to the iliac vein and diversion of the pancreatic ductal secretions to the urinary bladder (systemic-bladder technique). Starting in 1995, however, a seismic change occurred from bladder to bowel drainage of the pancreatic secretions that was spurred in part by the success of enteric conversion. In the new millennium, most pancreas transplants are performed as pancreatico-duodenal grafts with either iliac vein and bowel exocrine diversion (systemic-enteric technique) or portal-enteric drainage. With refinements in surgical techniques, exocrine drainage is no longer considered the “Achilles’ heel” of pancreas transplantation.

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INTRODUCTION

Since the inception of the International Pancreas Transplant Registry (IPTR) in 1984, data on > 48000 pancreas transplants has been captured in the ensuing 30 years^[1]. There exist 3 major types of vascularized pancreas transplantation; simultaneous pancreas-kidney (SPK), sequential pancreas after kidney (PAK), and pancreas transplantation alone (PTA). Solitary pancreas transplants refer to the PAK and PTA types. They are usually analyzed together because of similar outcomes coupled with the fact that these procedures are performed in the absence of uremia. However, the state of kidney function is quite different; post-uremic in PAK compared to non-uremic in PTA. In the past 3 decades, the results of SPK transplantation have been superior to solitary pancreas transplantation although the disparity in outcomes has decreased over time. In the United States, solitary pancreas transplants (PAK-17%, PTA-9%) represent the minority of activity while 74% are characterized as SPK transplants^[1-3].

In uremic patients with type 1 diabetes mellitus, SPK transplantation is a highly regarded treatment alternative because it addresses both kidney failure and diabetes^[3]. The number of United States annual pancreas transplants reached a high of 1484 in 2004 and had dropped to < 1000 by 2014^[1-3]. The number of annual pancreas transplants reported to the Eurotransplant Network has similarly declined in the past decade whereas annual activity in the United Kingdom has remained relatively stable and activity elsewhere in the world has increased^[1-3]. In spite of declining numbers, outcomes have continued to improve and include higher risk groups such as African-Americans, patients with a phenotype suggesting “type 2 diabetes” and solitary pancreas transplant recipients^[1-5]. Five year patient survival rates are now nearly 90% across all three transplant types and 10-year patient survival is > 70% in all three groups. Moreover, insulin independence is sustained at 5 years in 73% of SPK, 64% of PAK, and 53% of PTA recipients. The pancreas graft half-life is currently 10-15 years, which is amongst the lengthiest for extra-renal transplants^[2].

Evolution in surgical techniques has characterized and paralleled the growth and development of pancreas transplantation. In late 1966 at the University of Minnesota, Kelly *et al*^[6] reported the first human pancreas transplant. The initial case was an SPK transplant with a segmental pancreas graft implanted in the iliac fossa with ligation of the pancreatic duct. In the ensuing 13 cases performed between 1966 and 1973, however, Lillehei *et al*^[7] transplanted a pancreatico-duodenal graft with either an external ostomy/cutaneous fistula or connection between the recipient bowel and graft duodenum for exocrine drainage. Consequently, optimal management of the pancreatic ductal secretions was identified as a controversy very early in the development of pancreas transplantation. In the late 1970s and early 1980s, partial or segmental pancreatic grafts (based on the body and tail of the pancreas) with pancreatic ductal ligation or occlusion were the preferred methods of controlling the pancreatic secretions^[8,9]. During this developmental phase, exocrine drainage techniques were considered to be the “Achilles’ heel” of pancreas transplantation. The introduction of bladder diversion of the exocrine secretions into clinical transplantation in the mid-1980s revolutionized the safety and improved the success of pancreas transplantation^[10]. From this point in time onward, whole organ pancreaticoduodenal largely replaced segmental pancreas grafts as the preferred method of transplantation. However, segmental pancreas grafts remain the only surgical option in pancreas transplantation from living donors^[9,11]. From 1988 to 1995, > 90% of pancreas transplants in the United States were whole organ pancreatic grafts with iliac vein and bladder exocrine diversion (systemic-bladder technique), usually using a trimmed segment of donor duodenum inclusive of the ampulla of Vater as a channel for drainage of the exocrine pancreas^[12].

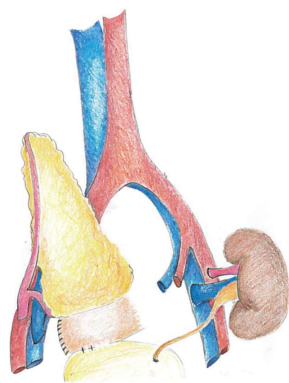


Figure 1 Technique of systemic-bladder drainage with creation of an anastomosis between the allograft duodenal segment and vesical dome of the recipient bladder.

To this day, there remains controversy regarding the optimal method for managing the pancreatic exocrine secretions. By review of data provided by the IPTR, it is evident that the overwhelming majority of pancreas transplants involve whole organ pancreatico-duodenal grafts with either bowel (systemic-enteric) or bladder diversion of the pancreatic ductal secretions coupled with systemic venous delivery of insulin^[1,2]. However, starting in 1995, a seismic change from bladder to bowel exocrine diversion transpired coincident with improvements in immunosuppression, preservation techniques, diagnostic monitoring, general medical care, and the success and frequency of enteric conversion^[13,14]. Enteric drainage usually refers to jejunal or ileal diversion of the exocrine secretions either as a direct anastomosis or in the presence of a defunctionalized Roux en y limb. By 1998, > 50% of SPK transplants were accomplished with bowel diversion and by 2003 this figure had risen to > 80% of cases in the United States although the systemic-bladder technique was still deployed in 50% of solitary pancreas transplants^[13,15]. At present, pancreas transplantation with primary enteric exocrine drainage is performed in 90% of cases in the United States from 2010-2014 although the systemic-bladder technique is a reasonable alternative in selected cases and a preferred option at specific centers^[1]. Roux limb diversion is performed in a minority of cases including 21% of SPK and 15% of solitary pancreas transplants^[1].

To mimic the natural physiology of the endocrine pancreas, an innovative method of portal vein delivery of insulin (by anastomosing the donor portal vein to the recipient superior mesenteric vein for venous outflow) and bowel diversion of the exocrine secretions (portal-enteric technique) was pioneered in the early 1990s and refined over the past ≥ 20 years^[16,17]. At present, the proportions of enteric-drained cases with portal venous delivery of insulin are 22% in SPK, 11% in PAK, and 13% in PTA cases. Consequently, > 80% of bowel drained pancreas transplants in the United States are performed without a decompressing Roux limb of small bowel and with systemic (iliac or vena cava)

venous delivery of insulin^[1]. Although the promise of the portal-enteric technique has not been achieved, it has spawned a number of newer and revisited techniques of enteric exocrine drainage including duodenal or gastric diversion^[18-32]. Previous reports have not shown any main variances in outcomes for bladder- or enteric-diverted pancreas transplants regardless of method of venous drainage^[33-55]. Although one of the three described techniques is deployed in nearly all pancreas transplants at present, the prevailing viewpoint is that the most appropriate procedure to be used is best determined both by recipient and donor anatomy as well as the practicing surgeon's comfort level and experience. A number of previous excellent reviews have emphasized technical aspects of pancreas transplantation but few have been published in the past 6 years^[52,56-64]. The purpose of this review is to examine the prevailing literature on exocrine drainage in the past 20 years (the purported "enteric drainage" era) with special attention to surgical techniques that have been introduced over time and with experience in pancreas transplantation.

Bladder drainage of the exocrine secretions (systemic-bladder technique)

Following the groundbreaking studies of Sollinger *et al*^[65] and Nghiem *et al*^[66] in the 1980s, bladder drainage with a donor duodenal segment became the preferred method of handling the pancreatic ductal secretions in pancreas transplantation until the mid- to late-1990s (Table 1)^[67-74]. With this technique, the donor duodenum functions as an exocrine conduit and is anastomosed to the vesical dome either using a 2-layer hand sewn technique or a circular stapled anastomosis^[75] (Figure 1). Bladder diversion gained wide acceptance owing to its safety, sterility, convenience, and ease of performance. In addition, bladder drainage enabled direct monitoring of the pancreatic secretions in the urine, permitted a direct approach for trans-cystoscopic biopsy of either the allograft duodenum or pancreatic parenchyma, and provided easy diagnosis and management of anastomotic problems with cystography and urethral catheter drainage^[76]. Similar to the use of low pressure cystography to diagnose urine leaks following kidney transplantation, cystography facilitated the detection of anastomotic or duodenal segment leaks following pancreas transplantation with bladder drainage. Prolonged urethral catheter drainage in effect decompressed the anastomosis and enabled control of the exocrine leakage while promoting healing.

Bladder diversion of the pancreatic ductal secretions avoided the inherent bacterial contamination (e.g., peritonitis) that occurred with bowel diversion leaks, contamination that lead to substantial morbidity and even mortality^[77]. Consequently, it was associated with a lower risk of intra-abdominal infections and sepsis (because of the sterility of the lower urinary tract) compared to previous techniques of either segmental or whole organ pancreas transplantation with enteric

Table 1 Bladder drainage: Literature review

Center, authors, year, ref., study design, and follow-up	Number and type of transplant	Complications	Enteric conversion	1 yr patient survival	1 yr pancreas graft survival
University of Minnesota, Hakim <i>et al</i> ^[67] , Retrospective, mean follow-up 55 mo	<i>n</i> = 425 with bladder drainage, SPK - 53%; PAK - 23%; PTA - 24%	Duodenal stump complications - 20%; Duodenal leak - 10%; Recurrent UTI - 9%; Hematuria - 6% (19% required surgery); Bladder stone - 0.5%; CMV duodenitis - 1.5%; Graft loss - 9%	16%	ND	ND
University of Nebraska, Stratta <i>et al</i> ^[68] , Retrospective, mean follow-up 44 mo	<i>n</i> = 201 with bladder drainage	Duodenal stump complications - 19%; Duodenal leak - 6% (all required surgery); Hematuria - 13% (30% required surgery); CMV duodenitis - 3%	13%	94%	80%
University of Wisconsin, Sollinger <i>et al</i> ^[69] , Retrospective	<i>n</i> = 500; 338 with bladder drainage, 112 with enteric drainage	Duodenal leak - 15.4%; Graft Thrombosis - 0.7%; Hematuria - 3%; UTI - 52.5%; Graft loss - 13%; Death with a functioning graft - 8%	24%	96.4%	87.5%
The Ohio State University, Henry <i>et al</i> ^[70] , Retrospective, mean follow-up 16 mo	<i>n</i> = 300 with bladder drainage	CMV - 2%; Intra-abdominal infection - 15%; Wound infection - 8%; Rejection - 55%; Hematuria - 14%; Bladder leak - 10%	4%	92%	82%
University of Maryland, Del Pizzo <i>et al</i> ^[71] , Retrospective, mean follow-up 35 mo	<i>n</i> = 140; SPK - 68%, PAK - 25%, PTA - 7%	Urological complication - 50%; Bladder stone - 10%; Duodenitis - 11%; Retained foreign bodies - 12%; Bladder tumor - 2%	21%	ND	ND
Mayo Clinic Rochester, Gettman <i>et al</i> ^[72] , Retrospective, mean follow-up 44 mo	<i>n</i> = 65	UTI - 59%; Hematuria - 26%; Allograft pancreatitis - 19%; Duodenal leaks 17%, (all required surgery); Ureteral lesions - 9%	ND	92%	86%
Hospital Universitario Spain, Medina Polo <i>et al</i> ^[73] , Retrospective, mean follow-up 52 mo	<i>n</i> = 107, all SPK, bladder drainage in 58, enteric drainage in 49	UTI - 72%; Hematuria - 20%; Bladder stone - 8%; Reflux pancreatitis - 48%	10%	92.7%	78.1%
University of Nebraska, Sudan <i>et al</i> ^[74] , Retrospective, mean follow-up 60 mo	<i>n</i> = 57, all with bladder drainage	UTI - 15%; Dehydration - 20%; Rejection - 1%	ND	95%	88%

SPK: Simultaneous pancreas-kidney; PAK: Pancreas after kidney; PTA: Pancreas transplantation alone; UTI: Urinary tract infection; CMV: Cytomegalovirus; ND: Not determined/no data.

diversion. In addition, bladder drainage also provided a means to monitor for pancreas allograft rejection by measuring urinary parameters such as amylase, insulin or cytology^[78]. However, bladder diversion created an abnormal linkage between the allograft pancreas with intervening donor duodenal conduit and the urinary bladder, which resulted in a number of unique metabolic, urologic, infectious, and miscellaneous complications. Disadvantages and advantages of bladder diversion are specified in Table 2.

With bladder drainage, anastomotic bleeding could be easily diagnosed by the presence of hematuria and usually managed non-operatively with urethral catheter drainage, alkalization of the urine, administration of blood products, and correction of coagulation parameters. In refractory or persistent cases of hematuria secondary

to anastomotic bleeding, however, administration of octreotide, bladder clot removal by cystoscopy with direct fulguration of bleeding sites, or enteric conversion might be indicated. Rates of hematuria are noted in Table 3.

In addition, bladder drainage resulted in obligatory fluid (up to 1-2 L/d of pancreatic exocrine secretions) losses and urinary bicarbonate wasting with consequent changes in the acid-base balance and enzyme-free environment of the lower genitourinary tract. Many patients were prone to dehydration, metabolic acidosis, erythrocytosis, and orthostasis, particularly in the setting of severe autonomic neuropathy secondary to diabetes. For these reasons, the length of donor duodenum transplanted with the pancreas was progressively shortened over time in an attempt to minimize protein

Table 2 Advantages and disadvantages of bladder drainage of the exocrine secretions**Advantages****Safety**

Reduced infection rate because of relative sterility of lower urinary tract

Control of anastomosis by urethral catheter decompression

Technical considerations

Relative simplicity because of favorable anatomic location of bladder

Bladder mobilization permits tension-free, multi-layer anastomosis

Bladder vasculature and urothelium promote healing

Direct access to exocrine secretions for monitoring pancreas allograft function

Detection of rejection by urinary parameters (amylase, lipase, insulin, cytology)

Cystoscopic access for either duodenal or pancreatic parenchymal biopsy

Disadvantages**Urologic problems**

Hematuria, dysuria, cystitis, urethritis, urethral stricture or disruption, balanitis

Increased risk of lower urinary tract infections, stone formation, and urine leaks (either from bladder or duodenum)

Metabolic and volume problems

Dehydration, orthostasis, constipation, erythrocytosis

Metabolic acidosis

Miscellaneous problems

Reflux-associated hyperamylasemia or pancreatitis

Transitional cell (urothelial) dysplasia

Need for enteric conversion for refractory, persistent, or recurrent problems

Medication burden (massive amounts of bicarbonate supplementation)

**Figure 2** Technique of conversion from bladder to enteric exocrine drainage (enteric conversion) for persistent metabolic, urologic, or other problems.

Most patients required daily oral sodium bicarbonate supplementation and some received chronic suppressive antibiotics to limit the morbidity attributable to the abnormal physiology. Alternative treatments to reduce exocrine drainage side effects included the use of oral pancreatic enzymes or long-acting somatostatin analogues. Other late complications comprised duodenal leaks, stone formation, and the risk of urothelial dysplasia.

At present, bladder drainage remains an important option in selected cases, such as those in which pancreas graft quality in general or viability of the allograft duodenum in particular is suspect. In cases of duodenal ischemia or severe reperfusion injury, the bladder anastomosis can be performed by invaginating the duodenum into the bladder in order to minimize leaks (Figure 1). In addition, if the recipient has severe adhesions from multiple previous intra-abdominal procedures or sclerosing peritonitis, then a bowel anastomosis may be risky. Moreover, until recently, bladder drainage was preferred by many centers in solitary pancreas transplantation (PAK, PTA) because of the increased incidence of acute rejection (early and late) in this setting coupled with the established difficulty in the timely detection of pancreas rejection in the absence of either a urinary marker (with bladder drainage) or serum creatinine monitoring (with an SPK transplant).

A number of centers have reported excellent long-term outcomes in pancreas transplantation with the systemic-bladder technique^[9,52,69,70,74,80,89]. For a period of time, the bladder drainage technique was also associated with lower incidences of thrombosis, early technical complications, and graft loss in IPTR reports compared to enteric drainage^[12,13,15]. Consequently, many new centers (including those in developing countries) elected to embark on their experience in pancreas transplantation with systemic-bladder drainage owing to its technical simplicity and purported lower technical complication rate. In some instances, centers have adopted a 2-stage approach in which primary bladder diversion is followed by planned enteric conversion in order to avoid the immediate complications of primary enteric diversion

and bicarbonate loss from the allograft duodenal mucosa. In some patients, intractable, recurrent, or refractory complications would occur, which were then treated with open conversion from bladder to bowel diversion (enteric conversion) (Figure 2). Paradoxically, the success of "enteric conversion" paved the way for renewed enthusiasm in primary enteric drainage. Enteric conversion frequency ranged from 10% to 40% (Table 3)^[79-86]. Several authors reported that enteric conversion resulted in superb long-term graft function coupled with marked symptom improvement even when performed several years following SPK transplant^[84,87,88]. Despite urological morbidity and the finite risk of enteric conversion, 5-year actuarial patient and graft survival rates with bladder drainage were excellent and most complications could be managed with conservative (non-operative) therapy.

For diabetic patients with neurogenic bladders, episodes of reflux pancreatitis (managed with urethral catheter drainage) and recurrent urinary tract infections were not uncommon. In the setting of urinary tract infection, the pH of urine would become more acidic, which led to pancreatic enzyme activation and a variety of complications including hematuria, duodenitis, cystitis, urethritis, urethral stricture or disruption, and balanitis. In severe cases, some investigators even reported reduction cystoplasty and bladder re-anastomosis in an attempt to control persistent urologic problems.

Table 3 Enteric conversion: Literature review

Center, authors, year, ref., and study design	Overall rate (%)	Urologic indications # (%)	Metabolic indications # (%)	Pancreatitis/other indications # (%)	Operative complications # (%)
University of Wisconsin, Van der Werf <i>et al</i> ^[79] , Retrospective	95/449 (21%)	90 (95)	1 (1)	4 (4)	21 (22)
Sollinger <i>et al</i> ^[80] , Retrospective	160/390 (41%)	93 (58)	1 (0.6)	47 (29)	ND
University of Minnesota, West <i>et al</i> ^[81] , Retrospective	79/500 (16%)	43 (54)	26 (33)	15 (19)	12 (15)
University of Nebraska, Sindhi <i>et al</i> ^[82] , Retrospective	25/195 (13%)	7 (28)	18 (72)	0	3 (12)
University of Barcelona, Spain, Fernandez-Cruz <i>et al</i> ^[83] , Retrospective	16/74 (22%)	0	0	16 (100)	Death 1 (6); Wound infection 2 (12); Anastomotic leak 3 (18)
Leiden University Medical Center, Netherlands, van de Linde <i>et al</i> ^[84] , Retrospective	51/ND	39 (76)	23 (45)	Pancreatitis 2 (3); Fistula 1 (1)	UTI 7 (13); Minor bleeding 1 (0.5); Phlebitis 1 (0.5); Paralytic ileus 1 (0.5); Relaparotomy 2 (3)
University of Cincinnati, Kaplan <i>et al</i> ^[85] , Retrospective	26 (32%)	13 (50)	13 (50)	0	Death 1 (3); Anastomotic bleeding 1 (3)
Beaumont Hospital, Ireland, Connolly <i>et al</i> ^[86] , Retrospective	6/ND	3 (50); 2 hematuria; 1 UTI	3 (50)	ND	Pulmonary edema 1 (16)

UTI: Urinary tract infection; ND: Not determined/no data.

(intra-abdominal infections, early graft loss) and the long-term metabolic and urologic problems related to bladder diversion^[84,87]. For example, Marang-van de Mheen *et al*^[87] routinely used a two-step approach in SPK transplant; primary bladder diversion followed by planned enteric conversion (Figure 2). They found that this approach resulted in urological complication rates similar to bowel-drained grafts with subsequent excellent survival rates. Conversions were performed by separating the graft duodeno-cystostomy, then re-establishing continuity and diversion by a side-to-side recipient jejunal-graft duodenal-anastomosis either without (most commonly) or with a diverting Roux limb.

The drawback to planned conversion is loss of urinary amylase as an immunological biomarker, especially in PAK and PTA recipients. In SPK transplant recipients, however, the renal allograft and serum creatinine can still be monitored as a biomarker for allograft rejection. Contrary to previous IPTR reports, however, there is no longer a survival, technical complication, or immunological monitoring advantage associated with bladder drainage, so the practice of "intentional" enteric conversion has been largely supplanted by primary bowel diversion^[1-3].

Bowel diversion of the pancreatic ductal secretions (systemic-enteric technique)

Initial attempts at bowel exocrine diversion in the 1970-80s were fraught with complications including intra-abdominal sepsis and mortality because of limitations in preservation techniques, immunosuppression, diagnostic monitoring, and general medical care. However, the introduction of University of Wisconsin solution (that was initially developed as a pancreas preservation solution), tacrolimus, mycophenolate mofetil, ganciclovir, newer

monoclonal and polyclonal antibody agents, biopsy-directed surveillance, and improvements in general medical and critical care (including higher resolution computerized tomographic scanning, more effective antibiotics, and the development of safe and more sophisticated percutaneous interventions) were pivotal in the re-emergence of primary bowel drainage as an alternative to bladder drainage. During the transitional phase from primary bladder to enteric drainage in the late 1990s to early 2000s, several studies (both prospective and retrospective) reported comparable outcomes with either technique although primary enteric drainage was not associated with the requisite long-term metabolic and urologic complications unique to bladder drainage (Table 4)^[90]. In addition, the success of enteric conversion corroborated the safety and feasibility of primary enteric drainage following pancreas transplantation, which in essence eliminated the need for re-operation in 10%-40% patients with urinary bladder diversion. Moreover, bowel diversion of the pancreatic ductal secretions was much more acceptable to the medical community at large because it was more "physiologic" and logical to drain the pancreatoduodenal secretions into the small bowel. Disadvantages and advantages of primary bowel diversion are noted in Table 5.

Potential risk variables for early bowel leaks include poor characteristics of the allograft duodenum (related to donor hemodynamic instability or trauma), ischemia-reperfusion and preservation injury (related to preservation solution as well as warm and cold ischemia), complications with either the vascular or bowel anastomosis because of adhesions or other technical issues, higher donor or recipient age or body mass index, peritoneal dialysis, and deconditioning in the recipient. In

Table 4 Bladder *vs* enteric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complication/enteric conversion	Acute rejection/graft loss	Reoperation and readmissions	1 yr patient survival	1 yr pancreas (and kidney) graft survival
University of Maryland, Kuo <i>et al</i> ^[35] , Retrospective	23 SPK ED	ED: Fewer UTIs and urologic complications	ND	ND	ED 100%; BD 96%	ED 88%; BD 91%
University of Chicago, Newell <i>et al</i> ^[33] , Retrospective	SPK; ED 12; BD 12	Acidosis and dehydration less with ED ($P < 0.005$); Hematuria; BD 25%; ED 0%; No anastomotic leaks in either group; No intra-abdominal infection in either group; Enteric conversion: 33%	ND	BD: 4 patients underwent enteric conversion	BD 100%; ED 83.3%	BD 91.7%; ED 83.3%
University of Wisconsin, Sollinger <i>et al</i> ^[60] , Retrospective	1000 SPK; BD 390; ED 610	Pancreas graft thrombosis; BD 2.3% ED 3.6%; Infection; BD 1.8% ED 0.8%; Pancreatitis; BD 1.3% ED 0.5%; Pancreatic leak BD: 12% ED: 5% ($P = 0.06$)	Kidney rejection; BD 29%; ED 19%; Pancreas rejection; BD 12.1%; ED 5.4%	ND	Similar in both groups	Similar kidney, and pancreas graft survival in both groups
Pirsch <i>et al</i> ^[37] , Retrospective	48 BD; 78 ED	Opportunistic infections; ED: 12% BD: 31% ($P = 0.002$); CMV; BD 21% ED 4% ($P = 0.04$); Fungal infection; BD 17% ED 4%; UTI BD 63% ED 20% ($P = 0.0001$)	Kidney rejection; BD 38%; ED 30%; Steroid-resistant rejection; BD 19%; ED 17%			
University of Washington, Friedrich <i>et al</i> ^[90] , Retrospective	34; ED 17; BD 17	ED 41%; BD 53%; Enteric conversion: 5%	ED 29%; BD 24%	Readmissions: ED 41%; BD 47%	ND	ND
University of Tennessee-Memphis, Stratta <i>et al</i> ^[41] , Prospective	BD 16; ED 16	UTI BD 50% ED 19%; Urologic complications; BD 25% ED 12.5%; Dehydration BD 100% ED 44%	BD 44%; ED 31% $P = NS$	BD 25%; ED 25%; Readmissions: BD 2.6 ± 1.8 ; ED 1.75 ± 1.2	BD 88%; ED 94%	Kidney survival; BD 92%; ED 93%; Pancreas survival BD 81%; ED 88%
Albert Einstein Medical Center, Bloom <i>et al</i> ^[34] , Retrospective	71 SPK; BD 37; ED 34	Dehydration BD 34% ED 3.4%; Acidosis BD 41% ED 0% Pancreatitis BD 40% ED 3.4% UTI BD 71% ED 27% ($P < 0.005$) Enteric conversion: 19%	BD: 13.5%; ED: 14.7%		Similar between groups	Pancreas allograft survival was similar between groups
Emory University, Pearson <i>et al</i> ^[36] , Retrospective	SPK; BD 55; ED 11	BD; UTI 78%; Hematuria 27%; Dehydration 38%; ED no complication				
University of Pittsburgh Corry <i>et al</i> ^[43] , Retrospective	BD 44; ED 199	Overall BD 41% ED 26%; Anastomotic bleeding; BD 16% ED 5%; Fistula BD 14% ED 6%		BD 24%; ED 16%		BD 44%; ED 69%
Toronto General Hospital, Catral <i>et al</i> ^[40] , Retrospective	SPK; BD 20; ED 20	UTI: Similar in both groups; CMV infections were significantly less in the ED group	BD 37%; ED 15%; ($P = 0.20$)	BD 1 patient to ligate an arteriovenous fistula in the pancreas graft; ED 4 patients; (bleeding in one, partial wound dehiscence in one, negative laparotomy in two)	BD 95%; ED 100%	Kidney graft survival; BD 95%; ED 100%; Pancreas graft survival; BD 95%; ED 100%

Wake Forest University, Stratta <i>et al</i> ^[46] , Retrospective	297 SPK; SE 171 (58%); PE 96 (32%); SB; 30 (10%)	No differences were seen in surgical complications including pancreas thrombosis; Infections: SE 49%; PE 85%; BD 63%	SE 19%; PE 26%; BD 30%	Readmissions: SE 61%; PE 63.5%; BD 63%	SE 97%; PE 99%; BD 97%	Kidney; SE 94%; PE 98%; BD 93%; Pancreas; SE 87%; PE 92%; BD 87%
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BD: Bladder drainage; ED: Enteric drainage; SB: Systemic-bladder; SE: Systemic-enteric; PE: Portal-enteric; UTI: Urinary tract infection; CMV: Cytomegalovirus; ND: Not determined/no data.

Table 5 Advantages and disadvantages of enteric drainage of the exocrine secretions

Advantages

Safety

Lower rates of urinary tract infections and urologic complications
More “physiologic”; fewer metabolic and volume problems
Fewer readmissions

Technical considerations

Treats exocrine insufficiency (in patients following total pancreatectomy or in patients with cystic fibrosis)
Avoidance of need for enteric conversion; lower relaparotomy rate
Can be used with either systemic or portal venous outflow

Disadvantages

Safety

Higher incidence of leakage of pancreatic enzymes, pancreatitis, peri-pancreatic fluid collections
Higher incidence of intra-abdominal abscess, peritonitis, sepsis
Anastomotic leaks, GI bleeding
Increased risk of wound infections, wound healing problems (contaminated case with GI tract breach)

Technical considerations

Selective need for enterolysis or diverting Roux en y limb
Loss of direct access to anastomosis and allograft for diagnosis and treatment

Miscellaneous problems

Inability to directly monitor exocrine secretions

GI: Gastrointestinal.

addition, late intra-peritoneal infectious complications may occur in bowel-drained transplants^[91-93]. In more recent series, however, the incidence of and outcomes associated with surgical complications following enteric diversion are similar to those following bladder drainage and the rates of early graft loss with either technique are comparable^[1-3,52,62-64]. The incidence of surgical complications is also similar by type of transplant (SPK compared to solitary pancreas transplantation)^[1-3]. Leaks from the allograft duodenum have been reported to occur in 5%-20% of bladder-drained and 5%-8% of bowel-drained pancreas transplants^[9,33-52,67-73,80,91-95]. Increasing experience with enteric exocrine drainage is likewise associated with a decreased rate of technical complications^[9,38,80,96-103].

Because of lingering concerns regarding the safety of enteric drainage based on historical precedent, the use of diverting Roux limbs was not uncommon in the late 1990s and many centers continued to direct the head and duodenum of the pancreas allograft toward the pelvis just in case “bladder conversion” was required.

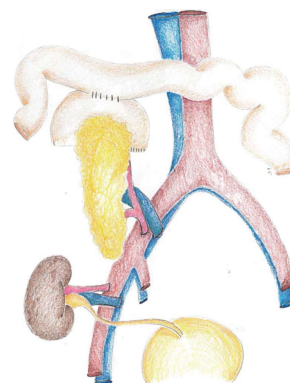


Figure 3 Technique of systemic-enteric drainage with side-to-side anastomosis between allograft duodenum and recipient small bowel.

Techniques that incorporated diverting Roux limbs with temporary external ostomies were also described in an attempt to permit direct endoscopic access and provide decompression of the enteric anastomosis and allograft duodenum^[23]. However, with time and experience, most pancreas transplant surgeons evolved to directing the head and duodenum of the pancreas allograft away from the pelvis to simplify the enteric anastomosis, which was typically performed side-to-side between the allograft duodenum and either the recipient proximal jejunum or ileum without a Roux limb (Table 6)^[104-108] (Figure 3). Safe techniques of using either the circular or linear stapler were described to simplify the enteric anastomosis^[109,110]. If a Meckel's diverticulum was identified, some surgeons would excise the diverticulum and then use this site for the bowel anastomosis^[111]. Placement ipsilateral of the kidney and pancreas allografts in SPK transplantation was also introduced to limit the dissection and expedite the procedure^[106]. A potential side benefit of enteric drainage was elimination of the need to construct a duodenal segment, which meant less dissection during back bench preparation, less risk of devascularizing the head of the pancreas or duodenum by collateral disruption, and less time spent with the pancreas *ex vivo* and exposed. By transplanting the pancreas as a complete pancreatico-duodenal graft, collateral circulation to the pancreas and duodenum was preserved. Maintaining full duodenal length also facilitated numerous possibilities for performing the bowel anastomosis in the recipient. In addition, the distal donor duodenum could be used as access for stapler

Table 6 Systemic-enteric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complications	Readmission/reoperation/length of stay	1 yr patient survival	1 yr kidney/pancreas survival
Medical University of South Carolina, Douzjian <i>et al</i> ^[105] , Retrospective	ED 16; BD 26	Recurrent/persistent urinary complications BD 46% ED 6% ($P = 0.01$); Dehydration BD 27% ED 6% ($P = 0.05$); Pancreatitis BD 8% ED 6% ($P = NS$); Wound infection BD 12% ED 19% ($P = 0.5$)	Readmissions BD: 1.7 ± 1.5 ; ED 1.2 ± 1.2 d ($P = 0.2$) Reoperations BD 23% ED 0 ($P = 0.04$); Length of stay BD: 12.9 ± 5.6 ED: 20.4 ± 9.6 d, $P = 0.007$	BD 96%; ED 94%; $P = 0.6$	Kidney BD 85%; ED 87%; Pancreas BD 90%; ED 85% ($P = 0.6$)
Institut de Malalties Digestives, Spain, Heredia <i>et al</i> ^[94] , Retrospective	205 SPK; ED 97	Duodenal leaks: ($n = 11$); Acute rejection ($n = 6$); CMV infection ($n = 3$); Technical failure ($n = 2$); Death: ($n = 2$) as a consequence of sepsis	Reoperation for duodenal leak: Roux-en-Y technique: ($n = 3$) DJ technique: ($n = 2$) Transplantectomy: ($n = 6$)	ND	ND
Toronto General Hospital, Spetzler <i>et al</i> ^[95] , Retrospective	Total 284; 191 SPK (67.3%); 93 PAK (32.7%)	Duodenal leak (incidence 6.3%), 12 (67%) occurred within the first 100 d after transplantation	Six grafts (33%) were rescued by duodenal segment resection;	ND	ND
Innsbruck University Hospital, Austria, Steurer <i>et al</i> ^[92] , Retrospective	40 ED	Intra-abdominal infection - 11 (27.5%)	Reoperation for intra-abdominal infection Pancreatectomy: 5 Necrosectomy and drainage: 5 Percutaneous drainage: 1	ND	ND
Ruhr-University Bochum, Germany, Ziaja <i>et al</i> ^[104] , Retrospective	30 SPK	Perioperative mortality 3.3%	Early relaparotomy was required in 20%; pancreatectomy in 10%	ND	ND
Indiana University, Fridell <i>et al</i> ^[106] , Retrospective	49; SPK; All ED	Death: ($n = 2$) (1 patient died from multi-system organ failure and a second from graft <i>vs</i> host disease); Pancreatic graft failures: (2); renal graft failure: (1)	Relaparotomies: ($n = 5$) bowel obstructions: (2) anastomotic leak: (1) ureteral stricture: (1)	96%	Kidney 94%; Pancreas
University of Pittsburgh, Corry <i>et al</i> ^[107] , Retrospective	104 SPK	Graft loss in 6 patients, Death in one patient	Splenic artery hemorrhage: (1) ND	98%	92%; Kidney 95%, Pancreas 83%
University of Maryland, Bartlett <i>et al</i> ^[108] , Prospective	27; Solitary pancreas transplants	One graft lost to acute rejection in the tacrolimus group because of patient noncompliance	ND	ND	90% in patients receiving tacrolimus, 53% in patients receiving cyclosporine ($P = 0.002$)

BD: Bladder drainage; ED: Enteric drainage; CMV: Cytomegalovirus; ND: Not determined/no data; DJ: Duodeno-jejunostomy.

placement to perform the enteric anastomosis^[109,110].

Bowel drainage of the pancreatic ductal secretions (portal-enteric technique)

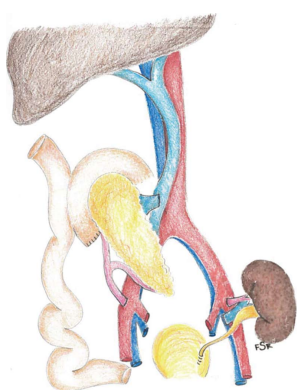
To address the unusual anatomy of pancreas transplantation, Gaber *et al*^[16] introduced a new technique in which an anterior intraperitoneal approach to the recipient superior mesenteric vein (SMV) was deployed for venous drainage. This procedure was later modified to a "retroperitoneal" approach to the SMV by Boggi's group in Pisa. Both of these techniques combined bowel drainage of the pancreatic ductal secretions with portal venous delivery of insulin (portal-enteric technique)^[16,17,112,113]. Alternative methods to achieve portal venous delivery of insulin have been reported using either the recipient portal vein directly, the inferior mesenteric vein, or splenic vein. However, in most cases, "portal venous" drainage usually infers that the

allograft has a vertical orientation with the body and tail directed towards the pelvis, the head and duodenum directed cephalad, and the recipient SMV as the site for the venous anastomosis^[18-22] (Figure 4). The bowel anastomosis is most commonly performed to a bowel loop that is not excluded from the transit of intestinal contents^[4,16,17,33,39-42,44-46,49-53,112-121]. Alternatively, the allograft duodenum can be connected directly into the native stomach or duodenum, to a diverting Roux limb without or with a venting jejunostomy, or to an omega loop^[23-32,122] (Table 7). Utilizing the native stomach or duodenum affords straightforward access to the allograft duodenum and pancreas for biopsy and surveillance by endoscopic techniques and also expands the possibilities for exocrine drainage sites, particularly in cases of pancreas retransplantation (Table 8)^[25-32,123]. However, because up to 5%-10% of transplanted pancreata are at risk for early technical failure that may lead to leaks,

Table 7 Portal-enteric drainage: Literature review

Center, authors, year, ref., study design and follow-up	Number and type of transplant	Complications	Readmissions, reoperation, length of stay	1 yr patient survival	1 yr kidney and pancreas graft survival
University of Tennessee, Stratta <i>et al</i> ^[122] , Retrospective, mean follow-up 3 yr	PE 126; 90 SPK; 18 PAK; 18 PTA; Era 1 (10/90-6/95); Era 2 (7/95-5/98); Era 3 (6/98-12/99)	In 3 successive eras, rates of acute rejection were 63%, 33%, and 39%, respectively; rates of major infection were 60%, 43%, and 44%, respectively	In 3 successive eras, rates of relaparotomy were 47%, 31%, and 33%, respectively; rates of thrombosis were 20%, 7%, and 6%, respectively. Mean length of stay: 12.5 d	In 3 successive eras, patient survival was 77%, 93%, and 100%, respectively	In 3 successive eras, kidney graft survival was 77%, 93%, and 94%, respectively; pancreas graft survival was 60%, 83%, and 83%, respectively
Università di Pisa, Italy, Boggi <i>et al</i> ^[17] , Retrospective, mean follow-up 21 ± 20 mo	PE 110	10 grafts were lost; 3 acute rejection, 2 chronic rejection, 2 venous thrombosis, 2 deaths, 1 late thrombosis (6 mo). Incidence of pancreas acute rejection was 6%	Relaparotomy rate was 13.6%; Mean length of stay was 26 ± 14 d; One month readmission rate was 13%	98%	Pancreas graft survival was 91%
University of Chicago, Bruce <i>et al</i> ^[116] , Retrospective, mean follow-up 16 mo	PE 70	Pancreas graft losses: Thrombosis (3), acute rejection (5), late duodenal perforation (2)	Total 1 st year hospitalization: 37 ± 28 d; Relaparotomy in 14 (70%)	88%	Kidney 78%; Pancreas 79%
Louisiana State University, Zibari <i>et al</i> ^[23] , Retrospective, mean follow-up 25 mo	PE 21	Postoperative Bleeding in 4, wound infections in 4, acute rejection in 9, pancreas graft loss in 2	Mean length of stay was 16 d	100%	Kidney 90%; Pancreas 90%
Wake Forest Baptist Medical Center, Rogers <i>et al</i> ^[4] , Retrospective, mean follow-up 6 ± 3 yr	202; SPK 162, PAK 35, PTA 5; PE 179; SE 23	Thrombosis rate was 8%; acute rejection rate was 28%; major infection rate was 50%	Mean length of stay was 13 d; Relaparotomy rate was 38%	Overall patient survival was 87%; one-year patient survival was 97%	Overall kidney and pancreas graft survival rates are 76% and 65%; death-censored graft survival rates are 84% and 72%, and one year graft survival rates are 94% and 88%, respectively
Monash Medical Centre, Victoria, Australia, Kave <i>et al</i> ^[118] , Retrospective, mean follow-up 2 yr	SB 37; PE 27	Pancreas graft thrombosis rates SB 10.8%, PE 7.4% (<i>P</i> = NS)		Two-year patient survival was SB 94.3% vs PE 96.0%	Two year kidney (SB 89.2% vs PE 85.2%); pancreas (SB 77.9% vs PE 71.4%)

SB: Systemic-bladder; SE: Systemic-enteric; PE: Portal-enteric.

**Figure 4** Technique of portal-enteric drainage with side-to-side anastomosis between allograft duodenum and small bowel; this technique is also amenable to using the native duodenum or stomach for exocrine diversion.

many centers are reluctant to perform enteric diversion either to the native stomach or duodenum. Following reperfusion of the transplanted pancreas, if the allograft duodenum does not appear well vascularized, bowel

drainage with creation of a diverting Roux limb may be preferred to bypass the enteric stream and promote healing even though this procedure mandates an additional bowel anastomosis.

Although the rate of bleeding at the may be higher, some surgeons prefer to use either a circular or linear stapling device to create the bowel anastomosis^[109,110]. However, most commonly, the connection between the allograft duodenum and recipient small bowel is performed using a 2-layer hand sewn technique that comprises a running continuous inner layer of interlocking absorbable suture coupled with an interrupted seromuscular outer layer of simple interrupted non-absorbable sutures to create a “watertight” and hemostatic closure^[121]. The bowel anastomosis can be located anywhere between the distal ileum and native stomach although most commonly is performed as a primary side-to-side connection to the proximal jejunum (Figure 4). Other methods of reconstruction may include either an end-to-side or end-to-end anastomosis between the allograft duodenum and recipient gastrointestinal tract. When using portal-enteric drain-

Table 8 Portal-duodenal/gastric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complications	Readmissions and reoperations	1 yr patient survival	1 yr pancreas survival
New York Medical College, Westchester Medical Center, Gunasekaran <i>et al</i> ^[28] , Retrospective	DJ: 36; DD: 21; stapled 14, hand-sewn 7	Thrombosis: None in DJ, 2 in DD ($P = NS$); Enteric leak and small-bowel obstruction: 3 in DJ, 2 in DD ($P = NS$); Gastrointestinal bleeding: None in DJ, 4 in DD ($P = 0.015$)	ND	94% with DJ, 95% with DD	89% with DJ, 86% with DD
Louisiana State University, Shokouh-Amiri <i>et al</i> ^[27] , Retrospective	Group 1: Allograft jejunum to stomach, $n = 30$; Group 2: Allograft duodenum to jejunum with Roux-en-Y venting jejunostomy, $n = 30$	In Group 1: Pancreatectomy in 3, CMV in 7, acute rejection in 4, death in 3; In Group 2: Pancreatectomy in 1, CMV in 2, acute rejection in 6, death in 2 (all $P = NS$)	Major complications: 4 in group 1, 10 in group 2	94% in group 1, 96% in group 2	85% in group 1, 83% in group 2
Bandeirantes Hospital, Sao Paulo, Brazil, Perosa <i>et al</i> ^[30] , Retrospective	43 PAK, 10 PTA with DD	Thrombosis in 5 (9%); 4 additional pancreas graft losses (including 2 deaths with functioning grafts); Acute rejection in 9 (17%); major infection in 24 (45%)	Readmissions: Mean 1.1; Mean length of hospital stay: 11.8 d; Reoperations in 9 (17%)	96%	83%
University Hospital Bochum, Germany, Walter <i>et al</i> ^[31] , Retrospective	DD in 125 (64% with portal outflow); DJ in 116 (12% with portal outflow)	GI bleeding in 14 with DD, 4 with DJ; Thrombosis in 5 with DD, 18 with DJ ($P = 0.002$); Acute rejection in 29% in DD vs 31% in DJ	2 anastomotic leaks with DD, 6 with DJ; Pancreatectomy in 14 with DD, 21 with DJ; Early relaparotomy in 42% DD vs 48% DJ, all $P = NS$	96% in both groups	82% with DD, 78% with DJ
Oslo University Hospital, Rikshospitalet, Norway, Horneland <i>et al</i> ^[32] , Retrospective	20 SPK, 17 PTA, 3 PAK with DD ($n = 40$); 30 SPK 7 PTA, 3 APK with DJ ($n = 40$); In sequential eras	Thrombosis in 13% DD vs 5% DJ; Acute rejection in 23% DD vs 28% DJ, both $P = NS$	Reoperations in 40% DD vs 30% DJ; Mean length of hospital stay 19 d DD vs 16 d DJ, both $P = NS$	97.5% DD vs 92.5% DJ	Overall pancreas survival was 80% with DD, 87.5% with DJ ($P = NS$)
Scientific-Research Institute of Sklifosovsky, Moscow, Russia, Khubutia <i>et al</i> ^[123] , retrospective	Group 1: 15 DJ; Group 2: 17 DD	Acute rejection in 13% DJ vs 12% DD; Major infections in 20% DJ vs 6% DD, both $P = NS$	Surgical complications in 20% DJ vs 23.5% DD, $P = NS$	93% DJ vs 94% DD	Pancreas survival 93% DJ vs 94% DD; kidney survival 93% DJ vs 88% DD

DD: Duodeno-duodenostomy; CMV: Cytomegalovirus; ND: Not determined/no data; DJ: Duodeno-jejunostomy; NS: Not significant.

age, the recipient ileum can be anastomosed to the distal graft duodenum whereas the recipient jejunum can be anastomosed to the proximal graft duodenum. We prefer the former technique with the location of the bowel anastomosis on the posterior aspect of the 3rd or 4th portion of the graft duodenum to promote dependent drainage of the atonic, denervated graft duodenum when the patient is either in the erect or supine position^[121]. Anastomotic length can be variable but usually ranges from 3-5 cm.

Unlike bladder drainage, however, anastomotic bleeding with enteric drainage is more occult and harder to diagnose in the absence of gastric, duodenal, or extreme proximal jejunal diversion or in the absence of a diverting jejunostomy. Because most enteric anastomoses are performed in the middle third of the gastrointestinal tract, endoscopic confirmation and treatment are not available. Consequently, the true incidence of anastomotic bleeding with enteric drainage is probably under-reported and the severity may be under-appreciated because of other causes of anemia in the immediate post-operative period. Fortunately, most cases are self-limited and

respond to supportive measures such as decompression of the gastrointestinal tract, administration of blood products, and correction of coagulation parameters. In cases of persistent and significant lower (or rarely upper) gastrointestinal bleeding, administration of octreotide may be helpful by inducing vasoconstriction. Rarely, re-operation with revision of the enteric anastomosis (with or without Roux limb diversion) may be indicated for anastomotic bleeding. For severe gastrointestinal bleeding that occurs more than one week post-transplant, however, one must not assume it is secondary to anastomotic bleeding. In this setting, it is imperative to rule out a leaking pseudoaneurysm, which is best diagnosed and treated with angiographic techniques^[124].

When using the retroperitoneal approach to the SMV for portal-enteric drainage, in order to perform an anastomosis to the small bowel, one must make a window in the mesentery of the right colon. Bowel drainage can then be accomplished without or with a diverting Roux limb in a standard side-to-side manner^[17,113]. If one initially performs a side-to-side bowel

Table 9 Systemic *vs* portal-enteric drainage: Literature review

Center, authors, year, ref., study design and follow up	Number and types of transplant	Complications	Length of stay, readmissions and reoperations	1 yr patient survival	1 yr kidney and pancreas survival
University of Tennessee, Memphis, Stratta <i>et al</i> ^[44] , Prospective, mean follow-up 17 mo	SE 27; PE 27	Incidences of acute rejection (33%) and major infection (52%) similar in both groups; Intraabdominal infections were slightly greater in the SE group (26% SE <i>vs</i> 11% PE); 2 deaths in SE group compared to one in PE group Pancreas Graft loss: 7 in SE compared to 4 in PE group, all <i>P</i> = NS	Readmissions (mean 2.8 SE <i>vs</i> 2.2 PE); Mean length of hospital stay: SE: 12.4 d; PE: 12.8 d; Relaparotomy: 8 in SE compared to 7 in PE group, all <i>P</i> = NS	SE 96%; PE 93%	Pancreas SE 74%; PE 85%; Kidney SE 96%; PE 93%
University of Maryland, Philopophe <i>et al</i> ^[45] , Retrospective	SE: 63 SPK, 42 PAK, 26 PTA	Acute rejection: At 36 mo, the pancreas rejection rates were 21% for PE <i>vs</i> 52% for SE (<i>P</i> < 0.0001); the kidney rejection rates following SPK were 26% PE <i>vs</i> 43% SE (<i>P</i> = 0.017)	ND	36-mo patient survival rates were similar in both groups, 89% for PE <i>vs</i> 93% for SE	36-mo graft survival rates for all pancreas transplants were 79% with PE <i>vs</i> 65% with SE (<i>P</i> = 0.008)
Hospital Juan Canalejo, Coruña, Spain, Alonso <i>et al</i> ^[49] and Quintela <i>et al</i> ^[51] , Retrospective, mean follow-up 23 mo	PE: 54 SPK, 55 PAK, 40 PTA; SE 18; PE 20	Incidences of intraabdominal infection and acute rejection episodes were not different between groups	Early relaparotomy no difference: SE: 34 d; PE: 20 d	PE: 80% <i>vs</i> SE: 86%	Death-censored pancreas (SKP and PAK) graft survival was 73% for PE and 81% for SE (<i>P</i> = NS)
Toronto General Hospital, Bazerbach <i>et al</i> ^[53] , Retrospective	SE 147; PE 45	In both groups, a complication occurred in 38% of patients in the first year; Major infections were not different between groups; 3-mo rejection rate was identical (6%) and the 1-yr rejection rate was 12.2% SE <i>vs</i> 13.3% PE; Most common reasons for pancreas graft loss in both groups were death with functioning graft (25%), graft thrombosis (13%), rejection (11%) and duodenal leak (9%)	Length of stay - mean 11 d <i>vs</i> 10 d in the SE <i>vs</i> PE; Most common causes of death in both groups were myocardial infarction (35%), cerebrovascular accident (13%) and cancer (13%); Most common causes of kidney graft loss in both groups were death with functioning graft (61%) and acute rejection (11%)	Patient survival did not differ at 5 yr (94% SE <i>vs</i> 89% PE) and 10 yr (85% SE <i>vs</i> 84% PE, <i>P</i> = NS)	Pancreas survival was similar at 5 yr (82% SE <i>vs</i> 76% PE) and 10 years (65% SE <i>vs</i> 60% PE); Kidney survival was similar at 5 yr (93% SE <i>vs</i> 84% PE) and 10 yr (82% SE <i>vs</i> 76% PE)
Medical University Innsbruck, Austria, Ollinger <i>et al</i> ^[120] , Retrospective, Mean follow-up 8.3 yr	509 transplants in 4 eras including 34 PE and 146 SE (with DJ) in most recent era (2004-2011)	Thrombosis: 9% PE <i>vs</i> 5% SE, <i>P</i> = NS		5-yr patient survival 94%	5-yr pancreas survival 77% PE <i>vs</i> 74% SE
Hôpital Edouard Herriot, Lyon, France, Petruzzo <i>et al</i> ^[50] , Retrospective	SE 36; PE 44; All SPK	No significant differences in long-term outcomes but the SE group had a higher incidence of pancreas graft loss secondary to thrombosis	No difference in total surgical complications	Patient survival rates 92% SE <i>vs</i> 95.5% PE	One-, 3-, 5-, and 8-yr pancreas survival rates were 75%, 60.6%, 56.7%, and 44%, respectively, in the SE group compared to 88.6%, 84.1%, 78.4%, and 31.3% in the PE group; One- 3-, 5-, and 8-yr kidney survival rates were 91.7%, 78.1%, 74.1%, and 57.9%, respectively, in the SE group compared to 93.2%, 88.6%, 78.4%, and 38.9% in the PE group

SE: Systemic enteric; PE: Portal enteric; ND: Not determined/no data; DJ: Duodeno-jejunostomy; NS: Not significant.

anastomosis, it is relatively straightforward to convert to a diverting Roux limb for whatever reason by separating the afferent limb with a gastrointestinal stapler just

proximal to the anastomosis. The stapled and divided proximal limb can then be placed 40 cm or more distal to the anastomosis on the efferent limb and the second

bowel anastomosis can be constructed either in a side-to-side or end-to-side manner with either sutures or a stapler. A potential advantage of accessing the SMV for venous drainage is that the procedure is no longer pelvic but rather mid-abdominal in location, which is helpful in cases of retransplantation or in patients who have had previous pelvic irradiation or procedures^[121].

With any method of enteric drainage, the efferent limb must be placed so as to remove any tension or traction on the bowel anastomosis. By careful positioning, an anastomotic “blow-out” or enteric leak can be averted by preventing bowel angulation just distal to the anastomosis. In addition, it is important close any mesenteric defects and to position the pancreas in such a way that the risk of internal hernia is minimized. Although some surgeons prefer to “wrap” omentum around the bowel anastomosis, we do not advocate this practice because of the concern for liquefaction necrosis that may develop from any fat that comes in direct contact with the pancreas following reperfusion. Fat necrosis may result in peri-pancreatic fluid collections that could subsequently require drainage or become infected.

Alleged gains of pancreas transplantation with portal venous delivery of insulin include immunological, technical, and metabolic, “advantages”. However, neither large registry analyses nor prospective cohort studies have been able to corroborate these purported benefits (Table 9)^[1,33,39-42,44-46,49-53,112-123]. Conversely, when comparing the three major techniques of pancreas transplantation, there are likewise no well controlled studies to suggest any major drawbacks of portal-enteric vs either systemic-bladder or systemic-enteric drainage.

One of most recent and exciting innovations in pancreas transplantation is the advent of laparoscopic pancreas transplantation with robotic support^[125-127]. With the da Vinci Robotic system, Boggi *et al.*^[125] reported the first three whole pancreas transplants performed by using this technology. Their experience constitutes a proof of concept for pancreas transplantation with robotic-assisted laparoscopic surgery. In these cases, enteric drainage of was accomplished using a circular stapler to create an anastomosis between the proximal recipient small bowel and donor duodenum^[126]. However, Boggi *et al.*^[127] have raised concerns regarding the influence of longer warm ischemia duration on viability of the graft because maintaining a cold graft temperature prior to reperfusion is difficult to accomplish laparoscopically. Although several “variations on a theme” exist in the procedural methodology of pancreas transplantation and novel approaches continue to be described, the prevailing viewpoint upholds that the technique with which the individual surgeon feels most confident and comfortable is the best one to be implemented based on donor pancreas quality and recipient anatomic considerations. With improved surgical outcomes over time, exocrine drainage techniques are no longer the “Achilles’ heel” of vascularized pancreas transplantation.

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Hepatoduodenal ligament dissection technique during recipient hepatectomy for liver transplantation: How I do it?

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Abstract

Accurate dissection of the hepatoduodenal ligament in the recipient is vital for the success of liver transplantation surgery. High incidence of anatomic variations at the hepatic artery, portal vein and biliary ducts in

the hepatoduodenal ligament is well known. Surgical experience is important to be able to foresee the most common anatomic diversities and the possible variations, in order to make a safe and accurate dissection in the hepatic hilum. Before anastomosis, all these hilar structures must be well identified, safely dissected and must also have a sufficient length for the coming implantation process. At the beginning of our program, we were starting the hepatic hilum dissection close to the liver. In time, however, we modified our surgical technique, preferring to start further away from the liver (closer to the duodenum). This length increased progressively over 1500 liver transplantations (80% living donor liver transplantation). During this process, our main purpose was the early control of the hepatic artery (artery first approach). In this paper, our aim is to share our latest version of the hepatoduodenal ligament dissection technique. We also describe alternative approaches used in extraordinary situations.

Key words: Liver transplantation; Living donor liver transplantation; Surgical technique

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Core tip: The hepatic artery is one of the main components of the hepatoduodenal ligament and exhibits high anatomic variability, which may change the outcome and success of liver transplantation. In our experience, early control of the hepatic artery (artery first approach) and by the guidance of the hepatic artery, dissection of the rest of the hepatoduodenal ligament components is more practical. In this paper, we share our latest version of the hepatoduodenal ligament dissection technique, developed over the course of 1500 liver transplantations (80% living donor liver transplantation) in our clinic.

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LAPAROTOMY

The Mercedes incision is probably the most widely preferred incision technique for liver transplantation in the world. When we first started performing liver transplantations, we used the Mercedes incision as well. However, we observed a high incidence of incisional hernia with this technique^[1]. In time, we reduced the size of the incision and started to use the "reverse L" right upper quadrant incision. Nowadays, we prefer the Mercedes incision only in special occasions (obesity, extensive adhesions due to previous surgery). The extension of "reverse L" incision on the right should extend laterally enough to permit the exposure of the segment VI of the liver. The tip of the incision on the midline extends up to the xiphoid process, high enough for exposure of the supra-hepatic vena cava. In some selected patients, we performed the liver transplantation only through a supra-umbilical median incision^[2]. After laparotomy, the falciform ligament is divided, trimmed and ligated. A sternum lifting mechanical retractor is placed after the suturing of the skin flap to the drape on the right.

MOBILIZATION OF THE LIVER

The left triangular ligament is divided and the gastro-hepatic ligament is examined for an accessory left hepatic artery (HA) arising from the left gastric artery; if there is one, it should first be controlled by a vascular bulldog clamp and then cut close to the liver. We do not prefer to use ligamentum Teres for traction of a cirrhotic liver, which usually tends to bleed from the liver capsule during traction. To achieve better exposure of the hepatoduodenal ligament in a cirrhotic liver, we prefer to first mobilize the right lobe of the liver and place a large piece of gauze behind the liver to move the hepatoduodenal ligament anteriorly. In other words, we position the hepatoduodenal ligament closer to the surgeon. Mobilization of the right liver lobe at the beginning of the procedure provides exposure of the retro-hepatic vena cava at full length. This also allows for total hepatic vascular occlusion when necessary, particularly in emergency conditions. One or two blades of the automatic liver retractors are placed on the visceral surface of the right and/or left lobes of the liver. Then we can easily and clearly expose the hepatoduodenal ligament. A dilated gall bladder may sometimes lay over the hepatoduodenal ligament, preventing good exposure, and its tractions can result in hemorrhage from the liver capsule. In these situations, a partial cholecystectomy may be useful^[3].

THE PHILOSOPHY OF THE ARTERY

FIRST APPROACH

At the beginning of our liver transplantation program, we began the hepatoduodenal ligament dissection as close as possible to the liver. This was done to avoid injuries to the proximal parts of the components of the hepatoduodenal ligament. However, we experienced some difficulties while working closer to the liver hilum. At first, it was difficult to perform a dissection in such a small area and increased risk of liver capsule bleeding. Secondly, there was difficulty in identifying the arteries from their distal ends, and we observed more intimal injuries during the dissection of these small caliber arteries if there was no proximal vascular control by a vascular bulldog clamp.

HA is one of the three main components of the hepatoduodenal ligament. It has the highest rates of variation, which may change the outcome and success of the liver transplantation. The surgeon performing the recipient hepatectomy is responsible for the protection of all the arteries that may have a potential use during the implantation process. It is obvious that the arteries that must be protected during the dissection are not limited to two (right and left). In every case, five potential arteries (right, left, segment IV, right HA from the superior mesenteric artery, left HA from the left gastric artery, Figure 1) must be encountered. In our experience, early control of the hepatic artery (artery first approach) and by the guidance of it, dissection of the rest of the hepatoduodenal ligament components is more practical.

STARTING NEAR TO THE DUODENUM

The hepatoduodenal dissection is started just above the duodenal margin (Figure 2). We proceed from laterally to medially with the ligation of the peritoneum and the vessels under the peritoneum (Figures 3 and 4). Trimming of the anterior-inferior leaf of the hepatoduodenal ligament makes it possible to identify the common bile duct with the help of 3 and 9 o'clock vessels. The direction of the dissection is towards the common HA, which is the main point of our hilar dissection at this stage. Above the duodenum, while dissecting medially, the right gastric artery that arises from the hilum and travels to the stomach must be ligated and transected. Inexperienced surgeons may worry about transecting this artery for fear of harming the common HA. However, the right gastric artery is more superficial than the HA, and it runs to the stomach and not into the hepatoduodenal ligament.

LYMPH NODES AS LANDMARKS

On the medial side of the hepatoduodenal ligament, just above the duodenum, the largest lymph node of

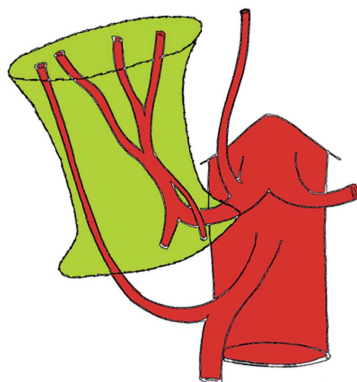


Figure 1 All the potential arterial branches that should be preserved during the recipient hepatectomy.

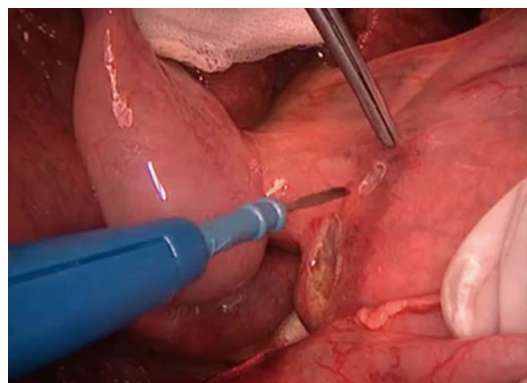


Figure 2 Opening small windows on the peritoneum of the distal hepatoduodenal ligament.

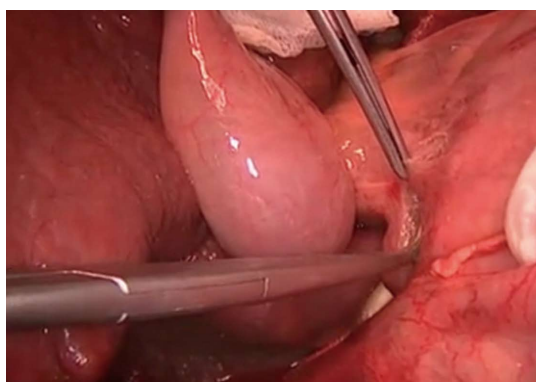


Figure 3 Dissection started just close to the duodenum.

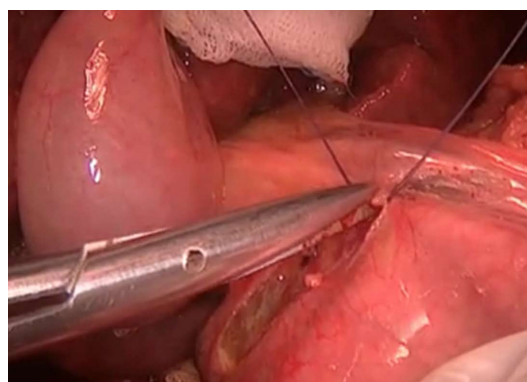


Figure 4 Vessels under the peritoneum were transected after ligation. Avoiding electrocautery at this stage protects the duodenum from thermal injury.

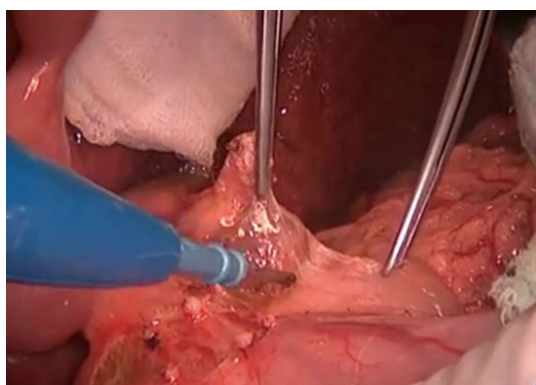


Figure 5 The lymph node located at the infero-medial part of the hepatoduodenal ligament is an important landmark. It locates along the upper border of the pancreas.

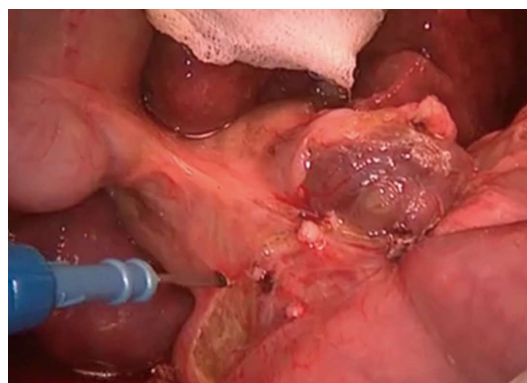


Figure 6 The landmark lymph node is one of the largest lymph nodes of the hepatoduodenal ligament. Its identification enables to find out the arteria hepatica communis which is just under this lymph node. The dissection is extended to the infero-lateral part of the hepatoduodenal ligament by jumping over the distal common bile duct. Another large counterpart lymph node is exposed near to the distal common bile duct.

the superficial hepatoduodenal lymph nodes can be seen (Figures 5 and 6). This lymph node is located on the trace of the common HA, acting as a landmark for hilar dissection. Once this lymph node is identified, it should be removed carefully after palpation of the common HA under this lymph node. In terms of the newest hemostatic technologies, like Ligasure or Ultrascission^[4], we generally prefer to use the suture ligation with low voltage adjusted (25 Watt) monopolar and bipolar electrocautery for hepatoduodenal ligament

dissection. Clamps and scissors can also be used for dissection of the common HA to avoid intimal injury due to thermal effects. The common HA is separated from the upper border of the pancreas and completely mobilized. At this stage, the gastroduodenal artery is searched for in the triangle formed by the medial aspect of the distal common bile duct, the trace of the

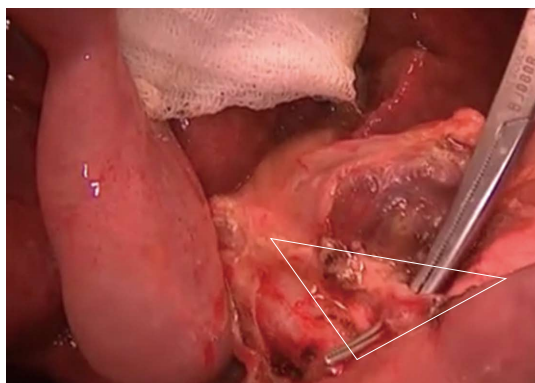


Figure 7 The gastroduodenal artery is identified in the triangle composed of; distal common bile duct, hepatic artery and upper border of the duodenum.

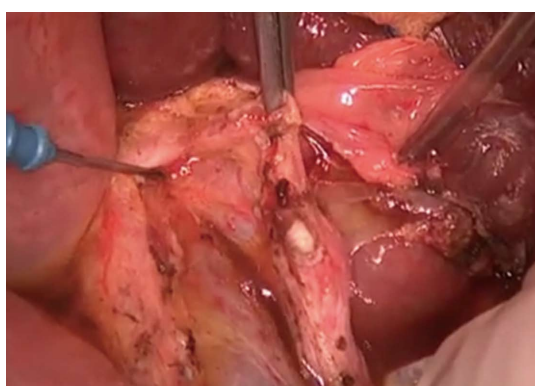


Figure 9 Division of the gastroduodenal artery makes the portal vein visible.

HA, and the upper side of the duodenum (Figure 7). Ligation and transection of the gastroduodenal artery makes the portal vein visible just beneath it (Figures 8 and 9). After this step, the main three components of the hepatoduodenal ligament can be partly identified. We place an atraumatic bulldog clamp to the common HA to decrease the intraluminal pressure in the arterial lumen and to prevent intimal dissection^[5] (Figure 10). The gastroduodenal artery is usually divided for several reasons, such as prolonging the HA for a living donor liver transplantation, avoiding steal syndrome through the gastroduodenal artery, and performing an arterial anastomosis to the bifurcation of the gastroduodenal artery and common HA during a deceased liver transplantation.

FOLLOW THE ANTERIOR SIDE OF THE COMMON HA

Generally, there are no main branches arising on the anterior side of the common HA. This knowledge is particularly valuable when dissecting of the hepatoduodenal ligament by the arterial route. Hanging the tissues on the common HA with the help of a right-angle clamp and cutting them *via* electrocautery will

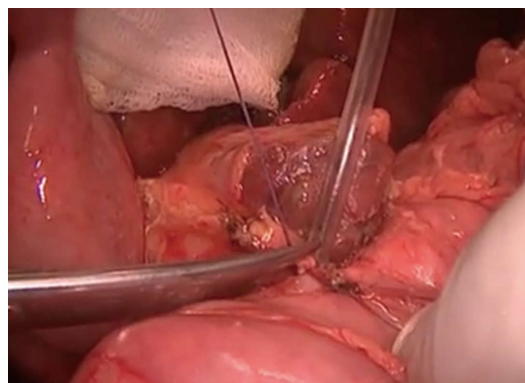


Figure 8 Ligation and transection of the gastroduodenal artery.

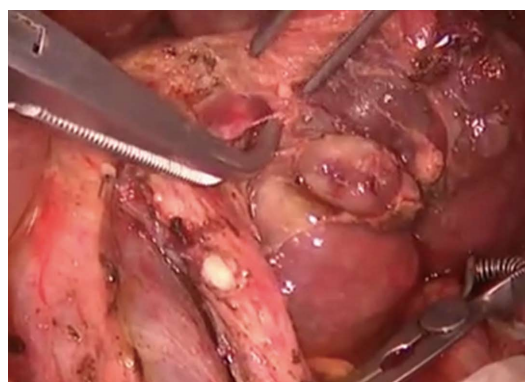


Figure 10 Bulldog clamp applied to the proximal common hepatic artery to prevent the intimal damage in the artery during further dissections.

help in visualizing the distal branches of the proper HA. This dissection meticulously moves toward the bifurcations of the right, left and segment IV hepatic arteries. There may be some small arterial branches going toward the lymph nodes. However, because the common HA is clamped by a bulldog, these will not cause any major bleedings. Even so, it is advisable to perform careful dissection to prevent hemorrhage. The left HA and segment segment IV hepatic arteries artery should be followed as far as their entrance into the liver parenchyma. The spatial relationship between the right HA and the bile duct should be evaluated. Generally, the right HA crosses posterior to the common bile duct (Figure 11). However, it sometimes crosses anteriorly and we cut this HA as closely as possible to the liver and continue on to bile duct dissection later. If the right HA is passing posteriorly, then the common bile duct dissection can be started before arterial transections.

BILE DUCTS

The cystic duct is identified, clipped and divided. The common bile duct and ductus choledochus can be identified with the help of the up traction of the cystic duct stump (Figure 12). The lateral side of the common hepatic duct is dissected caudally and cranially.

For better and safer exposure of the extra-hepatic

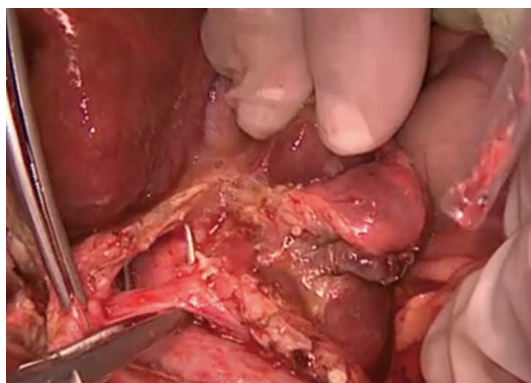


Figure 11 Right hepatic artery is crossing the common bile duct from the posterior.

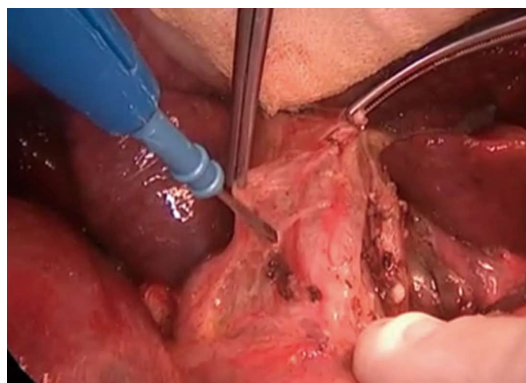


Figure 12 Traction of the cystic duct stump ensures a safer dissection of the lateral border of the common bile duct.

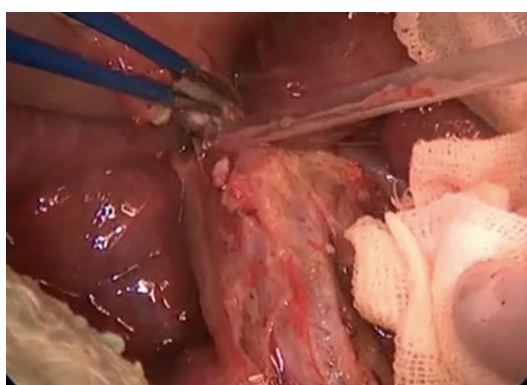


Figure 13 Removing the sheet over the extrahepatic bile ducts provides the identification of the medial and lateral borders of the bile ducts.

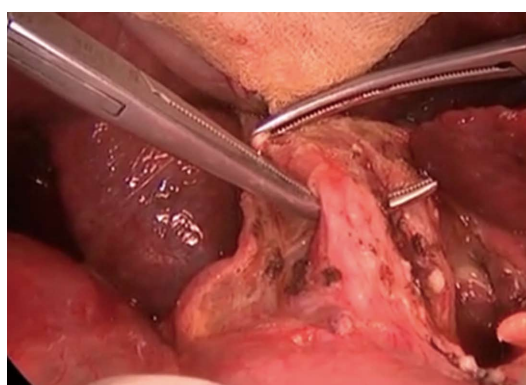


Figure 14 Common bile duct is further liberated and hanged by the right angle clamp.

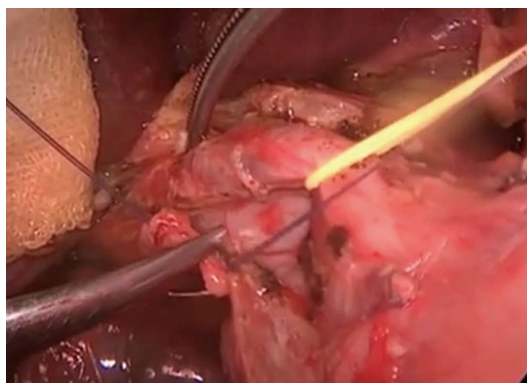


Figure 15 Medial traction applied to the bile duct by a rubber band and the lymphatics are transected. This transaction should be done after being sure that there is no accessory right hepatic artery arising from the superior mesenteric artery.

bile ducts, we peel back the peritoneal sheet that covers the common bile duct, starting from the side of the duodenum and moving to the liver hilum. In our daily surgical practice, we call this “undressing the coat” of the hepatoduodenal ligament. This allows us to safely go underneath the hilar plate that covers the main anatomic contents of the portal hilum. Here, so as not to damage the vascular supply of the choledochus AA and common bile duct, bleeding must be controlled with

fine suturing (Figure 13). In this way, the 3 and 9 o'clock arteries become more visible. These are the landmarks for the medial and the lateral margins of the common bile duct. The common bile duct can be lifted using a right-angle clamp (Figure 14). The peri-choledochal plexus supplying the bile ducts from the 3 and 9 o'clock arteries should be preserved. Electrocautery and excessive skeletonization must be avoided here in order to preserve the blood supply of the remnant bile duct, preventing future anastomosis strictures^[6]. The remaining tissues along the lateral part of the common bile duct and the portal vein should be divided (Figure 15). However, if there is an accessory right HA arising from the superior mesenteric artery, the surgeon must be careful not to harm it. We prefer to dissect all the lymph nodes around the hepatoduodenal ligament. In this way, the portal vein, bile duct and arteries can be better identified. It also preserves the length of bile duct, arterial branches and the portal vein, which is particularly important for living donor liver transplantations (Figure 16).

ALTERNATIVE APPROACHS

After identification of all hilar contents, we first cut the HA and then the main bile duct as closely as possible to the liver. The portal vein is then cut just before the un-

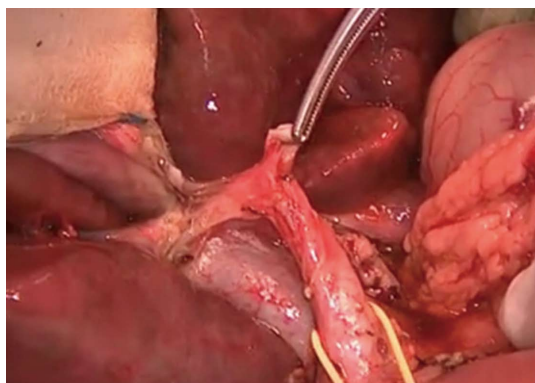


Figure 16 Removing the lymph nodes and the transection of the arteries enables a well visualized and prolonged common bile duct and portal vein.

hepatic phase. In cases in which the hepatoduodenal ligament cannot be dissected easily due to fibrosis or adhesions, we use alternative techniques. In such cases, we perform a double Pringle's maneuver to control the hepatoduodenal ligament, and then resect the ligament together with the contents as closely to the liver as possible. Next, we try to retrospectively identify the contents one by one. Once all of the contents have been identified, the Pringle's maneuvers are released. Also, in cases of portal vein thrombosis, we make available a Foley urinary catheter on the operating table for any unexpected bleeding from the porto-mesenteric veins during endovenectomy^[7].

CONCLUSION

Experience and expertise, especially in the surgical field, where many variations can be seen, is extremely

important for performing safe and successful dissection. We are still improving our technique for recipient hepatectomy day by day. We hope that these technical details will be helpful to our colleagues dealing with the liver transplantation and hepatobiliary surgery.

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Liver transplantation and the management of progressive familial intrahepatic cholestasis in children

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Abstract

Progressive familial intrahepatic cholestasis (PFIC) is a constellation of inherited disorders that result in the impairment of bile flow through the liver that predominantly affects children. The accumulation of bile results in progressive liver damage, and if left untreated leads to end stage liver disease and death. Patients often present with worsening jaundice and pruritis within the first few years of life. Many of these patients will progress to end stage liver disease and require liver transplantation. The role and timing of liver transplantation still remains debated especially in the management of PFIC1. In those patients who are appropriately selected, liver transplantation offers an excellent survival benefit. Appropriate timing and selection of patients for liver transplantation will be discussed, and the short and long term management of patients post liver transplantation will also be described.

Key words: Pediatric liver transplant; Progressive familial intrahepatic cholestasis; Familial intrahepatic cholestasis protein 1; Cholestasis; Multidrug resistance protein 3; Pediatric jaundice; Bile salt excretion protein

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Core tip: Progressive familial intrahepatic cholestasis is a rare disorder that predominantly affects young children. If left untreated, children develop debilitating cholestasis and eventually progress to liver failure. Liver transplantation is curative of symptoms related to liver disease but in some cases worsens the extrahepatic symptoms. A multidisciplinary approach is critical to obtaining good long-term outcomes.

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INTRODUCTION

Cholestasis in children is caused by many different entities. Progressive familial intrahepatic cholestasis (PFIC), which is also referred to as Byler's disease, Byler's syndrome, or Greenland-Eskimo familial cholestasis, is an autosomal recessive inherited disease that disrupts the genes encoding protein transporters responsible for bile formation^[1]. These mutant proteins result in the impairment of bile flow through the liver leading to severe intrahepatic cholestasis and progressive chronic liver disease^[2]. Recently, mutations in a gene important for the formation of tight junctions was also reported that leads to progressive intrahepatic cholestasis^[3].

Familial conditions of cholestasis were first reported in the 1950s with Ahrens *et al*^[4] reporting 4 patients with congenital absence of their intrahepatic bile ducts. These patients had persistent jaundice very early in life, severe growth retardation, malabsorption, pruritus and xanthomatosis with marked hypercholesterolemia. Liver biopsies of these patients revealed complete absence of interlobular bile ducts and bile stasis, despite a normal lobular architecture and extra hepatic biliary system. All four of these children died at an early age^[4]. Similarly, in 1966, Gray *et al*^[5] reported two sisters with jaundice, marked growth retardation, malabsorption, and pruritus. The course was progressive for both sisters and they died before the age 3^[5]. Clayton *et al*^[6], Juberg *et al*^[7], and Sharp *et al*^[8] also reported additional cases of children with progressive cholestasis and liver failure resulting in death. Similarities among these early reported cases were described in an early review on PFIC by Ballow *et al*^[9] and included: A familial occurrence, a clinical history of fluctuating jaundice, pruritus, malabsorption, growth retardation early in life and hepatosplenomegaly. Similar biochemical findings included conjugated hyperbilirubinemic obstructive cholestasis with normal blood cholesterol levels^[9].

SEARCH STRATEGY

A literature search of English language publications from 1990-2014 was used to identify published data on liver transplantation for PFIC using the Patients Intervention Comparator Outcomes outline (Table 1)^[10]. Databases searched were PubMed, Ovid MEDLINE, and Cochrane Reviews. Terms used in the search were "liver transplantation" AND one of the following terms "progressive familial intrahepatic cholestasis", "PFIC", "PFIC1", "PFIC2", "PFIC3", "Byler's Syndrome" or "Byler's Disease".

EPIDEMIOLOGY

The incidence of any of the defective genes involved in the development of PFIC is 1:50000-100000 births and has not shown predominance in any specific geographical area^[2,11]. However, there have been communities that have noted cohorts of patients including Faeroe Islands, Inuit (Eskimo) Indians (Greenland and Canada), and the Amish^[6,12-15]. PFIC is responsible for 10%-15% of cases of neonatal cholestasis syndrome and is one of the leading indications for pediatric liver transplantation^[16,17].

PATHOPHYSIOLOGY

Bile formation at the level of the hepatocytes involves active transport of bile salts, phospholipids, and cholesterol from the portal blood at the basolateral membrane. In PFIC, these transporters function abnormally (Figure 1). Bile then flows from the bile canaliculi lined by adjacent hepatocytes into the canals of Hering that are lined on one side by hepatocytes and one side by cholangiocytes. From there, bile drains into the larger bile ductules.

PFIC1

PFIC1 is an autosomal recessive condition. The mutant gene responsible for the disorder is the *ATP8B1* gene encoding the FIC1 protein^[18,19]. The gene locus for *ATP8B1* is located on chromosome 18 (18q21-22). FIC1 is a member of the type 4 subfamily of P type adenosine triphosphatase transporters and is involved in phospholipid translocation. The protein is located on the canalicular membrane of hepatocytes and facilitates movement of phosphatidylserine and phosphatidylethanolamine from the outer to inner leaflet of the plasma membrane of the hepatocyte. In addition, it helps to protect the membrane from high bile salt concentration in the canalicular lumen^[20].

Mutation of this protein significantly impairs bile salt secretion. The exact mechanism for how deficiency of FIC1 leads to cholestasis is not fully understood^[1]. Varying severities of PFIC1 are however noted^[11].

PFIC2

PFIC2 is caused by mutation of the ATP binding cassette family B member 11 (*ABCB11*) gene encoding the bile salt excretion protein (BSEP) protein. The gene locus is on chromosome 2 (2q24) and is similarly inherited in an autosomal recessive fashion. BSEP, like FIC1, is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids^[21]. BSEP malfunction leads to failure of bile salt secretion from hepatocytes into bile canaliculi and accumulation of bile inside the hepatocytes. This results in severe impaired bile flow and hepatocellular damage^[1]. On immunohistochemical staining, BSEP is usually not detectable in PFIC2, and if there is any protein present, it is usually non-functional^[22-26].

Table 1 Patients Intervention Comparator Outcomes table for assessment of progressive familial intrahepatic cholestasis

P	I	C	O
Pediatric patients with PFIC	Liver transplantation	Biliary diversion and medical management	Patients survival, graft survival, post operative morbidity

PFIC: Progressive familial intrahepatic cholestasis; P: Patients; I: Intervention; C: Comparator; O: Outcomes.

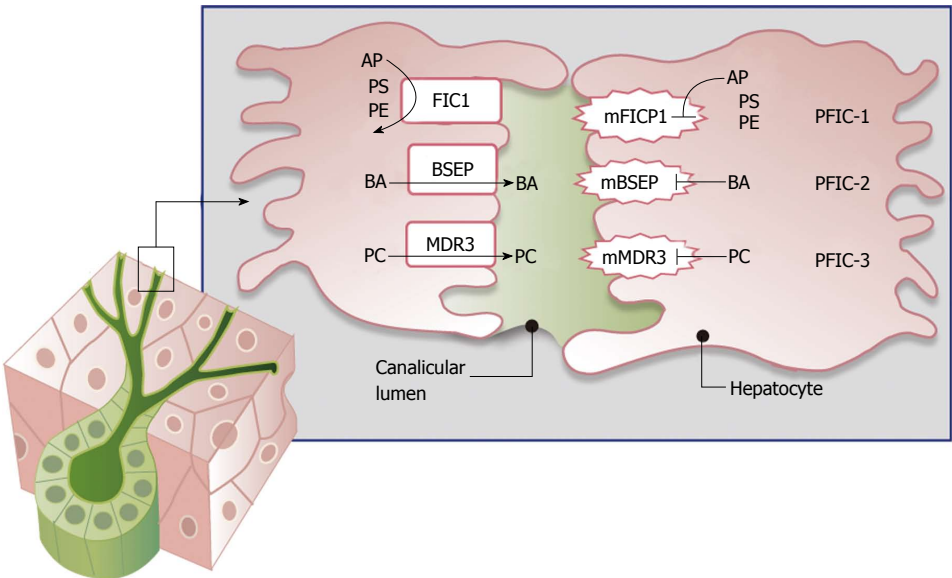


Figure 1 Disruption of bile flow and progressive familial intrahepatic cholestasis. AP: Aminophospholipids; PS: Phosphatidylserine; PE: Phosphatidylethanolamine; BA: Bile acids; PC: Phosphatidylcholine; FIC1: Familial intrahepatic cholestasis protein 1; BSEP: Bile salt exporter pump; MDR3: Multidrug resistance protein 3; mFIC1: Mutant familial intrahepatic cholestasis protein 1; mBSEP: Mutant bile salt exporter pump; mMDR3: Mutant multidrug resistance protein; PFIC: Progressive familial intrahepatic cholestasis.

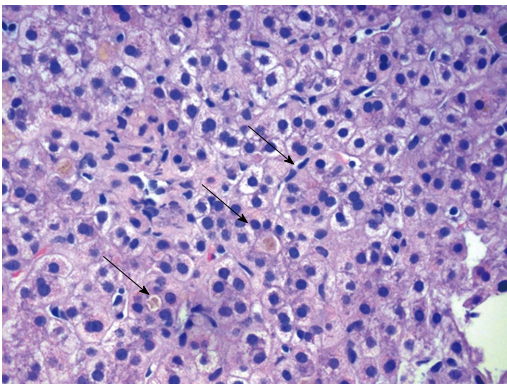


Figure 2 Progressive familial intrahepatic cholestasis type 1 with severe bland lobular cholestasis and lobular disarray. The image shows bile plugging with surrounding pseudorosette formation (arrows). In PFIC1, the canalicular bile is coarse on electronic microscopy and also referred to as “Byler bile”. Thick bile is seen within the pseudorosette here on H and E stain. There is an absence of lobular inflammation and typically no features of neonatal giant cell hepatitis. PFIC: Progressive familial intrahepatic cholestasis.

PFIC3

A mutation in adenosine triphosphate-binding cassette subfamily B member 4 (*ABCB4*) gene encoding the MDR3 protein leads to the development of PFIC3^[27,28]. The gene locus is on chromosome 7 (7q21). MDR3 protein is a p-glycoprotein that secretes phospholipids, primarily phosphatidylcholine within bile acid. Dys-

function leads to a decrease in phospholipid excretion^[28]. MDR3 defects results in biliary epithelium injury and bile canaliculi injury as well as cholestasis. In addition, there is destabilization of micelles and promotion of cholesterol crystallization that results in increased biliary lithogenicity. This subtype of PFIC is usually present on both alleles and yields complete loss of the MDR3 protein either from a truncated MDR3 from a premature stop codon or missense mutations. All mutations result in severe defective transport of phospholipids and intracellular misprocessing^[29].

PFIC4

PFIC4 is a recently described genetic mutation involving the *TJP2* gene that encodes for the tight junction protein 2^[3]. TJP2 is a cytosolic protein that interacts with several cytoskeletal proteins and integral membrane proteins and plays an important role in localizing proteins such as Claudins (*e.g.*, CLDN1) to these structures^[30]. Patients who presented with PFIC were found to have protein-truncating mutations that resulted in inappropriate localization and disruption of the tight junctions^[31].

HISTOLOGIC ALTERATIONS IN PFIC

Even within the different subtypes of PFIC, there are common features and some distinct features. Specific

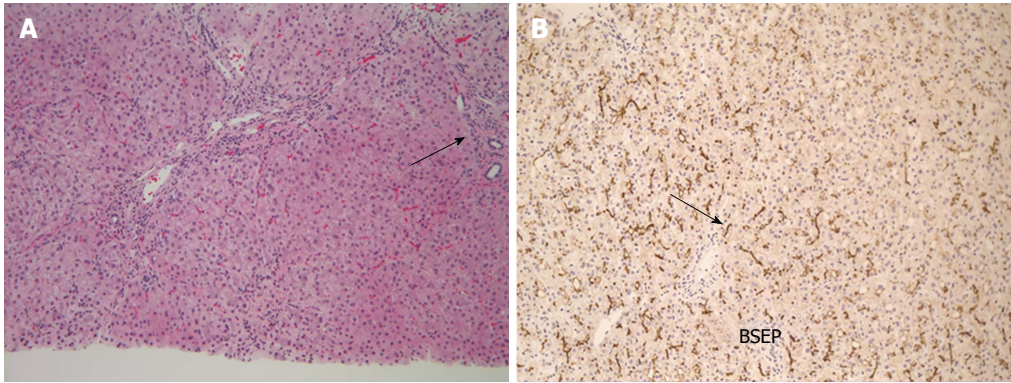


Figure 3 Progressive familial intrahepatic cholestasis type 2 is characterized by mutations in the *ABCB11* gene. A: Patients with progressive familial intrahepatic cholestasis type 2 (PFIC2) can initially present clinically similarly to PFIC1, but with more rapid progression of liver disease. Early on in the disease patients may present with neonatal giant cell hepatitis and lobular inflammation. However, there can be rapid progression with prominent duct reaction and progression to cirrhosis. This figure demonstrates prominent duct reaction in a patient with PFIC2 and advancing fibrosis (arrow). Duct reaction and cholestasis can also occur in patients with extrahepatic biliary obstruction so correlation with clinical findings is required; B: PFIC2 is also called BSEP disease and is characterized by mutations in the *ABCB11* gene. *ABCB11* encodes for the major canalicular bile salt exporter BSEP. Patients with normal BSEP expression show positive immunohistochemistry for BSEP with a canalicular pattern of staining (arrow). In some cases of PFIC2, there is complete lack of staining for BSEP. BSEP: Bile salt exporter pump.

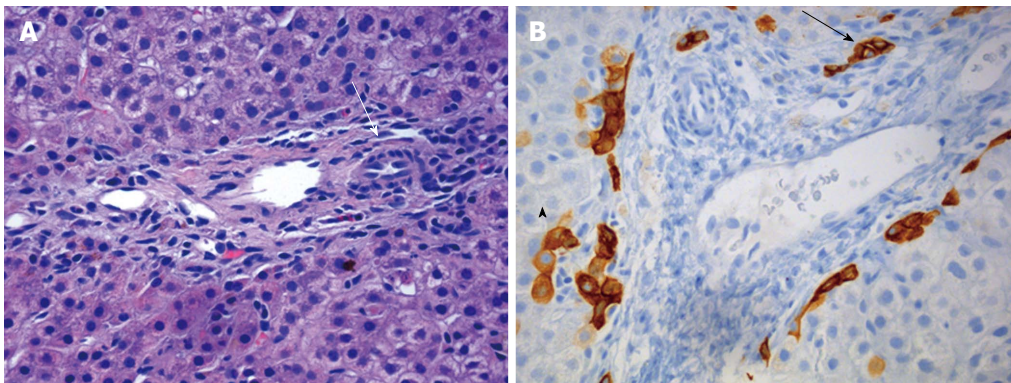


Figure 4 Progressive familial intrahepatic cholestasis type 1 and 2 can also present with duct paucity. A and B: The portal tracts show an absence of bile duct with periportal duct reaction; B: A higher power view of the portal tract with vein on the left artery on the right (arrow) and no appreciable bile duct. Keratin 7 is negative in this portal tract in B and positive in the bile duct reaction (arrow) with some bile duct progenitor cells (paler brown staining arrowhead).

signs on biopsy of PFIC1 (Figure 2) include bland cholestasis, mild lobular fibrosis, and centrilobular canalicular cholestasis with acinar or pseudo rosette formation^[1,32]. Early in the disease, the initial biopsy typically demonstrates hypoplastic and threadlike interlobular bile ducts. With progression of the disease, centrilobular hepatocyte loss occurs with resulting pericanalicular and periportal fibrosis. Over time, there is progression to portal-portal and portal-central bridging fibrosis that leads ultimately to micronodular cirrhosis. Interestingly, fibrosis progresses in the absence of significant inflammation and ductular reaction^[32].

Findings in the PFIC2 subtype (Figure 3) include cholestasis, giant cell hepatitis, hepatocellular necrosis, portal fibrosis and neonatal giant cell hepatitis with hepatocellular and canalicular cholestasis^[32]. The fibrosis begins both in the portal tracts and in centrilobular regions and progresses through a biliary pattern type cirrhosis leading to micro nodular cirrhosis with slight ductular reaction^[32]. Both PFIC1 and PFIC2 can show a paucity of bile ducts (Figure 4).

In PFIC3 (Figure 5), there is bile ductular proli-

feration, inflammatory infiltrate and biliary fibrosis with mild expansion of portal tracts due to a ductular reaction^[32]. Canalicular cholestasis is present in centrilobular areas, and biliary/micro nodular cirrhosis supervenes with a biliary halo around cirrhotic nodules. There is also often the presence of ductular reaction and bile plugs^[1].

Immunohistochemistry (IHC), electron microscopy (EM) and bile analysis can also provide important information regarding the different subtypes of PFIC. IHC for the different proteins associated with the different PFIC phenotypes is typically performed. However, normal IHC does not necessarily rule out a diagnosis of PFIC since some mutations are solely functional mutations and do not alter protein synthesis or expression^[22]. Immunohistochemical stains can be particularly helpful in the identification of the BSEP protein at the canalicular membrane^[22]. In PFIC3 patients, canalicular MDR3 immunoreactivity is typically detectable and the diagnosis of PFIC3 requires gene sequencing^[24].

EM is also useful in differentiating the different PFIC subtypes. In PFIC1, the EM is coarse and granular in

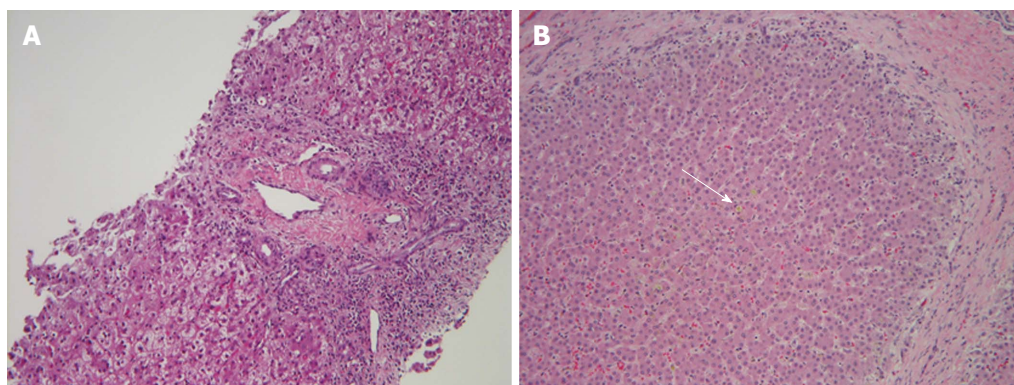


Figure 5 Clinical presentation of progressive familial intrahepatic cholestasis type 3. A: Progressive familial intrahepatic cholestasis type 3 (PFIC3) has a variable clinical presentation and may show nonspecific biliary pattern of injury that can mimic extrahepatic biliary atresia such as bile duct proliferation and cholestasis. In this patient with PFIC3 there is cholestasis, inflammation, and bile duct proliferation; B: Biliary type cirrhosis in a patient with PFIC3 with severe cholestasis (arrow) and micronodular cirrhosis.

appearance that is the characteristic “Byler’s bile”. In contrast, PFIC2 EM has an amorphous appearance^[33,34]. EM findings in PFIC3 patients have not been reported. In PFIC4, EM of the liver tissue of these patients demonstrated elongated tight junctions that lacked the densest part of the zona occludens^[3].

CLINICAL PRESENTATION

The hallmark sign and symptom of the disease is jaundice and pruritus. For children and their parents, pruritus is an extremely distressing manifestation of disease and its relief is often the goal of early therapy. Significant pruritus leads to cutaneous mutilation, loss of sleep, irritability, poor attention and impaired school performance. In addition to pruritus, other symptoms include icterus, hepatosplenomegaly, excoriations, hyperpigmentation of the skin, shiny nails, growth retardation, pale stools, and fat malabsorption^[1,11]. Most cases of PFIC present in infancy or early childhood with jaundice, and progress rapidly to fibrosis and end-stage liver disease. If left untreated, end stage liver disease will result in death.

There are many similarities and few distinct differences between the different PFIC subtypes^[35,36]. Signs specific to PFIC1 include presentation in early infancy as opposed to neonatal period or later in childhood. Foul-smelling, high volume stools and failure to thrive are also hallmarks for PFIC1^[35]. Gastrointestinal involvement even after liver transplant with secretory diarrhea can be significant^[35,37]. Hemorrhage is also a possible sequelae and is potentiated by vitamin K deficiency and similarly can be the first clinical manifestation^[1]. Classic biochemical signs include low or normal gamma-glutamyl transpeptidase (GGT), high alkaline phosphatase and a lower serum albumin as compared to PFIC2. Additionally, there is typically more severe cholestasis and recurrent jaundice, extrahepatic disease and portal hypertension. These sequelae often lead to decompensation in early childhood.

In contrast to PFIC1, PFIC2 tends to present in the

neonatal period rather than later in infancy or childhood and tends to progress more rapidly. Biochemically, patients generally have a low or normal GGT, higher serum aminotransferases, higher serum bile acids and higher α -fetoprotein^[35]. Patients present with severe cholestasis and persistent jaundice typically within the first month of life. Consistent with the restricted expression of ABCB11 to the liver, there are no extrahepatic manifestations of PFIC2. Progression to end stage liver disease results in portal hypertension and other manifestations of end stage liver disease. PFIC2 tends to progress to end-stage liver disease more rapidly, with cirrhosis, liver failure and death in the first decade of life, most commonly in the first year of life, if a liver transplant is not performed^[35].

PFIC3 usually presents in adulthood or late adolescence^[38,39]. It is characterized by cholestasis and gastrointestinal bleeds secondary to cirrhosis and portal hypertension. Gastrointestinal bleeding may be the first presenting symptoms in older children or young adults. Biochemically, PFIC3 patients tend to have an elevated GGT. There is also an increased risk of cholesterol and drug induced cholestasis in patients with MDR3 mutations and PFIC3^[40,41].

INVESTIGATIONS AND DIFFERENTIAL DIAGNOSIS

Initial investigations of the jaundiced child include a combination of clinical, radiological, and laboratory testing with the goal of ruling out biliary obstruction and extra hepatic causes of jaundice. In addition, infectious or metabolic etiologies should also be ruled out. Important screening and confirmatory laboratory tests include a complete blood count, chemistries including electrolytes, serum glucose, liver enzymes, total and direct bilirubin, GGT, thyroid function studies, C-reactive protein, ferritin, and coagulation studies. In addition to the above labs, serum bile acids, urinary bile acids, lactic acid, alpha-1-antitrypsin phenotype,

Table 2 Review of documented liver transplantation outcomes for progressive familial intrahepatic cholestasis patients (≥ 3 patients)

Ref.	PFIC type	Age at transplant (years old)	Previous management	Graft survival	Patient survival	Notes
Soubrane <i>et al</i> ^[45]	14 "byler disease" PFIC type unspecified	6.5 (0.4-13)	NR	93.3%	92.8%	Consanguineous to the 2 nd degree in 8 cases
Emond <i>et al</i> ^[91]	11 PFIC unspecified type	4.6 \pm 3.4	2 had previous partial biliary diversion procedures	76.9%	73%	LT performed on those with advanced cirrhosis (6 received diversion procedures only)
Ismail <i>et al</i> ^[80]	8 PFIC of unspecified type	Unknown	1 patient PEBD, all received cholestyramine, phenobarbital, rifampicin, UDCA	100%	85.7%	6 cadaver livers, 2 living donors
Kondo <i>et al</i> ^[63]	4 PFIC of unspecified type	2-7	NR	75%	75%	
Bassas <i>et al</i> ^[56]	5 PFIC3 8 "low GGT PFIC" PFIC1/2	10-40 mo	NR	84.6%	84.6%	Parents of 12 out of 13 were 1 st cousins
Cuttillo <i>et al</i> ^[57]	6 PFIC1/2 1 PFIC3	4-53 mo	NR	100%	75%	
Englert <i>et al</i> ^[44]	33 patients PFIC2 and 3	Unknown	UDCA 10 of 33 received biliary diversion then LT	100% with prior diversion 89% without prior diversion	100%	
Aydogdu <i>et al</i> ^[52]	10 PFIC1/2 2 PFIC3	43.2 \pm 27 mo	UDCA	69.2%	75%	Surviving patients show good quality of life, exacerbation of diarrhea as the exception, mix of LDLT and cadaveric
Hori <i>et al</i> ^[50,51] Miyagawa-Hayashino <i>et al</i> ^[54] , Egawa <i>et al</i> ^[53]	11 PFIC1 3 PFIC2	0.6-18.2 years old	Total external biliary diversion performed at time of re-transplantation in one PFIC1 patient	82.4% total graft survival (14/17)	PFIC1 - 90.9% at 5 yr, 72.7% at 10 yr, 54.5% at 15 yr; PFIC2 - 100% at 5 yr	Digestive symptoms in 10 out of 11 PFIC1; 8 out of 11 PFIC1 recipients exhibited steatosis; 9 out of 11 PFIC1 recipients exhibited fibrosis
Kaur <i>et al</i> ^[58]	2 PFIC3 2 PFIC1/2	2, 2.5, 6 and 9 years old males	UDCA, phenobarbital and ondansetron	100%	75%	

LT: Liver transplantation; PEBD: Partial external biliary diversion; UDCA: Ursodeoxycholic acid; PFIC: Progressive familial intrahepatic cholestasis; GGT: Gamma-glutamyl transpeptidase; LDLT: Living donor liver transplantation; NR: Not reported.

alpha-fetoprotein, ammonia, cortisol, viral serologies, carnitine and acyl carnitine profile, and plasma amino acids levels should also be considered^[14]. GGT levels not only assist in the differentiation of the type of PFIC, but may also be a helpful prognostic indicator^[42]. A serum albumin, which if low, may indicate advanced disease or malnutrition^[10]. The presence of coagulopathy may also increase the suspicion of advanced disease^[10]. Genetic studies for JAG1 mutations as well as for the described PFIC mutations should also be performed to clarify the etiology of cholestasis. Once a diagnosis of PFIC is made, differentiating between the subtypes, such as PFIC1 and 2 in newborns and young infants, is important since options for optimal treatment may differ between subtypes. Genetic testing is the gold standard for diagnosis using a "gene chip". One chip allows for the analysis of 27 coding regions and their splice junctions from 5 different genes known to be involved in inherited syndromes of intrahepatic cholestasis^[43].

In addition to laboratory testing, radiologic investigations are also critical and almost always include an initial abdominal ultrasound. In addition, magnetic resonance cholangiopancreatography can provide additional information especially in older children and help exclude other diagnoses such as primary sclerosing cholangitis that may be high on the differential list particularly in patients with high levels of GGT and cholestasis.

ROLE OF LIVER TRANSPLANTATION

Liver transplantation is currently the only definitive treatment available for PFIC. It corrects the genetic defect and reverses many if not all of the effects of chronic liver disease. Several series have been published examining the outcomes of liver transplantation for PFIC (Table 2). Of the cumulative 131 patients of all subtypes documented, graft survival and patient survival was 76.6% and 85.2% respectively with the

longest reported follow up interval being 19 years post-transplantation.

In the largest series by Englert *et al.*^[44], 23 patients (PFIC2 or 3) underwent orthotopic liver transplantation as their first line of treatment and 10 received liver transplantation after an initial biliary diversion procedure. The graft survival rate of those who received a liver transplant initially was 89%, whereas graft survival rates of those who first received biliary diversion and subsequent transplantation were 100%. Patient survival between the two groups was 100%^[44]. Soubrane *et al.*^[45] reported similar excellent outcomes. Of the 14 patients transplanted, 13 patients were alive at was an average follow up of 17 mo with normal family life and all children returning to school^[45].

Earlier transplantation for PFIC2 appears to be warranted as this subtype appears to progress to cirrhosis faster and also carries an increased risk for the development of primary liver cancers. Hepatoblastoma, hepatocellular carcinoma, and cholangiocarcinoma have all been reported in PFIC2^[46-48]. Transplantation in these patients is well tolerated with high graft and patient survival rates as well as great improvements in quality of life. Shimizu *et al.*^[49] reports two PFIC2 patients that were transplanted prior to the development of end-stage liver disease. Both siblings presented with jaundice and pruritus before 1 year of age. The elder sibling also demonstrated symptoms including acholic stools and failure to thrive. Histopathology revealed the classic findings of PFIC2 but no cirrhotic or malignant changes were identified. Neither sibling experienced major post-surgical complications.

Unlike in PFIC2, early transplantation in PFIC1 is controversial. Although liver transplantation corrects the *FIC1* gene in the liver and theoretically reverses the symptoms related to liver disease, the outcomes post-transplant are mixed. Hori *et al.*^[50,51] reported one of the largest series for patients that underwent liver transplantation for PFIC1. Eleven PFIC1 patients who received living-donor liver transplants were reported. Post-transplant steatosis was significant (moderate-severe) in 8 of the PFIC1 recipients (72.7%). Four of the 11 recipients eventually showed signs of cirrhosis post-transplant such as esophageal varices and splenomegaly^[50]. Two of the 11 PFIC1 patients suffered graft losses, and 10 of 11 patients (90.9%) reported digestive symptoms post liver transplantation. The survival rates of the PFIC1 patients at 5, 10 and 15 years liver transplantation were 90.9%, 72.7% and 54.5% respectively. Additional studies have also highlighted the presence or aggravation of severe digestive symptoms in addition to higher mortality rates following transplantation for PFIC1^[52-55]. Therefore, an attempt at medical management of symptoms and/or biliary diversion in PFIC1 patients should be considered prior to transplant. In additions, medical and/or surgical procedures to post liver transplantation should also be considered^[52-55].

In addition to considering delaying liver transplan-

tation in PFIC1 patients, the exact mutation specific to the PFIC1 patient may play a role in the development of steatohepatitis in the transplanted liver graft. Three of the 11 patients in this study had distinct mutations in the *FIC1* gene that did not result in persistent post-transplant diarrhea or steatosis^[54]. Lykavieris *et al.*^[37] reported two PFIC1 patients with specific mutations that both resulted in diarrhea exacerbation, appearance of liver steatosis and no catch-up of stature growth at 11 and 7.5 years post-transplant. Nicastro *et al.*^[55] similarly reported a PFIC1 patient upon whom gene analysis was done and was found to have double heterozygosity for two missense mutations. This mutation was associated with unremitting diarrhea, steatohepatitis and progressive fibrosis.

There is less data reporting on the outcomes of transplantation for patients with PFIC3. In patients that require transplantation, small series have reported excellent graft and patient survival^[44,52,56-58]. Like with PFIC2, liver transplantation is curative with resolution of pruritus and other manifestations of chronic liver disease. There are no reported cases of worsening of extrahepatic symptoms. The only post transplantation complications noted specifically for a PFIC3 patient was documented by Kaur *et al.*^[58] who noted grade 1 acute rejection in 1 post-operative patient. Greater than 80% patient survival rates in the groups that included known PFIC3 transplant recipients have been reported however post-operative quality of life for these patients needs to be further investigated.

In conclusion, liver transplantation is typically viewed as an option when patients have failed medical treatment and/or biliary diversion and have a poor quality of life due to refractory pruritus. Liver transplantation is also considered when patients have end stage liver disease or carcinoma. In regions where wait times potentially are shorter and/or living donation is available, liver transplantation can be considered earlier with excellent long-term survival and quality of life without the need to perform a biliary diversion. However, in cases of PFIC1, liver transplantation can be associated with an increase in extra hepatic manifestations, in particular chronic watery diarrhea and continued growth failure. Transplantation in this setting should be weighed against other options.

LIVING DONOR LIVER TRANSPLANTATION

Living donor liver transplantation (LDLT) has been shown to have outcomes equivalent to deceased donor liver transplantation^[59-61]. There is a significant survival advantage to patients transplanted with living donors as compared to those patients on the deceased donor waiting list by preventing death on the waiting list. This can be as high as 20% at some United States centers^[62]. In other parts of the world where deceased donation is non-existent, LDLT is the only option for patients with

ESLD. However, given that PFIC is an inherited disease, there was some concern that outcomes post transplant might be compromised when compared with deceased donor grafts from non-related donors. There have been several reports examining outcomes from LDLT that have refuted this notion.

All 13 PFIC patients who received a liver transplantation reported by Bassas *et al.*^[56] received a living related donor transplant. Eleven of the 13 patients survived and were without complications. The authors commented on the success of the grafts being due to adequate matching and graft size rather than the presence or lack of heterozygosity of gene variants in the donor. Similarly, of the 12 patients reported by Aydogdu *et al.*^[52], 6 received left lateral segment from living donors. All donors were biological parents. Four of the 6 patients were alive (66.7%) at 1 year follow-up. One patient death was due to hepatic artery thrombosis requiring re-transplantation and subsequent early post-operative death and the other patient developed post-transplant lympho-proliferative disease at 6 mo.

Several other smaller series and case reports have also corroborated these findings^[49,57,58,63]. Cutillo *et al.*^[57] reported 7 PFIC patients who received living related donor transplantation from parental donors. A previous family history of PFIC was found in three families and parental consanguinity in one family. Parental donors had normal liver functions tests and no personal past history of liver disease, gallstones, jaundice or cholestasis of pregnancy. They were alive and well at the time of follow up.

There is a natural concern for living related donor liver transplantation in patients with an inheritable intrahepatic cholestatic disease. However, grafts from related donors do not appear to be at higher risk for failure from PFIC-related causes. Living donation provides an excellent alternative to deceased donation and can provide timely liver transplant to patients.

ADVERSE OUTCOMES FOLLOWING LIVER TRANSPLANTATION

Like liver transplantation for other pediatric disorders, several well known complications have also been recorded such infection and rejection after transplantation for PFIC. These do not appear to occur at increased frequency post-transplant^[64]. In addition to the general complications associated with transplantation, there are some that are specifically associated with PFIC.

In patients with PFIC1, an undesired effect of liver transplant is the potential worsening of the extra hepatic manifestations like diarrhea and short stature^[52-55]. However, the manifestation and severity of these symptoms is unpredictable^[54]. The diarrhea is almost always associated with steatosis on liver histology as well^[50]. When these patients are treated with liver

transplantation, the impairment of bile salt secretion is corrected, and subsequently, there is a large increase in bile acid secretion relative to what the patient's body is accustomed to. The intestinal manifestations after transplant may reflect an important role for FIC1 in the intestine, where it is highly expressed. This increase in bile acids in the stool causes high volume osmotic diarrhea that has a significant impact on quality of life.

Bile acid resins and partial biliary diversion procedures have been shown to improve these symptoms. Cholestyramine has been reported to be very effective in these patients for managing post-operative diarrhea as well as aiding in overall growth progression^[37,50,53,54]. External biliary diversion post-transplantation in patients with PFIC1 who are experiencing an exacerbation of watery diarrhea has also been shown to improve symptoms as well as improve the steatosis on liver histology^[55,65].

PFIC2 patients with subtypes that have no immunodetectable BSEP in their native liver also appear to be at risk for the development of recurrent disease^[66,67]. Certain patients have developed antibodies against the BSEP protein in the donor liver^[66,68,69]. These antibodies cause similar symptoms of cholestasis, steatosis and fibrosis that were present in the original disease process. In some cases, these antibodies have resulted in recurrent graft failure^[70]. When allo-antibodies are detected, changes in immunosuppression and implementing plasmapheresis/molecular adsorbent recirculating system therapies have been shown to improve cholestatic episodes post-transplant in some of these PFIC2 patients^[70]. The use of rituximab has also been reported and shown to improve symptoms^[71].

ALTERNATIVES TO LIVER TRANSPLANTATION: MEDICAL AND SURGICAL THERAPIES

Both medical and surgical therapies play important roles in the management of patients with PFIC both as definitive therapy and as a bridge to transplant. In some cases, they have also been used to manage post-transplant complications.

Medical treatment for portal hypertension includes β -blockers and endoscopic management of esophageal and gastric varices when amenable. Fat-soluble vitamin supplementation and aggressive nutritional support with medium chain triglyceride - rich and high calorie concentrated formulas in infants is also important for the treatment of these patients as well.

Urso-deoxycholic acid (UDCA) increases hepatocyte excretion of bile acids and limits return to the liver by inhibiting their intestinal reabsorption. UDCA has been shown to improve symptoms and liver function tests in some patients with PFIC and is typically viewed as frontline therapy^[72-74]. Patients who experience the greatest benefit typically have milder forms of the disease, whereas patients with a total defect in MDR3

tend to be the non-responders to UDCA treatment. Recently, the degree of floppase activity in MDR3 was linked to response to UDCA treatment^[39]. In some cases, reversal of fibrosis with long term UDCA therapy has been noted^[75]. Combining 4-phenylbutyrate (4-PB) and UDCA treatment together has also been shown to be a promising pre transplant therapy for patients with PFIC2 in an effort to increase BSEP presence at the canalicular membrane^[76].

Cholestyramine and rifampicin are also used to provide symptomatic relief. Cholestyramine is a resin that binds bile salts in the intestinal lumen and thus reduces absorption and increases fecal bile salt excretion. Cholestyramine is the first line oral management for pruritus and is effective in up to 80%. Rifampicin aids in the excretion of bile salts and bilirubin in the urine, and aids in the treatment of pruritis.

Recently, Engelmann *et al*^[77] documented the use of steroids in PFIC2. These two patients were reported who were incidentally started on steroids for other medical reasons and who subsequently had complete resolution of symptoms and resolution of elevated bile salts.

Biliary diversion procedures

Biliary diversion procedures decrease the enterohepatic circulation of bile reducing its toxic effects. When offered early, biliary diversion is successful in reducing symptoms from pruritus and also slowing the progression of fibrosis^[11]. There are both partial external and internal biliary diversions that have been described. Nasobiliary drainage procedures when performed preoperatively can be helpful in the selection of patients that will have the highest success rate from the surgical diversion procedure^[17].

Partial external biliary diversion which was first described by Whittington uses a 10-15 cm jejunal conduit between the gallbladder and the abdominal wall creating a permanent biliary stoma^[78]. This procedure has been shown to improve growth, normalize liver function, reduce serum bile acids and improve liver histology^[79]. In many cases, this procedure is the first line surgical option and should be offered prior to the development of cirrhosis. However, once cirrhosis has been documented, these patients have poorer outcomes and should undergo liver transplantation^[80]. Success as documented by not progressing to liver transplantation is reported to be 23%-75%^[44,79-83]. This technique is also associated with significant complications including prolapse of the anastomosis, infection, and high volume bile excretion^[84]. Additionally, 1/3 of patients experience moderate to severe dehydration and hyponatremia^[84]. Modifications of this technique have included the use of a button cholecystostomy and also the use of the appendix in place of the jejunum as a conduit^[85,86].

Partial internal biliary diversion has the advantage in that it avoids an external stoma and the complications associated with it. The most common partial internal

biliary drainage links the gallbladder drainage to the colon^[87-89]. A modification of this procedure involving a laparoscopic cholecystocolostomy has also been described^[90]. Initial results from these techniques have been promising, but longer follow-up is needed. Internal diversion to bypass the distal 15% of the small intestine by creating an ileal colonic bypass has also been attempted but outcomes were poor^[82].

CONCLUSION

Until more research regarding targeted gene therapies and an increase in the development of the medical management for PFIC, liver transplant remains the most definitive treatment for those with PFIC. However, it is also important to consider current medical therapies and additional surgical interventions like biliary diversion that can potentially create a synergistic outcome. In particular, in patients with PFIC1, often the best clinical outcome and quality of life is an appropriate combination of all three of these therapies. Identification and better understanding of certain mutations in *FIC1* gene might lead to better patient selection. Similarly, in patients with PFIC2, the need for additional medical management can best be determined by pre-operative immunohistochemical studies which can help provide better clinical outcomes. Although the data for liver transplantation for PFIC3 is still lacking, it appears to be the preferred method of treatment with excellent long-term outcomes. There is currently no available clinical data regarding transplantation in the setting of mutations in *TJP2* gene (PFIC4).

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Massive haemorrhage in liver transplantation: Consequences, prediction and management

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Abstract

From its inception the success of liver transplantation has been associated with massive blood loss. Massive transfusion is classically defined as > 10 units of red blood cells within 24 h, but describing transfusion rates over a shorter period of time may reduce the potential for survival bias. Both massive haemorrhage and transfusion are associated with increased risk of mortality and morbidity (need for dialysis/surgical site infection) following liver transplantation although causality is difficult to prove due to the observational design of most trials. The blood loss associated with liver transplantation is multifactorial. Portal hypertension secondary to cirrhosis results in extensive collateral circulation, which can bleed during hepatectomy particularly if portal pressures are increased. Avoiding volume loading and maintenance of a low central venous pressure together with the use of vasopressors have been shown to reduce blood loss and transfusion during liver transplantation, but may increase the risk of renal impairment post-operatively. Coagulation defects may be present pre-transplant, but haemostasis is often re-balanced due to a deficit in both pro- and anti-coagulation factors. Further derangement of haemostasis may develop in the anhepatic and neohepatic phases due to absent hepatic metabolic function, hyperfibrinolysis and platelet sequestration in the donor liver. Point-of-care tests of coagulation such as the viscoelastic tests rotation thromboelastometry/thromboelastometry allow and more accurate and rapid assessment of these derangements in coagulation and guide the use of factor replacement and antifibrinolytics. Transfusion protocols guided by these tests have been shown to reduce transfusion rates compared with conventional coagulation tests, but have not shown

improvements in mortality or morbidity. Pre-operative factors associated with massive transfusion include previous surgery, re-do transplantation, the aetiology and severity of liver disease. Intra-operatively the use of piggy-back technique and avoiding veno-veno bypass has been shown to reduced blood loss.

Key words: Liver transplantation; Massive transfusion; Coagulopathy

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Core tip: The management of bleeding during liver transplantation requires an understanding of the unique coagulopathy of liver failure and the ability to recognize the risk factors for massive transfusion. By avoiding massive haemorrhage and transfusion, patients' outcomes after transplantation are likely to benefit.

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INTRODUCTION

The first human solid organ transplantation was performed in 1954 when Dr. Joseph Murray led a team in successfully transplanting a kidney between identical twin brothers^[1]. Liver transplantation proved far more difficult as patient decompensation was inevitable and the challenges of operating with massive and uncontrollable haemorrhage were substantial^[2]. In 1963, Starzl *et al*^[3] published the first case series of 3 patients, two of whom died shortly after the procedure and one bleeding to death on the operating table. Throughout the remainder of the 1960's liver transplantation was an experimental procedure with the first survival beyond a year not coming till 1967^[4]. Improvements in surgical outcomes became possible with the dramatic improvement in the graft quality due to the acceptance of the concept of brain death^[5], and with the introduction an effective immunosuppressive agent, cyclosporine^[4,6]. Survival after liver transplantation has steadily improved^[7,8], and orthotopic liver transplantation (OLT) is now an accepted treatment of advanced liver failure.

With the expansion of OLT programs in the early 1980's, there was an increasing demand on blood transfusion services. Butler *et al*^[9] reported red blood cell (RBC) transfusion rates in the range of 6-254 units per person in the first few years of their programme. With experience the same group was able to reduce their mean RBC, fresh frozen plasma (FFP) and platelet transfusion rates from 40 to 20 units per patient^[10],

which was comparable with other groups at the time^[11]. The reduction in transfusion rates was attributed to improved surgical technique and faster laboratory processing times to allow more rapid diagnosis and treatment of developing coagulopathies^[11]. Despite these advances, liver transplant recipients accounted for up to 25% of all the blood transfused in a hospital^[10] and had by far the greatest requirement for blood products of any solid organ transplants^[12].

Outcomes following liver transplantation have dramatically improved with 5-year graft survival rates in the United States of at least 70% despite transplants being performed on patients with a worse clinical condition due to the Model for End-Stage Liver Disease (MELD) score based organ allocation system^[13]. MELD was adapted by the United Network for Organ Sharing from a survival model used for patients undergoing transjugular intrahepatic portosystemic shunts^[14,15], and objectively predicts 3-mo mortality and therefore the need for transplantation^[16]. There has been an equally impressive decline in blood product use over the same period^[17,18] with case series describing OLT without the use of any blood products^[19-23]. Yet despite the notable improvements made in the management of blood loss and transfusion there remains a large variability in transfusion practices^[24]. This variability in transfusion practice of a precious resource is an important consideration as there may be implications for transplant morbidity and mortality^[25-29]. The impact of blood transfusion on surgical outcomes is an area of active debate, but the impact of massive transfusion is more convincing.

Recent reviews have discussed prediction of blood loss during liver transplantation^[30,31], and summarised strategies to reduce blood loss^[32,33]. This review will focus on massive haemorrhage in liver transplantation including consequence, prediction, and management as well as considering some of the lessons learned from other surgical specialties such as trauma and obstetrics.

DEFINITION

The classical definition of massive haemorrhage is the loss of one blood volume within a 24-h period^[34]. Correspondingly massive transfusion in an adult has commonly been defined as 10 or more units of packed red cells in a 24-h period, which approximates to replacement of one blood volume based on the approximate blood volume of a 70-kg male^[35].

These definitions are retrospective and often used as the basis for risk prediction models for massive blood loss and the implementation of resuscitative transfusion strategies and protocols. Their use has been questioned particularly in the setting of trauma as it excludes information regarding the patient's condition, institutional transfusion practices and the risk of survival bias as patients who die from exsanguination before receiving 10 units will not be included in the massive transfusion group^[36]. Haemorrhage is the main cause

of death following major injury in patients surviving to hospital admission with the highest incidence 1 to 3 h following admission^[37]. To address this researchers in trauma suggested more dynamic definitions of massive transfusion including the use of 4 red cell concentrates within one hour with likely on-going need^[38], 5-plus units within first four hours of admission^[39] or 10 units within 6 h^[35]. The PROMPTT trial investigators suggested two different approaches. Rahbar *et al.*^[35] demonstrated that resuscitation with four or more units (with 1 L crystalloid classed as 1 unit) of fluid within the first 30 min of admission for trauma was significantly associated with 6-h mortality and was a surrogate for sickness in severely bleeding patients. Alternatively Rahbar *et al.*^[36] using baseline admission characteristics (systolic blood pressure < 90 mmHg, HR > 120 bpm, pH < 7.25 and haemoglobin < 9) were able to develop a latent class model for those at risk of severe haemorrhage and in need a massive transfusion protocol (MTP). The British Committee for Standards in Haematology have suggested a similar dynamic definition as "bleeding which leads to a heart rate of more than 110 beats/min and/or systolic blood pressure less than 90 mmHg"^[40] in their most recent guidelines.

In obstetrics massive haemorrhage remains an important cause of morbidity and mortality with 13 death per 100000 maternities in the United Kingdom reported in the most recent confidential enquiry into maternal deaths. Post-partum haemorrhage (PPH) is defined as more than 500 mL from the genital tract within 24 h of birth and subdivided into minor (500-1000 mL), moderate (1000-2000 mL) and severe (> 2000 mL)^[41]. These definitions form the basis for activating protocols of resuscitation measures. The Royal College of Obstetrics and Gynaecology state that moderate PPH (1000 mL plus) with on-going bleeding or signs of shock should trigger such measures^[41].

Liver transplantation surgery in contrast to trauma and obstetrics is largely an elective or semi-elective procedure where blood loss can be anticipated and a strategized around. Death from exsanguination, common in the early days of transplantation is now a rare event and therefore the traditional definitions of massive haemorrhage/transfusion are less at risk of survivor bias. Defining massive transfusion as 6 unit or more in 24 h has been used in a number risk prediction studies for massive transfusion^[42-44].

EPIDEMIOLOGY OF HAEMORRHAGE DURING LIVER TRANSPLANTATION

Liver transplantation requires operating on patients with the pathophysiological changes associated with advanced cirrhotic liver disease. The presence of portal hypertension and the haemostatic changes that occur both as a consequence of hepatocyte death and during the stages of liver transplantation itself are important causes of bleeding that are unique to this procedure.

Portal hypertension

As chronic liver disease progresses hepatocyte death leads to inflammation and the subsequent generation of fibrosis that marks the onset of cirrhosis^[45]. Increased intrahepatic vascular resistance (HVR) with maintained portal blood flow requires increased portal pressures. Approximately, 70% of the portal hypertension is attributed to structural factors (fibrosis, vascular remodelling, vascular occlusion, nodule formation) whilst the remaining 30% is thought to be due to dynamic functional abnormalities in the liver microvasculature^[46]. A reduction in intrahepatic vasodilators (of which nitric oxide may be the most important) combined with an increased activity and sensitivity to endogenous vasoconstrictors contribute to the dysfunctional nature of sinusoidal endothelial cells with vasoconstriction of microvasculature and increased HVR^[46]. As portal hypertension develops portosystemic collateral vessels form and blood from the splanchnic circulation is diverted into these collateral vessels^[46]. In addition to increased portal blood flow, thinning of arterial walls in these circulatory beds increase the susceptibility for blood loss.

Coagulopathy of liver disease

The liver synthesises most of the circulating proteins of coagulation needed in haemostasis, therefore there is a decreased level of many of these proteins in liver failure^[47]. Conventional tests of coagulation are often deranged in advanced liver disease reflecting the deficiency in procoagulant factors. The prothrombin time (PT) and international normalised ratio (INR) are useful markers of hepatic synthetic function. The INR is also used in combination with recipient age, bilirubin and creatinine to calculate the MELD score.

Conventional coagulation tests are, however, poor predictors of peri-procedural bleeding in end-stage liver disease with no increase in bleeding seen in patients undergoing invasive procedures such as cardiac catheterisation^[48] or dental extraction^[49]. The main source of bleeding seen in liver disease pre-transplant is secondary to variceal haemorrhage, with portal hypertension and splanchnic haemodynamics the proposed mechanism for bleeding rather than coagulopathy.

The haemostasis in liver failure is neither shifted towards bleeding nor thrombosis, but has been referred to as a balanced coagulopathy^[50]. Thrombocytopenia and reduced platelet function is offset by elevated levels of von Willebrand factor (vWF) and decreased levels of ADAMTS 13 (a metalloprotease which cleaves vWF)^[51]. All pro-coagulant proteins are reduced in hepatic insufficiency with the exception of factor VIII, but so too are the levels of anti-coagulants antithrombin and protein C and S^[50]. It has been suggested that the relative excess of plasma coagulation factors in health provides a "margin of safety" to account for physiological or pathological stresses to the system^[50]. Without this excess of coagulation factors the balanced coagulopathy of liver failure can be thought of as more susceptible

to the perturbations associated with the perioperative period.

This revised understanding of the coagulopathy of liver failure challenges the ubiquitous use of plasma to correct abnormal blood tests and should focus the use of blood products to manage overt microangiopathic bleeding^[2]. In fact, the aggressive correction of derangements in INR without supportive evidence of impaired clotting may not only be unnecessary, but harmful in and of itself. In portal hypertensive rats subjected to a period of haemorrhage, replacing the exact volume lost with blood results in an increase in portal pressures by 20%^[52], higher rates of haemorrhage and worse outcome^[53]. This has subsequently been demonstrated in patients with severe acute upper GI bleeds. Those treated with a restrictive transfusion strategy had lower portal pressures, lower rates of further bleeding and higher rates of survival compared to those treated with a liberal strategy^[54].

Phases of transplantation

During the pre-anhepatic phase of transplantation the surgeon has to perform a hepatectomy whilst contending with the numerous porto-systemic collaterals and the hyperdynamic, dilated, thin walled splanchnic circulation. Adhesions from previous surgery can be another source of blood loss^[2]. During the anhepatic phase, hepatic synthesis and clearance is absent, and hyperfibrinolysis can increase rapidly with the accumulation of tissue plasminogen (t-PA)^[55]. Plasma t-PA increases the conversion of plasminogen to plasmin. The end result is that during the anhepatic phase fibrinogen production is stopped and the consumption of fibrin is promoted leading to a rapid consumption of the primary building block of clot formation and increased blood loss^[56]. In the neohepatic phase, fibrinolysis is further stimulated by the release of t-PA from the ischaemically injured endothelium of the donor liver^[57]. Platelet counts commonly decrease due to sequestration into the sinusoids, extravasation of platelets into disse spaces and phagocytosis by Kupffer cells^[55].

CONSEQUENCES OF MASSIVE BLOOD LOSS AND MASSIVE TRANSFUSION

Transfusion of RBCs and blood products has been linked to adverse outcomes in OLT patients^[28,58]. Even modest transfusion requirements have been linked to prolonged lengths of hospital stay, with the use of more than 6 units of red cells having the greater impact in decreased survival rates^[44]. de Boer *et al.*^[59] demonstrated a dose related effect in one year survival rates, with a HR of 1.37 per unit of platelets and 1.07 per unit of packed red blood cells, in their multivariate analysis of a cohort of 433 adult OLT patients.

Both short and long-term survival appears to be affected by intraoperative massive blood transfusion (MBT). Rana *et al.*^[28] found that an intraoperative blood

transfusion of > 28 units was as significant risk factor for decreased 3 mo survival in a study of 233 consecutive liver transplant recipients performed by the same experienced surgeon. Intraoperative blood transfusion greater than 5 units was independently associated with reduced 3 and 5 years survival in a study of 102 living donor liver transplant patients^[60].

Observational studies have demonstrated a link between blood loss and transfusion requirements and increased morbidity in OLT patients. Transfusion requirements of > 17.5 packed red cell units and > 3.5 platelet units in a study including 291 consecutive OLT patients were found to accurately predict the requirement for post-transplant renal replacement therapy^[29]. Transfusion of > 2 units of packed red cells was identified as a risk factor for development of surgical site infections in liver transplant recipients^[61]. Intraoperative blood loss was also found to be the main determinant of early surgical re-intervention after OLT^[62].

It is important to highlight that studies investigating outcomes following liver transplantation are limited by their observational nature in that they demonstrate association and not causality between blood loss, transfusion requirements and morbidity and mortality outcomes.

PREDICTION OF MASSIVE TRANSFUSION IN LIVER TRANSPLANTATION

A number of studies have identified factors associated with massive blood loss and transfusion requirements in liver transplant patient populations (Table 1)^[42-44,60,63-79]. Risk factors can be classified based on the perioperative period and surgical factors.

Preoperative risk factors

Patient, donor organ or other factors that increase the duration or technical difficulty of the surgical procedure such as previous abdominal surgery^[25,60,73,80] or redo transplantation^[42] are independently associated with higher blood loss and transfusion requirements. Observational studies suggest that haemostasis, coagulopathy and risk of bleeding differ according to the cause of liver failure. For instance, patients with primary biliary cirrhosis exhibit a preserved capacity for thrombin generation and less fibrinolytic activation during the anhepatic phase compared with other cirrhotic states^[81]. Case series of patient with portal vein thrombosis undergoing liver transplantation report greater operation times and consumption of blood products^[80,82]. Increasing age of the recipient has been reported as predictor of MBT in a number of studies^[42,72,79]. McCluskey *et al.*^[42] found age to be a weak predictor and the authors remarked that age is likely to be a surrogate for other unidentified risk factors.

Severity indexes of liver disease have been investigated as predictors of blood loss during liver transplant surgery. The Child-Turcotte-Pugh (CTP) score uses

Table 1 Studies evaluating red blood cell transfusion requirements and prediction variables in adult liver transplantation

Ref.	No. of patients	Population	Data methodology	Outcomes	Final model prediction variables	Performance of model
Motschman <i>et al</i> ^[63]	83	OLT	Retrospective univariate and multivariate	RBC transfusion requirement	History of previous GI bleed, Previous RUQ surgery	
Deakin <i>et al</i> ^[64]	300	OLT	Retrospective univariate and stepwise multivariate	≥ 7 units RBC	Urea levels and platelet count	Specificity 62%
Findlay <i>et al</i> ^[65]	583	OLT	Retrospective univariate and multiple linear regression analysis	RBC transfusion requirement	Age, creatinine and bilirubin	Sensitivity 68% R = 0.22
Steib <i>et al</i> ^[66]	410	OLT	Retrospective univariate and stepwise multivariate analysis	High blood loss ≥ 12 units RBC	Preoperative Hb, previous abdominal surgery, preoperative FDP	Sensitivity 18% Specificity 98%
Pirat <i>et al</i> ^[67]	40	OLT	Bivariate and multiple linear regression	RBC transfusion requirement	Preoperative albumin	R = 0.48
Ramos <i>et al</i> ^[44]	122	OLT	Univariate and multivariate regression	> 6 units RBC	UNOS class and placement of caval shunt	
Massicotte <i>et al</i> ^[68]	206	OLT	Retrospective univariate and multivariate logistic regression	> 4 units RBC	Starting INR, platelet count and duration of surgery	
Yuasa <i>et al</i> ^[69]	635	LDLT (adult and pediatric)	Univariate	Arbitrary high blood loss	Univariate = age < 1 yr, Hct $< 30\%$, T-Bil > 20 mg/dL, BUN > 30 mg/dL. Dx Pre-op atresia, Re transplantation	
McCluskey <i>et al</i> ^[42]	460	OLT	Multivariate regression. Risk index internally validated	> 6 units RBC in 24 h	Age > 40 , Hb < 10 g/dL, NR 1.21-1.99 and > 2 , platelet < 70 , creatinine > 110 mmol/L female and > 120 mmol/L males, albumin < 28 h/L and redo transplant	C statistic model = 0.79
Mangus <i>et al</i> ^[70]	526	OLT "piggy back"	Univariate and multivariate regression	RBC transfusion requirements	Pre-op Hb MELD score, Initial CVP	
Massicotte <i>et al</i> ^[71]	505	OLT	Nomogram risk model based on multivariate regression analysis		FFP transfusion. High starting Hb and phlebotomy protective for blood loss	Bootstrapped AUC prediction model = 89.8%
Araújo <i>et al</i> ^[72]	758	OLT	Retrospective univariate and multivariate regression	RBC requirements	PT, Hb, age, liver malignancy	R = 0.30
Bang <i>et al</i> ^[73]	555	LDLT	Multivariate regression	Intraoperative blood loss > 1000 mL	MELD, albumin, ascites and previous abdominal surgery	
Roullet <i>et al</i> ^[74]	148	OLT	Univariate and multivariate regression	> 8 units RBC and loss of > 1 blood volume	Preoperative Hb and Child-Pugh A protective for blood loss > 1 blood volume	
Esmat Gamil <i>et al</i> ^[43]	286	OLT	Univariate and multivariate logistic regression	> 6 units RBC	INR > 1.6 , Ascites	
Li <i>et al</i> ^[60]	181	LDLT	Univariate and multivariate regression	> 6 units RBC	Platelet count $< 70 \times 10^9/L$, Hb < 100 g/L, fibrinogen < 1.5 g/L and previous abdominal surgery	
Wu <i>et al</i> ^[75]	522	LDLT	Univariate and multivariate regression	Re-exploration for hemostasis	> 10 mL/kg FFP transfusion	
Varotti <i>et al</i> ^[76]	219	OLT	Univariate and multivariate regression	RBC transfusion requirements	MELD	
[77]	291	OLT (no malignancy or re-transplant)	Multivariate logistic regression	RBC transfusion requirements	Baseline Hb and Fibrinogen	
De Santis <i>et al</i> ^[78]	166	OLT "piggy back"	Univariate and multivariate regression	Blood product requirements	Child-Pugh, preoperative Hb and INR, graft ischemia time	
Cywinski <i>et al</i> ^[79]	804	OLT	Multivariate regression Bootstrapping for prediction model	RBC and cell saver requirement, > 20 and > 30 RBC units usage	MELD and preoperative platelet count	RBC + CS > 20 units c = 0.70 (RBC + CS > 30 units c = 0.67)

OLT: Orthotopic liver transplantation; LDLT: Living donor liver transplantation; RBC: Red blood cells; GI: Gastrointestinal; RUQ: Right upper quadrant; UNOS: United Network for Organ Sharing; INR: International normalized ratio for prothrombin activity; FFP: Fresh frozen plasma; Hct: Hematocrit; Hb: Hemoglobin; PT: Prothrombin time; MELD: Model for End-Stage Liver Disease; CVP: Central venous pressure; AUC: Area under the curves.

levels of serum bilirubin, albumin, PT and the presence of ascites and encephalopathy to quantify of disease severity. Multiple studies have included the CTP score in multivariate analyses of factors associated with increased blood loss during liver transplantation with diverging results^[44,60,68,78]. De Santis *et al.*^[78] found in a population of 166 "piggy-back" OLT that the CTP score together with haemoglobin and graft ischaemia time to be associated with blood and blood products transfusion requirements. A CTP class A was found to be a protective risk factor for bleeding more than one blood volume in a study including 148 OLT patients^[74].

Multivariate analysis found an association between pre-operative MELD scores and blood products usage or massive blood loss in different liver transplant patient populations such as hepatitis B related cirrhosis^[83], living donor^[73], piggyback^[70] and mixed OLT populations^[76,79]. MELD was significantly associated with patients requiring the use of blood products, but failed to predict those requiring massive blood transfusions^[79]. MELD was also a poor predictor of blood loss or blood transfusion requirements in a series of 350 patients with mean MELD scores of 20 ± 10 ^[71]. It is important to note to mention that the reported mean transfusion requirement was only 0.5 ± 1.3 unit which is lower than the reported by other studies in similar populations^[76].

Preoperative haemoglobin is an important predictor of blood transfusion in a number of multivariate models^[42,60,66,70,72,77,83]. Preoperative haemoglobin of more than 12.6 g/dL was found to be a protective factor for blood loss of one blood volume or more in a series of 148 patients receiving OLT^[74]. Thrombocytopenia pre-transplant is also associated with massive blood transfusion requirements^[60,80].

Coagulation variables such as the INR and fibrinogen are predictors of blood loss and transfusion requirements. A cut-off INR of ≥ 1.6 was found to be predictor of > 6 units blood transfusion requirement in an study of 286 patients receiving OLT^[43]. Preoperative INR values were also found to be independent predictors of risk for MBT in a study of 460 liver transplant recipients^[42]. Fibrinogen levels below 1.5 g/dL were associated with increased risk for transfusion of > 6 units of RBC in living donor related transplant patients^[60].

The presence of ascites was found to be predictive of a transfusion requirement of > 6 units RBC^[43] and of high intraoperative blood loss (> 1000 mL)^[73]. The development of ascites may serve as a marker of portal hypertension with an associated increase in collateral circulation and dilated blood vessels that may be transected during surgical dissection.

Models to improve prediction of blood loss and MBT requirements have been developed from preoperative risk predictor variables that are readily accessible to the clinician during the preoperative assessment. The McCluskey risk index for MBT includes seven preoperative variables: Age > 40 years, haemoglobin concentration (≤ 10.0 g/dL), INR 1.2-1.99 and > 2), platelet count $\leq 70 \times 10^9$ /L, creatinine (> 110 μ mol/L

for female subjects and > 120 μ mol/L for male subjects,) and repeat transplantation. The model was internally validated achieving a high c statistic (0.79)^[42]. External validation of the McCluskey index attained reasonable sensitivity (80%) and specificity (84.21%)^[84]. However, more recently, Cywinski *et al.*^[79] also attempted to create a prediction model for intraoperative blood product requirements based on preoperative variables. The authors used several advanced statistical techniques to analyse data from 804 primary OLTs performed during a 9-year period. Although, they found a strong relationship between transfusion and postoperative mortality, the model proved to be an unreliable predictor of transfusion requirements^[79].

Surgical factors

Advances in surgical techniques and experience have been crucial for the reduction in blood loss. The piggy-back technique involves a single anastomosis of the donor vena cava to the recipient inferior vena cava and a shortened warm ischemic time^[85]. Additionally, the preservation of the recipient's vena cava reduces the requirement for extensive resection of the retroperitoneum. Large case series of patients undergoing OLT using the piggyback technique report a reduction in transfusion requirements^[86-88] compared with the classic technique or use of veno-venous bypass. Veno-venous bypass has been found to be an independent predictor for increased blood loss and transfusion requirements^[44,89]. It is thought that the contact with the bypass circuits triggers fibrinolysis, haemolysis and platelet activation, thus impairing or worsening haemostasis. Despite the encouraging data from case series, a Cochrane review that included two trials with high risk of bias comparing the piggyback with the conventional method of liver transplantation did not find enough evidence to recommend or refute the use of the piggy-back method^[85].

MANAGEMENT OF MASSIVE BLOOD LOSS

Lessons from the Battlefield

Many of the developments in the management of the exsanguinating patient have come from the trauma literature and the experience gained by treating military casualties in the Iraq and Afghanistan wars. Haemorrhage is the leading cause of death in the first hour following traumatic injury and causes 40% of all trauma deaths^[90]. Treatment of massive haemorrhage was historically concerned with restoration of the circulating volume using crystalloids until a transfusion trigger was met (commonly 6 g/L) after which packed red cells were to be given. Both British and American guidelines advised only giving FFP after the loss of approximately one blood volume and aiming for an INR < 1.5 ^[34,91]. Coagulation abnormalities with trauma patients were thought to be as a result of closed head injury or iatro-

genic due to massive blood transfusion or excessive fluid resuscitation. Two papers from 2003 challenged this concept and demonstrated that patients presenting with major trauma commonly had a significant coagulopathy that was present before resuscitation had commenced and was an independent predictor of mortality^[92,93]. This coagulopathy was termed acute coagulopathy of trauma.

Acute coagulopathy of trauma is characterised by ooze-type bleeding from mucosal regions, serosal surfaces and vascular access sites distinct from simple massive bleeding^[94]. It consists of endogenous primary pathologies - disseminated intravascular coagulation (DIC) and acute coagulopathy trauma shock (ACOTS), and exogenous secondary pathologies that mimic DIC and ACOTS - hypothermia, acidosis, anaemia and dilutional coagulopathies^[95]. Similarities between the pathophysiological changes that occur in liver transplantation have been discussed in a recent review on haemostasis in liver transplantation^[96]. Derangements in thrombin-thrombomodulin-protein C system lead to anticoagulation in both trauma and liver transplantation patients^[96]. Catecholamine release during traumatic injury is thought to directly damage the endothelium resulting in progressive de-endothelialisation. High levels of syndecan-1, a marker of endothelial degradation is association with inflammation, coagulopathy and increased mortality in trauma patients^[97], and patients with end-stage liver disease have recently been demonstrated to have significantly higher levels than controls^[98]. These levels are further elevated following graft reperfusion during liver transplantation.

MTPs with fixed ratios of red cells to plasma more closely approximating whole blood transfusions came to the fore following a retrospective analysis of United States army combat patients requiring massive transfusion. Those that were treated with a high plasma to RBC ratio had a significantly improved survival to hospital discharge compared with those treated with low ratio transfusion, primarily through decreasing death from haemorrhage^[99]. These results led to a proliferation of studies reporting beneficial outcomes from high plasma:RBC ratio MTPs in trauma^[100,101] as well as obstetrics^[102,103]. Part of the benefit must be attributed to the decreased delay in obtaining blood products and improved communication between the laboratory and the team treating the patient. One criticism of the studies investigating MTPs is that they are largely retrospective before and after studies that are subject to survivor bias. Given the lack of high quality trials the Canadian National Advisory Committee on Blood and Blood products took the decision in 2011 that fixed ratio formula based care could not be recommended as a standard of care^[104]. In an attempt to address these concerns two large concurrent prospective multicentre trials have been conducted in severely injured adult civilian trauma patients.

The observational trial PROMMTT, demonstrated reduced 30-d mortality in patients treated with a higher

FFP/Platelet to red cell ratio early in resuscitation and went on to inform the design of the randomised control trial PROPPRR^[105,106]. Here, while 30-d mortality was not improved in patients treated with a 1:1:1 ratio vs 1:1:2 (plasma:platelets:red cells), fewer patients died from exsanguination in the first 24 h^[107]. Criticism of the use of fixed ration protocols cite the potential waste of blood products and the one-size fits all approach to massive haemorrhage. MTPs promote the early use of plasma and platelets, which might otherwise be delayed if waiting for conventional laboratory coagulation test results to guide treatment. The increasing availability of point of care (POC) haemostatic tests such as the viscoelastic assays, rotational thromboelastometry (ROTEMTM) and thromboelastometry (TEGTM), provide an alternative. Tapia *et al.*^[108] demonstrated that TEGTM guided resuscitation was superior to standardized MTP resuscitation of penetrating trauma patient and Karkouti *et al.*^[109] were able to demonstrate a significant reduction in transfusion rates for all blood products for patients undergoing cardiac surgery through a ROTEMTM based algorithm. Recent state of the art papers on the management of traumatic haemorrhage have viscoelastic tests integrated into MTPs^[38,110-112]. In the presence of uncontrolled haemorrhage, fixed ratio transfusion packages are instigated converting to viscoelastic test-guided goal-driven resuscitation once bleeding slows^[110]. While trials comparing fixed ratio-guided resuscitation with viscoelastic test-guided in liver transplantation are lacking it is usually a well-controlled procedure and most centres have access to POC coagulation monitors to guide transfusion, the fixed ration MTP's are possibly only required in the most uncontrolled of settings.

Fluid management

Another strategy to reduce blood loss is fluid restriction similar to liver resection surgery. However, excessive fluid restriction may have deleterious consequences including hemodynamic instability and postoperative renal impairment. Schroeder *et al.*^[113] conducted a retrospective record review comparing two liver transplant centres using "low" central venous pressure (CVP) (< 5 mmHg) and "normal" CVP (7-10 mmHg) targets during liver transplant. Even though transfusion rates were reduced, increased rates of postoperative renal failure and 30 d mortality were observed in the "low" CVP group.

Reduction of blood loss through maintenance of a low CVP must be balanced against adequate tissue perfusion. Static pressure measurements such as CVP are unreliable indicators of volume status and adequacy of organ perfusion^[114]. Dynamic (pulse and stroke volume variation) and thermodynamic (Intrathoracic Blood Volume Index) have demonstrated superior performance compared to static pressure measurements in terms of volume status assessment and preload dependence prediction in critical care and perioperative settings^[115]. Studies looking at the performance of dynamic parameters during liver transplant surgery have

produced mixed results^[116,117] and their impact on liver transplantation outcomes requires further research.

Vasopressors

A variety of pharmacological agents can produce selective vasoconstriction of the splanchnic vascular bed and reduce portal blood flow. Vasopressin, octreotide and phenylephrine are examples of agents that have been studied as potential interventions for blood loss reduction during OLT. Use of low dose vasopressin (0.04 U/min) infusion during the dissection phase was associated with reduce blood loss compared with control group in a retrospective non randomised study of 110 OLT patients^[118].

The effect of an octreotide infusion was studied in a randomised controlled trial of 79 patients undergoing OLT. The study found that an octreotide infusion was associated with an increased urine output during the operation compared to control, but it failed to show any significant difference in terms of blood loss or blood transfusion requirements^[119].

Phenylephrine administration was found to be associated with decreased blood loss and lower lactate levels compared to patients receiving inotropes (dobutamine or dopamine) for cardiovascular support during liver transplant^[120]. Phenylephrine was also found to be useful in restoring systemic arterial pressure following phlebotomy aimed at reduced portal venous pressure and thus blood loss during the dissection phase of OLT^[121].

Transfusion thresholds and coagulation monitoring

There is significant variability among liver transplantation centres in methods of coagulation monitoring, transfusion triggers and transfusion protocols^[24]. There is no evidence supporting specific haemoglobin or haematocrit triggers for packed RBC transfusion in OLT. However, data from other surgical and critical care populations indicates that transfusion strategies targeting lower perioperative haemoglobin levels are safe and can lead to a reduction in RBC transfusion. A transfusion threshold of 70 g/L for hemodynamically stable critically ill is suggested by data from the Transfusion Requirements in Critical Care trial^[122]. The Transfusion Reduction Threshold Reduction Trial (TITRe2) compared the outcomes of a large population of cardiac surgical patients finding no evidence of harm with the use of a restrictive threshold of 75 g/L compared with a "liberal" threshold of 90 g/L^[123]. Similarly, results from a randomized surgical trials of hip surgery patients with pre-existing cardiovascular disease indicate that a restrictive RBC transfusion strategy is not associated with harm^[124]. Some guidance can also be extrapolated from a randomize study performed in the setting of severe acute gastrointestinal bleeding excluding massive exsanguinating bleeding, concurrent acute coronary syndrome, stroke or peripheral vascular disease. All patients received endoscopic and treatment for bleeding

within 6 h if required. Patients were randomized to a "liberal" RBC transfusion threshold of 90 g/L or "restrictive" of 70 g/L. Thirty-one percent of patients in both groups had cirrhosis and bleeding was due to oesophageal varices in 21% of the patients. The authors observed improved mortality rates, reduced risk of further bleeding, and less complications such as pulmonary oedema, in patients randomised to the restrictive strategy.

There is some evidence that erythrocytes stimulate thrombin generation and play a concentration dependant role in accelerating the initial coagulation reaction^[125]. Therefore, higher haemoglobin concentrations may be desirable during acute bleeding associated with hemodynamic instability.

Blood loss during liver transplant surgery can occur in a slow and protracted manner or can be rapid and cause severe hemodynamic instability limiting the applicability of haemoglobin thresholds. During exsanguinating blood loss transfusion should be guided by the rate of bleeding and the likelihood of surgical control: Guided by transfusion indicators and POC testing where possible and guided by fixed ratio transfusion of RBC, plasma and platelets when bleeding is acute and time does not permit real time assessment of the coagulation status.

Viscoelastic tests of coagulation (TEGTM, ROTEMTM) provide a dynamic picture of the interaction of the whole blood coagulation and fibrinolytic systems. Viscoelastic methods have faster turnaround times compared to traditional tests and are POC or bedside tests, performed in close proximity to the patient in the operating room or critical care areas.

The use of POC viscoelastic methods of coagulation monitoring and their inclusion in blood and blood products transfusion algorithms has been found to be associated with reduced blood and blood products requirements in cardiac surgery^[126]. A Cochrane review including 9 RCTs concluded that the use of ROTEMTM or TEGTM to guide transfusion strategies in patients with massive bleeding appears to reduce the amount of bleeding and requirement for blood and blood products, but found no evidence of benefit in terms of morbidity and mortality^[127].

Another Cochrane review studying interventions to reduce blood loss in liver transplantation analysed two randomised studies using thromboelastography in liver transplant populations^[128]. The studies were both single centre and included a population of adults undergoing OLT^[129,130]. The authors concluded that thromboelastography-guided transfusion was associated with a reduction in FFP transfusion requirements but had no impact on 3-year survival rates, RBC or platelet transfusion requirements. The trials were however deemed to have a high risk of bias by the Cochrane reviewers.

Viscoelastic tests can detect the presence and degree of fibrinolysis at different stages of the transplant procedure and can be used effectively to guide the need

for and response to anti-fibrinolytic therapy^[131].

Antifibrinolytics

There are 2 major classifications of antifibrinolytic agents, the lysine analogues [aminocaproic acid, Amicar and cyclokapron, tranexamic acid (TXA)], and the trypsin inhibitor (aprotinin, Trasylol). Hyperfibrinolysis may lead to significant blood loss due to diffuse micro-vascular bleeding, however, much of the fibrinolysis is self-limiting which might help to explain why our ability to predict massive transfusion is difficult and it calls into question the routine prophylactic use of anti-fibrinolytic therapy. In most circumstances the risk of thromboembolic complications with an antifibrinolytic is low providing an excellent therapeutic index, but in liver failure our inability to identify thromboembolic risk is also limited^[132] and therefore the judicious use of these agents is recommended. Patients with a pro-thrombotic state, such as primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular carcinoma, portal vein thrombosis and Budd-Chiari syndrome, may be at particularly increased risk of thromboembolic complications.

In 1987 Royston demonstrated a dramatic reduction in blood loss with aprotinin in patients undergoing repeat open heart surgery and its use in cardiac surgery was approved by the Food and Drug Administration in 1993. Concerns regarding an increased risk of renal dysfunction were raised in several observational trials^[133,134]. The publication of the Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) trial raised additional concerns where patients undergoing high risk cardiac surgery were shown to have a significantly higher 30-d mortality when given aprotinin vs tranexamic acid or aminocaproic acid^[135] led to its licence being withdrawn in a number of countries. A number of concerns regarding the methodology of the BART trial have subsequently been raised and a review by Health Canada found that the trial was too small to reliably assess mortality and concluded that the benefits of aprotinin outweighed its risks^[136]. Studies investigating the aprotinin ban on blood loss in liver transplantation give mixed results with both an increase in blood transfusion rates following its withdrawal^[137] and no change^[138] being reported.

Several systematic reviews have investigated the use of antifibrinolytics in liver surgery. A recent Cochrane review focused on methods to decrease blood loss and transfusion requirements in liver resection surgery including 33 trials involving 1913 patients with interventions comparing aprotinin vs control, TXA vs control and TXA vs aprotinin^[139]. There was no significant difference in 60-d mortality or thromboembolic episodes and while aprotinin was associated with a significantly lower allogenic blood transfusion requirements, it did not confer any outcome benefit. Importantly, the reviewers deemed all the trials to have high risk of bias thus further weakening the strength of the conclusions^[139].

In liver transplantation recipients a systematic review

and meta-analysis of 23 studies including 1407 patients analysed the effect of either TXA or aprotinin on blood loss, transfusion requirements and incidence of thromboembolic^[132]. Blood loss and transfusion requirements were lower with TXA compared to controls, but the thromboembolic risk was unchanged in groups of patient receiving anti-fibrinolytic therapy^[132].

In OLT, thromboembolic events are relatively rare and as such trials studying TXA lack statistical power to detect clinically significant important increases on thromboembolic risk^[140]. However, it would be prudent to treat with TXA only in presence of fibrinolysis, observed clinically as microvascular bleeding or evidenced by POC test such as TEGTM or ROTEMTM. Routine use is no longer recommended in international guidelines^[141] and should be carefully considered in patients at risk of thromboembolic complications.

Cell salvage

Intraoperative cell salvage has been adopted in a variety of surgical settings in an effort to reduce allogeneic blood transfusion rates and thus potential complications and cost associated with the transfusion of allogeneic blood^[142]. Controversy exists surrounding the use of cell salvage in liver transplantation. The washed RBCs are devoid of clotting factors and platelets and there is potential for accumulation of fibrinolytic factors released by the processed RBC or the transplanted liver. Older studies appeared to substantiate these concerns suggesting that transfusion of salvaged blood was associated with increase blood loss and requirement for blood products^[143]. The cost effectiveness of cell salvage has also being questioned^[144]. More recent studies have demonstrated the efficacy of cell salvage in reducing the need for allogeneic blood transfusion for both OLT^[145] and living donor liver transplantation^[146]. The cost effectiveness of cell salvage was also established in a large prospective study including 660 liver transplant patients where a total cost saving of \$188618 United States dollars was achieved over the study period^[147].

Malignant disease is a relative contraindication for cell salvage due to the risk of metastasis arising from cancerous cells that are not eliminated by the cell salvage process. Intraoperative cell salvage has however been used in the setting of hepatocellular carcinoma with no apparent increase in recurrence rates^[148]. Leucocyte depletion filters incorporated into cell salvage circuits have shown to effectively remove malignant cells when used during liver transplantation of patients with non-ruptured hepatocellular tumours^[149].

Bacteria can contaminate salvaged red cells when suctioned blood is mixed with biliary, bowel secretions or is in contact with the skin. A study analysing bacterial contamination of salvaged blood during liver transplant found that even though micro-organisms can be observed in to up to 70% of the processed and reinfused units, none of the postoperative blood cultures revealed growth of the same micro-organisms^[150]. It is however, advisable to avoid aspiration of blood after initiation

of the biliary anastomosis stage of the liver transplant procedure.

CONCLUSION

The management of bleeding associated with liver transplantation remains an important area of investigation and no one change in clinical practice will have a dramatic impact. What is required is a concerted effort including the identification of patients at risk for massive blood loss, POC evaluation of medically manageable bleeding, and cost effective blood conservation strategies designed specifically for each patient. The beneficiaries of our efforts will be the transplant recipients in prolonged disease free survival and our health care systems in reduce cost per patient by both reducing blood product utilization and hospital length of stay.

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Loco-regional therapies for patients with hepatocellular carcinoma awaiting liver transplantation: Selecting an optimal therapy

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Abstract

Hepatocellular carcinoma (HCC) is a common, increas-

ingly prevalent malignancy. For all but the smallest lesions, surgical removal of cancer *via* resection or liver transplantation (LT) is considered the most feasible pathway to cure. Resection - even with favorable survival - is associated with a fairly high rate of recurrence, perhaps since most HCCs occur in the setting of cirrhosis. LT offers the advantage of removing not only the cancer but the diseased liver from which the cancer has arisen, and LT outperforms resection for survival with selected patients. Since time waiting for LT is time during which HCC can progress, loco-regional therapy (LRT) is widely employed by transplant centers. The purpose of LRT is either to bridge patients to LT by preventing progression and waitlist dropout, or to downstage patients who slightly exceed standard eligibility criteria initially but can fall within it after treatment. Transarterial chemoembolization and radiofrequency ablation have been the most widely utilized LRTs to date, with favorable efficacy and safety as a bridge to LT (and for the former, as a downstaging modality). The list of potentially effective LRTs has expanded in recent years, and includes transarterial chemoembolization with drug-eluting beads, radioembolization and novel forms of extracorporeal therapy. Herein we appraise the various LRT modalities for HCC, and their potential roles in specific clinical scenarios in patients awaiting LT.

Key words: Liver transplantation; Loco-regional therapy; Transarterial chemoembolization; Radioembolization; Hepatocellular carcinoma

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Core tip: Hepatocellular carcinoma has increased in incidence in recent decades. Liver transplantation is an excellent therapy for carefully selected patients. Due to the risk of tumor progression while awaiting liver

transplantation, loco-regional therapy is frequently used in this setting. An expanding array of treatment options exist and are herein characterized, including descriptions of which modality may be ideal in various settings.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common human malignancy and the third leading cause of cancer-related death^[1,2]. Driven largely by the hepatitis C virus (HCV) epidemic, the age-adjusted incidence of HCC in developed nations has approximately tripled since the early 1970's^[3]. Cirrhosis is the major risk factor in HCC formation and is present in the vast majority of cases.

Therapy for HCC has evolved during recent decades. While some small HCCs may be fully eradicated with percutaneous ablation^[4], surgery with resection or liver transplantation (LT) is considered the only curative option in most situations. That cirrhosis is present in the majority of patients diagnosed with HCC may explain this, since localized ablation would not address the diseased non-cancerous liver which still harbors the potential for hepatocarcinogenesis.

Resection and LT both achieve favorable survival in selected patients with early-stage and/or unifocal HCC^[5,6]. However, a review of a large North American cohort (> 20000) of liver cancer patients using the Surveillance, Epidemiology and End Results 1973-2003 database showed a dramatically superior actuarial survival for LT compared to resection or ablation^[7]. Resection is associated with a relatively high rate of recurrence^[5], with 3-year recurrence frequency above 60% in some series^[8]. Recurrence of HCC following resection - at least in cirrhotic patients - is due to de-novo hepatocarcinogenesis in the diseased remnant liver and/or unseen micrometastases. The rationale for LT in the setting of HCC is that it removes not only the cancer but the diseased (and cancer-promoting) liver parenchyma surrounding the tumor(s).

EXPERIENCE WITH LT FOR HCC

Initial experience with LT for HCC as reported in early series was extremely poor^[9,10]. Such was the pessimism regarding LT for liver cancer that in many centers HCC was considered a contraindication to transplant. In this era there were no standardized transplant eligibility criteria based on tumor size or number, and imaging ability was limited compared to today. Thus

the poor outcomes were likely related to the inclusion of patients with large and/or multifocal tumors, with correspondingly high rates of HCC recurrence after LT. HCC recurrence itself is a leading cause of mortality in this patient population.

Despite the disappointing early experience, there was simultaneous awareness that patients who had small, incidental HCCs found at explant tended to have low rates of recurrence with favorable long-term survival after LT^[11]. This in turn led to consideration of LT in patients with limited tumor burden. In 1996 Mazzaferro published his landmark series demonstrating that patients whose pre-LT tumor burden was limited to a single lesion ≤ 5 cm, or 2 to 3 lesions each ≤ 3 cm, enjoyed excellent disease-free survival after LT (> 80% at 4 years)^[12]. These size parameters have become known as the "Milan criteria" and are widely endorsed as the most common eligibility criteria for LT among patients with HCC.

TUMOR PROGRESSION ON THE TRANSPLANT WAITING LIST

In the United States organ transplantation is regulated by the United Network for Organ Sharing (UNOS). By UNOS classification the Milan criteria include stage T1 (1 tumor < 2 cm) and stage T2 (1 tumor 2-5 cm or 2-3 tumors ≤ 3 cm). Current UNOS policy allows patients with Milan T2 to receive priority listing for LT^[13]. Historically, however, HCC patients pursuing LT still face reduced survival by intention-to-treat analysis^[14]. This is due to tumor progression while awaiting LT, resulting in waitlist dropout. For waiting times up to 1 year, historical dropout rates of 10%-30% are encountered, with 5-year survival reduced by as much as 20%^[14]. In some UNOS regions, expected waiting time for priority-listed HCC patients exceeds 1 year.

Neo-adjuvant loco-regional therapy (LRT) for HCC is widely utilized by transplant centers internationally. The specific types of LRT available for use have expanded in the last decade, and are discussed later in this manuscript. For patients meeting Milan criteria, the intent of LRT is to serve as bridging therapy to LT by preventing tumor progression and waitlist dropout. For another group of patients who exceed Milan criteria, but fall within expanded criteria allowing a cumulative total diameter for all lesions ≤ 8 cm, the intent of LRT is "downstaging". Successful downstaging implies that LRT has resulted in tumor shrinkage and/or devitalization (tumors no longer exhibit arterial phase enhancement on imaging), such that upon re-measuring the active tumor burden at some future time point after LRT, the patient falls within Milan criteria.

Advocates of these expanded downstaging criteria - particularly Yao and colleagues at the University of California San Francisco (UCSF) - have reported favorable outcomes for successfully downstaged patients, with a recent paper showing a 56.1% 5-year intention-

to-treat survival for 64 patients assigned to downstaging, not statistically different from a 63.3% 5-year intention-to-treat survival in 488 patients with Milan stage T2^[13]. However, expanded downstaging criteria have not been universally accepted and remain controversial in the face of already-present severe organ shortage.

LRT FOR HCC PATIENTS AWAITING TRANSPLANT

To date a post-transplant survival advantage for LRT prior to LT has not been definitively proven^[15,16]. However, given what is known about the risk of waitlist dropout, a randomized controlled trial comparing LRT to no LRT in patients awaiting transplant may be difficult to justify. An emerging concept is that tumor biology - as observed by imaging over time - is a more useful surrogate marker of tumor biology than size and number based on an initial imaging study. Patients with HCCs that display radiographic progression over relatively short time periods such as 3-6 mo - without LRT or despite it - are more likely to possess cancers that are inherently aggressive. Such patients are more likely to experience tumor recurrence and diminished survival after LT^[17].

Favorable response to LRT - whether used as bridging therapy for Milan criteria, or with downstaging intent for expanded criteria patients - has thus been proposed as a surrogate marker of more favorable tumor biology^[13,18-20]. In this paradigm, a mandatory waiting period of 3-6 mo after LRT is required before LT can be offered, in order to observe tumor response to LRT. Presumably, patients whose cancer progressed during the observation period - despite LRT - would not be offered LT. This strategy has been termed "ablate and wait"^[21]. The expanded downstaging criteria used and advocated by UCSF requires a minimum 3 mo waiting period after LRT before LT can occur^[13], and some UNOS regions (including Region 5 within which UCSF resides) impose a 6-mo delay of the assignment of priority points for listing of Milan stage T2 patients, in order to observe tumor behavior and response to LRT.

A number of different LRT options exist. Transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) have historically been the first and second most commonly utilized neo-adjuvant treatments before LT, respectively^[15]. TACE using drug-eluting beads (DEBs) - DEB-TACE - has become more widespread in recent years^[22]. Percutaneous ethanol ablation - once common for small tumors - and cryotherapy have declined markedly in use and are not further described here. Other forms of LRT include radioembolization with Yttrium-90 (Y-90), for which emerging literature suggests a favorable efficacy and tolerance^[23], and a novel mode of radiation therapy which may be effective as bridging therapy to transplant^[24]. The remainder of the manuscript appraises the types of LRT being used as neo-adjuvant therapy before LT, as well as

their respective efficacies and roles in various clinical situations.

INTRA-ARTERIAL CHEMOTHERAPY

Traditional TACE involves catheterization - as selectively as possible - of the artery branch(es) supplying the tumor(s) with blood, followed by the infusion of liquid chemotherapy agents into the branch(es). Specific chemotherapy agents different across institutions, but often a mixture of doxorubicin, cisplatin and mitomycin-C is delivered. The liquid chemotherapy is often pre-mixed with ethiodized oil, which serves as both a drug-delivery vehicle as well as a radiopaque marker of where in the liver the mixture has been delivered^[25]. The oily nature of the emulsion itself contributes to embolization effect on small vessels, though transiently so. Many centers add embolic particles either to the oily emulsion or as a separate infusion immediately following release of the emulsion^[26]. Embolic agents include polyvinyl alcohol particles or Gelfoam. The duration of arterial occlusion is shorter with Gelfoam, with recannulization of flow occurring in about 2 wk. The intended duration of arterial occlusion is not permanent since this would interfere with future chemoembolization if it became clinically desirable. The combination of cytotoxic chemotherapy and embolization achieves varying degrees of tumor necrosis^[26,27], but achieving even complete necrosis has not necessarily been predictive of post-LT survival^[16].

The outcome of TACE must be assessed with two questions in mind. First, does TACE prior to LT improve survival after LT? And second, is TACE effective as a bridge to LT by preventing tumor progression and waitlist dropout. Both questions are problematic. As mentioned previously, there have been no large prospective trials comparing LRT to no-LRT in patients with HCC awaiting LT. And the evidence to date for pre-transplant TACE does not establish a clear post-transplant survival benefit. The waiting time to LT varies across regions, and a very short duration from TACE to LT does not allow sufficient time for observation of tumor behavior. This in turn will lead to some patients with biologically unfavorable tumors proceeding to LT, likely contributing to increased HCC recurrence and reduced survival. Those limitations notwithstanding, it does appear from a number of studies that TACE is associated with waitlist dropout rates of 3%-13%^[18,20,28,29], which is lower than expected based on historical data^[14] and supports TACE as an effective bridge to LT. TACE also has a favorable safety profile, and in the case of inoperable disease (non-transplant candidates), is associated with improved survival vs supportive care^[30].

DEB-TACE is similar to traditional TACE as an intra-arterial therapy for HCC administered selectively in the hepatic arterial circulation. The beads themselves are microspheres impregnated with a chemotherapeutic substance (most commonly doxorubicin), ranging in size from 100 to 700 μm . The amount of delivered doxorubicin is typically 100-150 mg/session^[22]. The

proposed advantage of DEB-TACE vs traditional TACE is a more concentrated delivery of chemotherapy in the targeted area, and for a longer duration, since traditional TACE results in a more transient drug concentration. This is because there is a delay from release of the oily therapeutic solution and the actual embolization in traditional TACE, causing some release into the systemic circulation (with systemic toxicities, and diminished activity at the intended tumoral site)^[31].

The safety of DEB-TACE has been validated in large studies as at least comparable to traditional TACE^[31], and the PRECISION-V study showed a statistically significant lower incidence of alopecia, degree of post-treatment aminotransferase elevation, and frequency of decreased left ventricular function with DEB-TACE vs conventional TACE^[22]. In clinical practice, since there is less induced arterial ischemia with DEB-TACE compared to conventional TACE, the former is an attractive consideration in patients with partially or completely thrombosed portal vein branches, since such patients may not tolerate a new, substantial arterial ischemia. For the same reason, many groups favor DEB-TACE for patients with worse liver function at baseline. In terms of efficacy and survival, there is insufficient data to claim that either TACE or DEB-TACE clearly outperforms the other^[22,31]. DEB-TACE has not been widely studied specifically for use as a bridge to transplant, though some published reports suggest its efficacy in this role^[32].

RADIOEMBOLIZATION

Transarterial radioembolization (TARE) has emerged as a viable strategy for solid liver tumors. The most commonly used form of TARE for HCC involves Y-90 microspheres delivered intra-arterially. Y-90 has a physical half-life of 64.2 h and decays to stable zirconium-90^[33]. A staging visceral angiography with injected technetium-99 is necessary to detect clinically relevant shunting to the gastrointestinal (GI) tract or lung, the latter assessed by measuring lung-shunt percentage on imaging^[34]. If shunts to the GI tract cannot be embolized (and closed), or if the lung-shunt fraction is elevated, Y-90 is not offered due to concerns about intestinal and pulmonary toxicity, respectively. If no such problems are encountered, Y-90 microspheres are delivered either to the right or left lobe, usually allowing at least 1 mo before treating the opposite side if bi-lobar disease is present, in order to monitor for toxicity.

Overall tolerance and safety appears comparable to TACE, although the amount of published experience with Y-90 is vastly less than with TACE. Due the hypervascularity of HCCs, radioactive microspheres theoretically flow preferentially - by a factor of 3 to 1^[35] - to tumors rather than hepatic parenchyma, limiting toxicity. Nonetheless, post-embolization syndrome following TARE - with nausea, abdominal pain and anorexia with or without fever - occurs with roughly the same frequency as with TACE, though severity may be

less^[36]. Some unique toxicities of Y-90 therapy must be appreciated. Radiation-induced liver disease (RILD) is a potentially serious sequela of TARE. RILD involves the emergence of varying degrees of liver decompensation with jaundice and ascites occurring 2-8 wk after treatment, with series suggesting a frequency of 4% to as much as 20%^[36,37]. The risk of RILD appears to increase significantly with repeated Y-90 administrations^[38]. Radiation-induced biliary stricturing is another potential consequence of TARE, though the incidence appears to be less than 10%^[39]. As with TACE, care must be taken to avoid inadvertent embolization of the cystic artery, which could cause gall bladder necrosis. Radiation induced pneumonitis and GI ulcerations are rare if standard precautions are undertaken^[36], but may occur with unrecognized shunting to lung or bowel.

Efficacy of radioembolization in terms of radiographic response and survival in non-operative candidates appears comparable or possibly superior to TACE^[23], acknowledging that the cumulative amount of experience with Y-90 is less. Its utility as a bridge to LT is similarly less defined, but selected series show that TARE is effective in this role^[34,40]. Lewandowski published a series comparing TACE (35 patients) to TARE (43 patients) for downstaging of HCC beyond Milan criteria, and reported successful downstaging to Milan T2 was superior with TARE (58% vs 31%, $P = 0.023$)^[41]. One theoretical concern with Y-90 as a bridge to LT is the risk of radioactivity affecting surgical or pathology team members handling the explanted organ. However the decay properties of Y-90 are such that unless LT happens within 4 wk of TARE, the risks should be trivial.

ABLATION THERAPY

Except for TACE, RFA has been the most widely utilized and reported LRT for patients awaiting LT. RFA involves the insertion of one or more narrow probes - under ultrasound or computed tomography guidance - into a target liver lesion, usually with the patient anesthetized. Occasionally more than one tumor is treated in a given session. The probes are connected to an alternating current that generates heat at their tip, causing thermal injury to tissue. Some technical limitations of RFA involve a relatively long time (16-18 min) to achieve adequate thermal injury to fully ablate a 3-4 cm lesion, as well as the potential loss of heat energy (and thus treatment effect) if large blood vessels are near the treatment zone. In such cases, the vessels act as heat sinks dissipating energy. In view of these limitations, some centers have begun to utilize microwave ablation (MWA). MWA achieves much more rapid heating with shorter treatment time, as well as a larger zone of ablation. However, neither RFA nor MWA is ideal for lesions high in the dome of the liver or near the gall bladder, due the risk of pulmonary insult or gall bladder necrosis, respectively.

Complications of ablation include abdominal pain and anorexia with or without fever, not necessarily different

from the symptoms of post-embolization syndrome. Serious bleeding is possible but uncommon (< 2%), as is the rate of abscess formation, portal vein thrombosis, thoracic injury, and severe liver decompensation^[42,43]. The risk of tumoral seeding by ablation probes (2%) and overall mortality (< 1%) is low, and seems comparable between RFA and MWA^[43-45].

For very small (≤ 3 cm) HCCs, it is recognized that RFA can achieve complete eradication and is viewed by many as equivalent in efficacy to resection for this scenario^[46,47]. Two large series published by Lu *et al.*^[48] and Mazzaferro *et al.*^[49] respectively, demonstrated the effectiveness of RFA as a bridge to LT, with very low dropout rates of 6% and 0%, respectively. A large Canadian study reported a higher rate of dropout with RFA (21%) as compared to an untreated cohort (12%), but this was in part driven by longer median waiting time to LT in the RFA cohort (9.5 mo vs 5 mo), as well as 9% of RFA-treated patients (vs 1% untreated) voluntarily seeking de-listing after achieving complete radiographic response^[50]. The role of RFA/MWA for downstaging - at least of larger diameter tumors - is limited in that ablation zones are not ideal to treat tumors > 3-4 cm.

NOVEL EXTRACORPORAL THERAPY

Stereotactic body radiation therapy (SBRT) has emerged as a treatment for solid liver and lung tumors, and is occasionally used for cancer in other sites such as the pancreas, prostate and kidney. SBRT involves highly confocal beams of energy delivered at a narrowly defined site. Prior to treatment, 4-dimensional imaging is used to map the target area as it moves during breathing. Occasionally gold seed fiducials are placed into the target tumor to assist with imaging. Whereas conventional external beam radiation - generally ineffective for HCC - delivers relatively small daily doses over the course of several weeks, SBRT can deliver a much larger dose of radiation per session - usually lasting 30-60 min - such that treatment is completed in 1-5 d. Due to the ability to deliver the radiation in a highly targeted and localized manner, SBRT may have advantages over ablation since it can be used to treat lesions high in the dome of the liver (sparing the lung), near the gall bladder (sparing it), or near large blood vessels (no heat sink effect).

SBRT has been studied in HCC both as a bridge to LT and for inoperable patients. O'Connor *et al.*^[24] reported in a small study that SBRT (used because patients were deemed ineligible for further standard LRT) was successful as a bridge to LT in 10/10 patients, with none experiencing HCC progression between SBRT and LT^[24]. Explant analysis from this series showed a 27% complete necrosis rate in treated tumors, with 75% of the incompletely necrotic tumors measuring smaller than pre-LT imaging size^[24]. In two sequential studies using SBRT in 102 patients with Child's class A liver disease and locally advanced HCC, Bujold *et*

al.^[51] reported a median survival of 17 mo^[51], which is substantially higher than the median survival of the cohort receiving placebo in the SHARP study of sorafenib, which also was restricted to patients with mostly preserved liver function^[52].

Toxicity from SBRT has been limited, and mostly grade 1 or 2 GI toxicity (nausea, vomiting, pain)^[24,53], though Bujold's study reported grade 3 toxicity in up to 30%^[51]. Rare GI ulcers have occurred following SBRT^[53]. The role of SBRT is still evolving, and studies comparing SBRT directly to other forms of LRT for bridging therapy to LT are in progress.

High-intensity focused ultrasound (HIFU) is a novel extracorporeal therapy that induces thermal injury to tumors using high frequency sound waves. Experience with HIFU is limited to date, but early experience with HCC patients has suggested a favorable radiographic response rate and safety profile^[54]. A recent pilot study from Hong Kong comparing TACE and HIFU as bridging therapy to LT showed comparable degrees of tumor necrosis for both modalities when assessed at explant^[55]. While more investigation is needed, the focused, extracorporeal nature of HIFU may permit its use in patients with Child-Pugh C liver disease. Reported side effects have included localized bruising and first-and second-degree skin burns on skin overlying treatment zones^[54].

CHOOSING THE OPTIMAL LRT FOR HCC IN THE PRE-TRANSPLANT SETTING

An ongoing difficulty for the transplant community is the lack of consensus regarding when/whether to use LRT for HCC prior to LT. There is further lack of consensus regarding which LRT to use for a given tumor. Even within each LRT category there is variation among institutions regarding the specifics of treatment. For example, "TACE" may involve different specific chemotherapeutic agents and/or embolic materials at different centers. And for small lesions, choice of TACE or ablation may come down to institution- or clinician-preference.

Despite these limitations, some general principles may assist decision-making. First, for Milan stage T2 HCC and preserved liver function, TACE has an excellent track record of safety and efficacy as a bridge to LT, with substantial lowering of dropout rates from historical standards^[14]. TACE is also effective as a downstaging modality for larger lesions^[13], though consideration for DEB-TACE is reasonable if there is portal venous thrombosis and/or decompensated liver function. Y-90 or TACE may be considered for larger (> 4 cm) tumors, the latter only if waiting time to LT is expected to exceed 1 mo.

Ablation (RFA/MWA) continues to be an effective bridge to LT for lesions < 3-4 cm, if the lesion is not located near the dome of the liver (lung), gall bladder or large vessels. For such lesions, ablation or TACE may

be equivalent in efficacy, though explant histological analysis suggests RFA has a higher rate of complete tumor necrosis for very small (< 3 cm) HCCs^[56]. For lesions 4–6 cm in sensitive areas such as the dome of the liver or near the gall bladder, SBRT appears to be a safe, targeted therapy with early success reported as a bridging therapy. Lesions these sizes are generally too large for successful ablation. SBRT and novel HIFU may also be compelling considerations for patients with greater liver decompensation, as such patients may not tolerate TACE or TARE. More study is needed and planned.

CONCLUSION

The incidence of HCC has substantially increased in many regions during the past 3–4 decades. For all but very small HCCs, surgery (resection or LT) is necessary for long-term survival or cure. As most HCCs occur in the setting of cirrhosis, resection leaves behind diseased (and presumably prone-to-cancer) tissue, and thus LT appears to strongly out-perform resection in actuarial survival.

Given the risk of tumor progression and waitlist dropout, LRT is routinely offered to patients on the transplant waiting list. TACE and RFA are the most widely studied modalities, and are effective as bridging therapy to LT in appropriate settings. TACE is also used for downstaging in patients whose initial tumor burdens exceed Milan criteria. Other forms of LRT include DEB-TACE, Y-90 and more recently, extracorporeal treatments such as SBRT. Each may have a “niche” role in the pre-transplant setting, and ongoing investigation will be critical in the development of widely accepted treatment paradigms to guide the use of LRT in waitlisted patients.

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Potential approaches to improve the outcomes of donation after cardiac death liver grafts

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Abstract

There is a growing discrepancy between the supply

and demand of livers for transplantation resulting in high mortality rates on the waiting list. One of the options to decrease the mortality on the waiting list is to optimize organs with inferior quality that otherwise would be discarded. Livers from donation after cardiac death (DCD) donors are frequently discarded because they are exposed to additional warm ischemia time, and this might lead to primary-non-function, delayed graft function, or severe biliary complications. In order to maximize the usage of DCD livers several new preservation approaches have been proposed. Here, we will review 3 innovative organ preservation methods: (1) different *ex vivo* perfusion techniques; (2) persufflation with oxygen; and (3) addition of thrombolytic therapy. Improvement of the quality of DCD liver grafts could increase the pool of liver grafts for transplantation, improve the outcomes, and decrease the mortality on the waiting list.

Key words: Biliary complications; Donation after cardiac death; Organ preservation methods

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Core tip: As the demand for more organs increases, the transplant community searches for new approaches to expand the pool of organs. Recently developed methods to improve the condition of donation after cardiac death (DCD) livers look promising. During the past decade, *ex vivo* machine perfusion method has demonstrated positive results and it is considered as a new potential preservation method for DCD organs. This paper provides an overview of the attempts to ameliorate the quality of DCD liver grafts and transplant outcomes by improving preservation techniques.

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INTRODUCTION

Liver transplant is considered as the only available treatment for patients with end stage liver disease. Liver transplantation has been performed with success since 1963 and the outcomes continue to improve achieving 1-year graft survival superior to 90%^[1,2]. At the same time the demand for liver transplant has increased and many patients die on the waiting list. The organ shortage has led to an increase in the use of grafts with inferior quality such as from donation after cardiac death (DCD) donors, also called non-heart-beating donors. DCD livers undergo additional warm ischemia time (WIT) which is associated with inferior liver function and poor outcome after transplant. Therefore, searching for potential approaches to ameliorate the quality of organs from DCD donors and minimizing injury is of special importance for the transplantation field.

In this paper we discuss about the attempts to ameliorate the quality of DCD liver grafts by improving preservation techniques.

CHARACTERISTICS OF DCD DONORS

DCD donors are characterized by the termination of ventilation and blood circulation before cold flushing of organs^[3]. The idea to use DCD liver grafts was reintroduced in the 1990s after achieving success in kidney transplantation^[3]. The use of grafts from DCD donors in the United States has exponentially increased from 0.95% in 2000 to 5% in 2010 (1 UNOS).

In general DCD donors are divided into uncontrolled and controlled donation groups. In the uncontrolled group, the organ suffers from prolonged WIT, as the potential donor is dead on arrival or has been undergoing unsuccessful resuscitation. In this group, the organ suffers from long WIT which is a detrimental factor in organ quality. In the controlled group, cardiac arrest is planned and it happens following withdrawal of ventilator in the operating room or intensive unit care^[3,4]. It is generally accepted that DCD grafts have less energy stores and undergo more damage during the storage time^[5].

Biliary complications are much more common in patients that received grafts from DCD donors (20%-40% compared to 5% in grafts from brain-dead donors)^[6]. Post transplant biliary complications could lead to a number of serious complications such as graft loss, high morbidity which requires re-transplant or could result in patient's death^[7]. The most critical type of biliary complications are the so-called ischemic-type biliary lesions (ITBL), also called ischemic cholangiopathy, with an incidence varying between 5% and 15%^[8,9]. The risk of ischemic cholangiopathy with grafts from DCD donors is 10 times higher than for brain dead donors because

of severe warm ischemia suffered by these grafts^[6]. The reason why they develop more biliary complications is that bile ducts (cholangiocytes) are more sensitive to ischemia-reperfusion injury than hepatocytes^[10]. Many of DCD liver grafts are not used because longer warm or cold ischemia times have been associated with poor outcomes^[6,11,12]. Most of the transplant centers accept livers from DCD donors that have a maximum WIT, the period between extubation and cold flushing, of less than 30 min and short cold ischemia time (in general less than 6 h)^[11].

OXYGENATED COLD STORAGE (PERSUFFLATION)

Simple cold storage (SCS) is the currently widely used organ preservation method in the clinical setting because of the low cost and simplicity. The idea of CS is to decrease the metabolism level to provide protection from ischemia. However, even at 4 °C there is approximately 5% active metabolism in the organ which eventually leads to ATP depletion and accumulation of waste product^[13,14]. In order to improve organ preservation method, persufflation (PSF) had been introduced as an alternative method with the capacity of delivering oxygen during cold preservation. PSF has been used in rat livers for the first time between 1980 and 1990 by the Fischer group. They first established the model on rodent liver and continued with large animals (pig) and were able to demonstrate the benefits of PSF by improving the quality of liver grafts^[15]. They also showed the feasibility of this method by publishing the outcome of five patients transplanted with persufflated livers^[16]. The livers underwent WIT between 20-60 min and they were rejected by all the other transplant centers for transplant. They were flushed with University of Wisconsin (UW) or histidin-tryptophan-ketoglutarate and after arriving to the transplant center were subjected to retrograde PSF (R-PSF) at 18 mmHg for 70-200 min before the implantation. The results were promising and during the two years follow-up period, all the recipients showed good graft function. Later, in a study done by Minor *et al*^[17], it was shown that PSF of Wistar rat liver grafts with 18 mmHg of oxygen for 48 h at 4 °C could lower the activity of Kupfer cells compared to simple SCS.

Following these preliminary results, the studies were extended to study the effect of PSF in DCD livers. Minor *et al*^[18] introduced venous systemic oxygen PSF in DCD rat livers following 30 min of WIT. In a following study, the same group transplanted livers after 24 h PSF preservation, which showed that it improved mitochondrial function, and normalized ATP level^[19].

Following the increasing concern on potential reactive oxygen species (ROS) production during PSF, Minor *et al*^[20] preserved DCD rat livers for 24 h with R-PSF and compared with the result of the livers that were preserved in UW solution. The ATP level, bile production

and perfusion flow was improved in R-PSF livers. The outcome of this study demonstrated the beneficial role of R-PSF in eliminating ROS and lipid peroxidation production. In another study by Minor *et al.*^[21] it was also demonstrated that treatment with anti-oxidants such as superoxide dismutase or allopurinol during normothermic R-PSF could eliminate lipid peroxidation and restore the energy level in liver grafts after 60 min of WIT and 60 min of SCS in Euro-Collins solution. They also reported that PSF alone could induce some oxidative damage^[21]. Recently in a study done by Lürer *et al.*^[22], it is shown that pulsatile PSF of DCD rat livers is beneficial in early graft recovery after reperfusion. In this study livers that were procured from male Wistar rats were subjected to 30 min WIT and then 18 h of cold ischemia. Later the grafts ($n = 5$ each group) were preserved with either nonpulsatile or pulsatile gaseous oxygen PSF. Pulsatile PSF demonstrated better parenchymal preservation, higher nitric oxide levels in perfusate, and decreased portal vein resistance^[22].

In the next step, PSF was tested on pig livers, and subsequently to human DCD livers. In 2001 Saad *et al.*^[23] showed that R-PSF with antioxidant treatment in a transplant model is a promising method in improving the quality of the porcine DCD livers. DCD livers underwent 60 min WIT followed by 4 h SCS in UW solution or R-PSF with antioxidant treatment. In R-PSF group all animals survived, while animals in SCS group died 3 h after transplantation^[23]. After successful animal experiments, the first clinical study was started in 2004 in Germany using R-PSF in 5 DCD livers. Liver grafts underwent R-PSF at 18 mmHg at least one hour before transplantation. Evaluation of the histological biopsies taken before and after R-PSF showed that ATP level was enhanced by 2-5 times after R-PSF treatment, and all the patients survived during the two years of observation period with good graft function panel^[16].

HYPOTHERMIC MACHINE PERFUSION

Hypothermic machine perfusion (HMP) is considered as one of the alternative preservation methods to SCS which have recently been increased in use for DCD grafts preservation. HMP is a continuous or pulsatile circulation of the cold preservation solution in an organ at 4 °C-8 °C, and it has already been shown that HMP can resuscitate DCD liver grafts in different rat models^[24-26]. Schlegel *et al.*^[27] demonstrated that hypothermic oxygenated perfusion (HOPE) in a rat model could impact down regulation of the immune system after transplantation, in addition to protecting against ischemia injury. In this study, using an acute rejection model, livers from the Lewis Rats were used to be transplanted into the Brown Norway Rats. Rat livers underwent one hour HOPE before implantation with or without low dose (0.03 mg/kg) tacrolimus treatment in the recipients during the four weeks of observation. The combination of tacrolimus with HOPE resulted in 100% survival in the recipients without any sign of rejection. As it was mentioned prior,

one of the important issues in using DCD liver is to overcome biliary complications, in particular ischemic cholangiopathy related to strictures. In 2013, in a study done by Schlegel *et al.*^[25], it was demonstrated that HOPE is a sufficient method to protect DCD livers from biliary complications. The rat livers underwent to 30 min warm ischemia and it was followed by 4 h SCS. In the HOPE group, livers underwent one hour HOPE prior to implantation. Subsequently, livers were implanted and the recipients were observed for four weeks. Kupffer cell and endothelial cell activation was reduced. Moreover, cholestasis parameters were also improved in the HOPE group. In another study, Op den Dries *et al.*^[28] in a DCD pig model indicated the efficacy of oxygenated hypothermic machine perfusion in decreasing and limiting arteriolonecrosis injury of the peribiliary vascular plexus of the bile ducts. After 30 min of warm ischemia, the livers were preserved by SCS or oxygenated hypothermic machine perfusion using dual perfusion machine for 4 h. Next step was liver reperfusion for two hours at 37 °C with oxygenated autologous blood to simulate transplantation. Studying the bile duct histology disclosed reduced arteriolonecrosis of the peribiliary vascular plexus in the livers that were subjected to HMP perfusion vs SCS.

The feasibility of HMP study on human livers of brain dead donors was performed by Guarrera *et al.*^[29] at Columbia University. They used dual perfusion to perfuse 20 livers and successfully transplant them. They reported reduced early graft dysfunction, peak transaminases and improved renal function^[29]. The first use of HMP for DCD livers was reported in 2014 by Dutkowski *et al.*^[30]. Eight DCD livers with median of 38 min WIT were included. Liver grafts underwent 1-2 h HOPE with perfusion pressure at 10 °C, 3 mmHg. After transplantation the grafts revealed good hepatic function and no evidence of ITBL. Using HMP in other organs such as kidney is more common. There have been several clinical trials done on kidney HMP and it has become routine to use this method to preserve the human kidney in some part of Europe and some states in United States. Cold static storage is still the most common method of preservation in liver since cannulation and perfusion is more complicated in liver, and currently there is no Food and Drug Administration approved liver perfusion machine for clinical use.

SUBNORMOTHERMIC MACHINE PERFUSION

Another new potential method to replace SCS is subnormothermic (SNP) machine perfusion. Olschewski *et al.*^[31] presented that SNP perfusion is more beneficial in DCD rat liver which were subjected to one hour warm ischemia and reperfused at body temperature. Berendsen *et al.*^[32] established a rat liver transplant model. In this study the livers underwent 3 h of SNP perfusion at 21 °C with Williams Medium E solution

after one hour of WIT^[32]. The survival rate was 83.3% in a one month observation period. In another study performed by the same group, they perfused 7 human discarded DCD livers at 21 °C for 3 h with oxygenated Williams Medium E^[33]. This study found that oxygen uptake and ATP content was improved with an increase in bile production, and better bile quality. They suggested that SNP perfusion is effective in improving DCD livers quality and hepatobiliary cellular parameters.

NORMOTHERMIC MACHINE PERFUSION

Normothermic machine perfusion (NMP) is one of the innovative organ preservation techniques. NMP consists of a pulsatile flow of oxygenated perfusion solution in the organ which supports cellular metabolism at body temperature, restores the energy content of the organ and washes out waste products prior to the reperfusion in the recipient body. Another advantage of this method is to provide the opportunity of assessing the organ viability prior to implantation. In 2001, Friend *et al.*^[34] published a paper in which they described maintaining viability of DCD livers for a minimum of 24 h by applying NMP. After 60 min of WIT the liver grafts were stored for 24 h in UW solution or were immediately subjected to NMP. To mimic the anastomosis time, the livers were not perfused for 45 min after flushing with cold preservation solution. After 45 min, livers were reperfused for another 24 h. The continuous bile production, lower resistance in portal flow, reduced alanine transaminase level in the NMP group suggested that the quality of preservation can be enhanced by NMP perfusion.

op den Dries *et al.*^[35] was the first group to report the feasibility of this method in human DCD livers. They perfused 4 DCD discarded livers for 6 h using a dual perfusion system. The perfusion fluid consisted of packed blood cells with fresh frozen plasma to provide a sufficient support for high metabolism activity at 37 °C. Reduced lactate level to the normal value, bile production, and well preserved hepatocytes and biliary sinusoids suggested that NMP is beneficial in improving the quality of DCD livers. One year later the same group published a study on criteria of assessing the graft viability during *ex vivo* NMP perfusion^[36]. They investigated whether bile production and the quality of the produced bile during NMP would be a reliable biomarker for viability assessment. Twelve discarded DCD livers with median cold storage of 6.5 h were included and subjected to 6 h NMP at 37 °C with plasma and red blood cells. Liver grafts were divided into two groups; high bile production (more than 30 g in 6 h, and low bile production (less than 20 g). Higher bilirubin and bicarbonate concentration in the bile samples and lower hepatic necrosis in the high bile production group suggested that bile production might be a potential biomarker to assess the organ viability during warm perfusion. In a recent case report, Watson *et al.*^[37] from Addenbrooke's Hospital revealed the effect of Normothermic perfusion on a DCD liver graft

before implantation. The liver graft was retrieved from a 57-year-old donor. Circulatory arrest occur 150 min after stopping of life-supporting treatment and the graft underwent 5 h cold storage. Later the graft was perfused at 37 °C for 132 min with a plasma free solution. During the first 74 min of perfusion, the lactate was decreased from 7.2 to 0.3 mmol/L. after implantation the liver biochemistry was normal and during 6 mo posttransplant observation, there was no evidence of cholangiopathy^[37].

GRADUAL REWARMING MACHINE PERFUSION

Minor *et al.*^[38] for the first time introduced the concept of thermally controlled oxygenated rewarming (COR) of the liver grafts prior to reperfusion. In this study, Porcine livers were subjected to 18 h SCS and then were perfused 90 min by COR perfusion, HMP and SNP. In the COR group, during the first part of the perfusion temperature was stabilized at 8 °C and then was gradually enhanced to 12 °C, 16 °C, and 20 °C after 30 min, 45 min and 60 min, respectively. The perfusion pressure was kept at 4 mmHg in the portal side and at 25 mmHg at the hepatic artery side. In order to mimic the anastomosis time, the liver grafts were not perfused and were kept for 30 min in room temperature and then were reperfused with autologous blood for 4 h. The liver in the COR group demonstrated increased ATP, decreased lipid peroxidation, enzyme leakage and improved bile production. Minor *et al.*^[39] suggested that starting reoxygenation in a low temperature could reduce oxidative stress injury during reperfusion, and improve mitochondrial function^[40]. Following the previous study Westerkamp *et al.*^[41] investigated COR in a rat DCD model. In this study, the rat DCD livers were subjected to SCS at 4 °C for 6 h and then subjected to COR, HMP or SNP. After 45 min mimic anastomosis time, they were reperfused 2 h with red blood cells and Williams Medium E solution. In the control group, livers were immediately reperfused at 37 °C. Reduced transaminase enzymes level and lipid peroxidation level, superior mitochondrial function, higher bile production, improved bile quality and better preserved bile duct epithelium was observed in the COR group. The COR represented superior liver function compare to the SCS groups but comparable to the HMP and the SNP group.

ABDOMINAL REGIONAL PERFUSION

The main concept of abdominal regional perfusion is to limit deleterious effect of warm ischemia in DCD organs by the abdominal organ perfusion with continuous flow. Abdominal regional perfusion is being done *via* cannulation of femoral artery and vein using cardio pulmonary bypass machine or extracorporeal membrane oxygenation machine. For the first time regional perfusion was performed by a Spanish surgeon at 1989^[42]. The perfusion is being used in two categories

Table 1 Disadvantages of the different methods

Preservation methods	Disadvantages
Persufflation	Not able to assess the viability of the grafts
Hypothermic perfusion	High cost, not able to assess the viability of the graft
Subnormothermic perfusion	High cost, clinical challenging, not able to assess the viability of the graft
Normothermic perfusion	High cost, clinical challenging, the blood supply, the risk of losing the graft in the case of emboli in the system
Gradual rewarming	High cost, clinical challenging, need an accurate machine to be able to change the temperature and pressure
Abdominal regional perfusion	Ethical challenging
Thrombolytic therapies	Risk of severe bleeding

as hypothermic or normothermic perfusion^[43]. One group from West forest University describes perfusing of six DCD livers with hypothermic regional perfusion which was performed at 22 °C. In this study they showed good initial graft survival^[44]. The hospital clinic in Barcelona started using normothermic regional perfusion protocol on human category 2 DCD donors. The recipients were subjected to a median 45 mo follow-up. One year graft survival was 73% while patient survival rate was 81%. In another study a group from La coruna in Spain included category 2 DCD liver donors, they subjected 7 donors to hypothermic regional perfusion and 10 donors to normothermic regional perfusion. The results demonstrated high biliary complication in the recipients (25%) with low rate of five years graft survival^[45].

THROMBOLYTIC THERAPIES (TISSUE PLASMINOGEN ACTIVATOR)

Hashimoto *et al*^[46] suggested that the higher incidence in biliary complications of DCD livers may be related to microthrombi in the peri-biliary plexus. In this study they included 22 patients and assessed the effect of tissue plasminogen activator (TPA) injected into the hepatic artery of donor's during back table. Fourteen recipients out of 22 developed excessive post reperfusion bleeding and 2 patients developed ITBS. The TPA level was investigated in all the patients to find out if there was a correlation between the TPA level and excessive bleeding. They found that TPA level in the patients with bleeding was comparable with those who did not develop bleeding. The patients with excessive bleeding had history of higher previous laparotomy done in the past and higher body mass index, which might be associated with incidence of massive bleeding. In another study Seal *et al*^[47] recently showed that TPA treatment in DCD liver grafts decreases ITBSs occurrence and improves one- and three-year graft survival after transplant. TPA injection was delivered into the hepatic artery during liver transplant in 85 patients and compared to 33 patients who did not undergo TPA treatment. They reported lower occurrence rate of ITBL (16.5% vs 33.3%) and lower intrahepatic constriction in the group that received TPA treatment (3.5% vs 21.2%).

THE DISADVANTAGES OF DIFFERENT PRESERVATION METHODS

The disadvantages of each method are listed in the Table 1.

CONCLUSION

Because of exponential increase in the demand of liver grafts and high mortality on the waitlist, the interest of expanding the suitable organs for transplant has been increased. The optimized use of DCD liver grafts, different *ex-vivo* preservation interventions have been proposed achieving high rates of success. There is enough evidence that these new techniques have potential to improve graft function. Now, it is time for randomized controlled trials and a cost-effective analysis to determine if these techniques will become standard clinical practice.

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First line vs delayed transplantation in myeloma: Certainties and controversies

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of young patients with multiple myeloma (MM). In the last decade the introduction of novel agents such as immunomodulatory drugs (IMiDs) and proteasome inhibitors (PI), has dramatically changed the therapeutic scenario of this yet incurable disease. Due to the impressive results achieved with IMiDs and PI both in terms of response rates and in terms of progression free and overall survival, and to the toxicity linked to high dose therapy and autologous stem cell transplantation (ASCT), a burning question nowadays is whether all young patients should be offered autotransplantation up front or if this should be reserved for the time of relapse. This article provides a review of the data available regarding ASCT in MM and of the current opinion of the scientific community regarding its optimal timing.

Key words: Autologous stem cell transplantation; Immunomodulatory drugs; Proteasome inhibitors; High dose therapy; Multiple myeloma

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Core tip: Autologous stem cell transplantation (ASCT) is the cornerstone for the treatment of young multiple myeloma patients. This review summarizes the current knowledge on ASCT, with a special focus on the role of ASCT in the era of novel agents for multiple myeloma treatment.

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Abstract

Since the middle of 1990s autologous stem cell transplantation has been the cornerstone for the treatment

INTRODUCTION

Multiple myeloma (MM) is the second most common

hematologic malignancy, accounting for approximately 13% of all blood neoplasm and for approximately 1% of all cancers. The number of new cases diagnosed every year is of approximately 86000 worldwide^[1]. MM is mainly a disease of the aging population, however young individuals below 65 years of age can also be affected^[1].

Traditionally MM patients have been divided in two groups, based on their eligibility and fitness to receive high dose therapy (HDT) and autologous stem cell transplantation (ASCT). Fit patients, usually younger than 65-70 years of age, were offered HDT (with doses ranging from 200 to 100 mg/m² based on age and clinical conditions) and ASCT, while conventional treatment with lower doses of chemotherapy (mostly Melphalan) and steroids was given to elderly or unfit patients^[2-9].

In the last decade major advances in the management of MM have been made thanks to the introduction of novel agents such as immunomodulatory drugs [the immunomodulatory drugs (IMiDs), such as thalidomide, lenalidomide and pomalidomide] and proteasome inhibitors [the proteasome inhibitors (PI) bortezomib and carfilzomib]^[10-15]. The introduction of these drugs as part of the frontline treatment in both transplant eligible and non-eligible patients translated into a markedly increased rate of complete remission (CR), time to progression (TTP), progression-free survival (PFS) and overall survival (OS)^[11,13,16-18]. In patients ineligible to ASCT, the addition of bortezomib to the conventional melphalan and prednisone (MP) treatment translated into a rate of CR of 30%, with an OS at 5 years of 56.4 mo^[19,20]. These impressive results, comparable to the rate of CR and OS achieved with ASCT, have raised the question whether autologous transplant is nowadays still needed to treat MM patients or if it should be replaced by new drug containing regimens with or without chemotherapy. In this latter case ASCT would be used as a salvage treatment at the time of progression in patients initially treated with novel agents. This review will focus on the current role of ASCT for the treatment of MM patients.

UP-FRONT TRANSPLANTATION

High dose melphalan supported by ASCT for the treatment of fit MM patients was first developed in the 1980s, and it has been considered the standard of care for this group of patients since the middle of 1990s^[21,22]. The infusion of harvested and cryopreserved autologous stem cells, first introduced in the relapsed-refractory setting, proved to be able to reduce the prolonged myelosuppression caused by high doses of melphalan^[23,24]. In consideration of the good results seen in this subset of patients, ASCT was translated in the newly diagnosed setting, and also in this group of patients HDT ASCT demonstrated its superiority in

comparison to conventional chemotherapy^[4,5]. At present 7 randomised trials have compared ASCT with conventional chemotherapy, and results largely confirm the benefit of a transplant treatment approach (Table 1)^[4,5,9,25-28]. The majority of the studies demonstrated that treatment with ASCT was associated with a longer PFS^[4,5,9,25-27]; conversely, the benefit in terms of OS was less clear^[4,5,9]. This finding can be partly explained by the fact that patients initially treated with only chemotherapy were later rescued with ASCT, thus providing a rationale for reserving ASCT at a later time point in patient's history^[29]. Similar results were shown in a meta-analysis of 2411 patients, in which a benefit in terms of PFS, but not of OS, was observed^[30].

The introduction of novel agents in the induction phase before and in a consolidation or maintenance phase after ASCT, has further improved the outcomes of MM patients, increasing response rates, PFS and OS (Table 2). The combination of thalidomide and dexamethasone (TD) or of thalidomide with conventional chemotherapy has significantly increased the rate of responses compared to chemotherapy alone^[10,17,31-33]. TD incorporated into double ASCT was able to improve PFS and OS (median PFS 48 mo, OS 65% at 5 years) compared to standard chemotherapy with vincristine, adriamycin and dexamethasone^[10,31].

Bortezomib in the context of ASCT gave even more impressive results^[16,34-36], with the best combinations being those of bortezomib plus dexamethasone and an IMiDs^[13,37,38]. The combination of bortezomib, thalidomide and dexamethasone incorporated into ASCT resulted in a PFS of 68% at 3 years^[13], and a OS that reached 82% at 2 years^[37].

Even more interesting seems the combination of bortezomib and dexamethasone with lenalidomide (VRD) followed by ASCT. A phase I / II study investigating this combination in newly diagnosed MM patients reported impressive results, with an overall response rate of 100% and an estimated PFS and OS at 18 mo of 75% and of 97% respectively. This results have however to be carefully interpreted and confirmed, considering the short follow up that at the time of reporting of only 21 mo^[38].

The high rate of good quality responses seen with the incorporation of PI and IMiDs as induction before, and consolidation and maintenance after ASCT translated into an increase of both PFS and OS; in consideration of these results, and of the toxicity associated with HDT and ASCT, a burning question nowadays is whether new treatments alone, without the use of upfront ASCT, would be sufficient to treat young MM patients^[39]. In this scenario it is worth noting that the majority of patients enrolled in clinical trials that were not treated with ASCT upfront could still receive it at the time of relapse. Furthermore impressive results were seen with the introduction of novel agents in the treatment of MM patients not suitable for ASCT.

Table 1 Phase III clinical trials of chemotherapy *vs* transplantation

Ref.	Publication year	Random	Patients <i>n</i>	ORR (%)	CR (%)	PFS/EFS	OS
Attal <i>et al</i> ^[41]	1996	ASCT	100	81	¹ 22	28 mo	57 mo
IFM90		CCT	100	57	5	18 mo	44 mo
				<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.01	<i>P</i> = 0.03
Child <i>et al</i> ^[5]	2003	ASCT	200	86	44	32 mo	54 mo
MRC VII		CCT	201	48	8	20 mo	42 mo
				<i>P</i> = NR	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.04
Ferland <i>et al</i> ^[25]	1998	ASCT	91	78	57	39 mo	64.6 mo
MAG90		CCT	94	58	20	13 mo	64 mo
Barlogie <i>et al</i> ^[28]	2006	ASCT	261	93	17	17%	38%
S9321		CCT	255	90	15	14%	38%
						At 7 yr	At 7 yr
Ferland ^[27]	2005	ASCT	94	62	36	37 mo	79 mo
MAG95		CCT	96	58.5	20	16 mo	43 mo
Bladé <i>et al</i> ^[26]	2005	ASCT	81	82	30	42 mo	66 mo
PETHEMA		CCT	83	83	11	33 mo	61 mo
					<i>P</i> = 0.002		
Palumbo <i>et al</i> ^[9]	2004	ASCT	95	72	¹ 25	28 mo	58 mo
MMSG		CCT	99	66	6	16 mo	42 mo
					<i>P</i> = 0.002	<i>P</i> < 0.001	<i>P</i> < 0.001

¹ ≥ nCR. Only statistical significant *P* is reported. CCT: Conventional chemotherapy; ASCT: Autologous stem cell transplantation; ORR: Overall response rate; CR: Complete remission; nCR: Near CR; PFS: Progression free survival; EFS: Event free survival; OS: Overall survival; NR: Not reported; IFM: Intergroupe Francophone du Myélome; MRC: Medical Research Council; PETHEMA: Programa Para El Estudio y Tratamiento De Las Hemopatías Malignas; MAG: Myélome Autogreffe.

RATIONAL FOR DELAYED TRANSPLANTATION: NEW DRUGS COMBINATIONS WITH OR WITHOUT CHEMOTHERAPY FOR PATIENTS NOT CANDIDATE TO ASCT

The advent of new drugs has dramatically changed the outcomes not only of young MM patients, but also, and maybe even more impressively, those of older transplant ineligible patients. Already the implementation of thalidomide into the classic combination of MP was able to improve patients outcomes compared to MP alone^[40]. The addition of bortezomib to MP led to even more impressive results, increasing the response rate of elderly MM patients to rates previously seen only in patients that received ASCT. Patients treated with Bortezomib, Melphalan and Prednisone (VMP) showed a TTP of 24 mo and a 3- and 5-year OS of 68.5% and 46%, respectively. The addition of bortezomib to MP was able to increase the OS of patients of 13 mo^[19,20,41].

Another interesting combination is the one of lenalidomide and dexamethasone. The combination of lenalidomide and dexamethasone was first evaluated both in young and elderly MM patients, identifying the association of lenalidomide with low dose dexamethasone (Ld) as the combination to bring forward in further trials^[11]. This combination has been proved to be extremely beneficial in the elderly population. A continuous treatment with lenalidomide and dexamethasone was found to be superior not only to MP plus thalidomide, but also to the same regimen given for

a fixed number of cycles (18 cycles); continuous Ld significantly reduced the risk of death (HR = 0.78; *P* = 0.02) and the authors speculate that for the first time a regimen without chemotherapy can be considered as a standard of care for the treatment of MM patients^[42]. The knowledge that ASCT can be given also as a salvage treatment, together with the data coming from the aforementioned trials resulted in the treatment strategy comprehensive of upfront ASCT now being questioned by some centres^[43].

DELAYED TRANSPLANTATION

The best timing of ASCT, whether it should be given as an upfront treatment or as salvage therapy at the time of relapse, was already a burning question before the novel agents era. From 1990 to 1995, Fermand *et al*^[25] randomly assigned 185 patients to receive early ASCT or conventional chemotherapy with vincristine, melphalan, cyclophosphamide and prednisone (VMPC). In this latter group ASCT was reserved for the time of relapse. Although median event free survival was longer for patients treated with early ASCT (39 mo vs 13 mo) the median OS was not significantly different between the two groups (64.6 mo vs 64 mo, *P* = 0.92), and 90% of the patients randomised to the VMPC arm were able to receive the planned delayed ASCT at the time of relapse^[25].

Several analyses, summarised in Table 3, have investigated the role of ASCT as a salvage therapy for MM^[29,44-51]. These works are not always comparable, due to the different nature of the works (both prospective and retrospective) and to the fact that ASCT was

Table 2 Improved outcomes with the introduction of novel agents in the upfront treatment of multiple myeloma

Ref.	Publication's year	Therapy	Patients <i>n</i>	≥ VGPR (%) preASCT	≥ VGPR (%) postASCT	≥ PR %	CR/ nCR %	PFS/EFS OS
Thalidomide								
Rajkumar <i>et al</i> ^[32]	2006	TD <i>vs</i> D	200	63 <i>vs</i> 41 (≥ PR)	NR			NR
Cavo <i>et al</i> ^[10]	2009	TD <i>vs</i> VAD	270	30 <i>vs</i> 15	68 <i>vs</i> 49			PFS 51% <i>vs</i> 31% at 4 yr OS 69% <i>vs</i> 53% at 5 yr EFS 56% <i>vs</i> 44% at 3 yr OS 65% <i>vs</i> 65% at 5 yr EFS 34 mo <i>vs</i> 22 mo OS 73 mo <i>vs</i> 60 mo
Barlogie <i>et al</i> ^[17]	2006	TT2 + Thal <i>vs</i> TT2	668	NR	62 <i>vs</i> 43			
Lokhorst <i>et al</i> ^[33]	2010	TAD <i>vs</i> VAD	402	32 <i>vs</i> 15	49 <i>vs</i> 32			
Lenalidomide								
Richardson <i>et al</i> ^[38]	2010	VRD	35			100	57	NR
Palumbo <i>et al</i> ^[56]	2014	MPR <i>vs</i> HDM Maintenance R <i>vs</i> No maintenance	402 202 200 198 204			NR NR 78 77	NR NR 23 19	PFS 22.4 mo <i>vs</i> 43 mo OS 65.3% <i>vs</i> 81.6% PFS 41.9 mo <i>vs</i> 21.6 mo OS 79% <i>vs</i> 88% PFS at 3 yr
McCarthy <i>et al</i> ^[66]	2012	Lenalidomide <i>vs</i> placebo	460					66% <i>vs</i> 39% OS at 3 yr 88% <i>vs</i> 80% PFS at 4 yr
Attal <i>et al</i> ^[67]	2012	Lenalidomide <i>vs</i> placebo	614					43% <i>vs</i> 22% OS at 4 y 73% <i>vs</i> 75%
Bortezomib								
Harousseau <i>et al</i> ^[16]	2010	*VD <i>vs</i> VAD	482	38 <i>vs</i> 15	54 <i>vs</i> 37			36 m <i>vs</i> 27 m
Sonneveld <i>et al</i> ^[34]	2012	*Induction PAD + maint VEL <i>vs</i> induction VAD + maint Thal	626	NR	75 <i>vs</i> 61			46% <i>vs</i> 42% at 3 yr
Cavo <i>et al</i> ^[13]	2010	*VTD <i>vs</i> TD induction and consolid	480	62 <i>vs</i> 28	82 <i>vs</i> 64			68% <i>vs</i> 56% at 3 yr
Rosinol <i>et al</i> ^[37]	2012	*VTD <i>vs</i> TD	202	29 <i>vs</i> 14 (CR)	59 <i>vs</i> 40 (CR)			82% at 2 yr (OS)
Moreau <i>et al</i> ^[35]	2011	*VD <i>vs</i> vtD	199	49 <i>vs</i> 39	74 <i>vs</i> 58			30 mo <i>vs</i> 26 mo
Leleu <i>et al</i> ^[36]	2013	VTd-ASCT + consolid VTd <i>vs</i> VTd-ASCT	217	After treatment: 83 <i>vs</i> 64				TTP: 62% <i>vs</i> 29% at 4 yr

VGPR: Very good partial response; TTP: Time to progression; PFS: Progression free survival; NR: Not reported; Thal: Thalidomide; TD: Thalidomide dexamethasone; VAD: Vincristine adriamycin dexamethasone; TAD: Thalidomide adriamycin dexamethasone; MPR: Melphalan prednisone lenalidomide; VTD: Bortezomib thalidomide dexamethasone; VD: Bortezomib dexamethasone; PAD: Adriamycin bortezomib dexamethasone; vtD: Reduced doses bortezomib thalidomide dexamethasone; R: Lenalidomide; VRD: Bortezomib lenalidomide dexamethasone; OS: Overall survival; ASCT: Autologous transplantation; nCR: Near CR; HDM: High dose melphalan; Consolid: Consolidation; Maint: Maintenance.

Table 3 Major studies of delayed autologous stem cell transplantation (for randomised trials only data regarding delayed autologous stem cell transplantation are reported)

Ref.	Publication's year	Patients <i>n</i>	Type of trial	Median interval between diagnosis or first ASCT and delayed ASCT	Previous ASCT	ORR (%)	PFS (mo)	OS (mo)
Cook <i>et al</i> ^[49]	2011	106	Retrospective	19 mo (relapse from first transplant)	Yes	63%	NR	37
Jimenez-Zepeda <i>et al</i> ^[51]	2012	81	Retrospective	39 mo (relapse from first transplant)	Yes	97.4%	16.43	53
Sellner <i>et al</i> ^[44]	2013	200	Retrospective	NR	Yes	80.4%	15.2	43.2
Cook <i>et al</i> ^[46]	2014	89	Prospective	2.7 yr	Yes	83%	19	80.3% at 3 yr
Gertz <i>et al</i> ^[29]	2000	64	Prospective	NR	No	97%	11.4	19.6
Michaelis <i>et al</i> ^[45]	2013	187	Retrospective	32 mo	Yes	68%	5% at 5 yr	29% at 5 yr
Shah <i>et al</i> ^[68]	2012	44	Retrospective	30 mo	Yes	90%	12.3	31.7
Kumar <i>et al</i> ^[48]	2012	112	Prospective	> 12 mo	No	32% (≥ VGPR)	16 (TTP)	73.4% at 4 yr
Dunavin <i>et al</i> ^[47]	2013	65	Retrospective	17.7 mo	No	NR	23 (TTP)	63% at 5 yr

VGPR: Very good partial response; TTP: Time to progression; PFS: Progression free survival; ASCT: Autologous stem cell transplant; OS: Overall survival; NR: Not reported; ORR: Overall response rate.

in some cases given as a salvage treatment after a previous ASCT^[44-46], whilst in others patients received ASCT after relapsing from a treatment not including transplantation^[29,47,48].

One of the biggest records is the one published by Sellner *et al*^[44], in which 200 MM patients retreated with ASCT at the time of relapse were retrospectively analysed. In the study a prognostic score was created, based on the International Staging System (ISS) at the time of relapse and on the duration of response after the first ASCT. The analysis showed that the biggest benefit of salvage ASCT was achieved in those patients with a low ISS (ISS 1) and with a first PFS longer than 18 mo. Another interesting finding of the study was that about 50% of the patients presented at the time of relapse with cytogenetic features of high risk, such as the presence of del(17p), t(4;14) or amp(1q), and that these patients had a worst outcome as compared to patients that relapsed with standard risk features^[44]. These findings are of primary importance in the decision of when to perform an ASCT (upfront or at relapse), taking into account that patients may relapse with a more aggressive disease, and that cytogenetic abnormalities known to confer a dismal outcome are seen more often in patients in advanced stages of disease, probably as the result of an increasing biological risk and clonal selection^[52-54].

Most of the studies available were published before IMiDs and PI became available for upfront treatment. In the era of novel agents two studies have retrospective analysed the role of early vs delayed ASCT^[47,48] and one study prospectively evaluated a second ASCT after relapse from a previous one^[46]. One study reported the outcomes of 290 patients treated with IMiDs based therapy (thalidomide or lenalidomide) and that received early (within 12 mo of diagnosis) or late ASCT; PFS was similar irrespective of when ASCT was performed (early or late) and no significant difference could be observed in OS, with both groups experiencing a 4-year OS of 73%^[48]. In a similar study Dunavin *et al*^[47] retrospectively reviewed the outcome of 167 patients treated with novel agent-based therapy (IMiDs or PI) and receiving early or delayed ASCT. The 5-year OS from diagnosis was similar in the two groups (63% both in early and late ASCT, $P = 0.45$), in accordance with the data reported by Kumar *et al*^[48]. The English group prospectively evaluated the role of salvage ASCT after relapse from a previous one; patients relapsing after ASCT were randomised between treatment with a second ASCT or chemotherapy with cyclophosphamide (Cy). With a median follow-up of 31 mo, although patients randomised to a second ASCT experienced a longer PFS compared to patients treated with Cy (19 vs 11 mo for ASCT and Cy respectively, $P < 0.0001$) no difference in terms of OS could be seen. It also has to be noted that the comparator chemotherapy arm, comprehensive of only weekly Cy, might not be the standard of care in a time when multiple drugs, such as third generation IMiDs, second generation PI, spindle

kinase inhibitor or monoclonal antibodies are available for the treatment of relapsed MM.

NEW DRUGS IN THE CONTEXT OF UP-FRONT VS DELAYED TRANSPLANTATION: PHASE III CLINICAL TRIALS

As already stated the advent of new drugs has dramatically changed the therapeutic scenario of MM patients. Not only an induction treatment comprehensive of new drugs significantly increased the rate of high quality responses and improved survival outcomes^[11,13,16,34], but the manageable toxicity of these compounds make them suitable for a long term and continuous treatment^[42,55]. In the above mentioned phase I / II VRD trial, a post hoc landmark analysis showed that the risk of progression after one year was low irrespective of whether patients had received or not an ASCT and that in patients who did not wish to undergo transplantation, responses increased prolonging therapy from 4 to 8 cycles^[38].

The impressive results obtained with first line treatment comprehensive of IMiDs and PI prompt the investigation of upfront vs delayed transplantation in the context of specifically designed phase III randomised trials.

The Italian Gruppo Italiano Malattie Ematologiche dell'Adulto conducted a phase III clinical trial aimed at comparing melphalan, prednisone and lenalidomide (MPR) vs two courses of HDT with melphalan (melphalan 200 mg/m²). All patients had previously received an induction treatment with four courses of Lenalidomide and low dose dexamethasone (Ld). With a median follow-up of 51.2 mo the results showed a clear advantage of the ASCT arm both in terms of PFS (43 vs 22 mo, $P < 0.001$) and of OS (82% vs 65% at 4 years, $P = 0.02$)^[56]. Another factor that might have influenced the outcome of the study was that 41% of the patients randomised in the late transplant arm did not receive the planned salvage ASCT^[56]. High dose melphalan (HDM) after 4 cycles of induction with Ld was also compared to cyclophosphamide, lenalidomide and dexamethasone (CRD). Similarly to what already seen with the MPR treatment, HDM was superior to CRD in terms of PFS (27 mo vs not reached for CRD and HDM, respectively, $P = 0.012$), whilst no advantage was seen in terms of OS (estimated 3-year OS 81% vs 84% for CRD and HDM, respectively, $P = 0.891$)^[57]. A pooled analysis the two trials showed that in newly diagnosed MM patients, HDM followed by ASCT significantly improved PFS and OS in comparison to MPR or CRD. Patients with favourable baseline conditions, such as a good baseline performance status (PS) (Karnofsky PS $\geq 80\%$), a low ISS (ISS 1), the absence of high-risk cytogenetic abnormalities [del(17p), t(4;14), t(14;16)] and those that had achieved at least a very good partial response

after induction had the most significant benefit in terms of OS^[58].

The reported trials seem to favour upfront ASCT, however a possible caveat of these studies is the not-optimal induction treatment, with the rate of complete responses reported after consolidation (with MPR or HDM) that were lower than those reported at the same time point after other chemotherapy-free induction regimens, such as bortezomib-thalidomide-dexamethasone^[13,37,56]. The most promising induction combinations to be tested in the context of upfront vs delayed transplantation are triplet combinations including two novel agents or a novel agent and a chemotherapeutic drug associated with Dexamethasone^[13,34,37,38]. Two multicentre randomised phase III trials are currently ongoing, evaluating the role of upfront vs delayed ASCT in the context of a new drug based therapy. The European Myeloma Network (EMN) on one side and the Intergroupe Francophone du Myélome (IFM) in association with the Dana-Farber Cancer Institute (DFCI) on the other, are conducting two trials aimed at assessing the role of ASCT in comparison to a novel agent based consolidation. The EMN02 trial randomises transplant eligible newly diagnosed MM patients, after an induction with 4 cycles of bortezomib, cyclophosphamide and dexamethasone, to receive a consolidation therapy with 4 cycles of VMP or with ASCT to support one or two cycles of HDM. Patients are further randomised to a second consolidation treatment with VRD vs observation; all patients will receive maintenance treatment with lenalidomide. The IFM/DFCI 2009 trial compares VRD with or without transplantation in a subset of patients similar to those included in the EMN02 study. As for patients in the EMN02 study, patients enrolled in the IFM/DFCI 2009 trial will receive maintenance lenalidomide. Both trials are currently closed to recruitment and definitive results with a long follow up results are eagerly awaited.

CONCLUSION

In the era of novel agents the appropriate timing for performing ASCT, whether upfront or at relapse, is still a burning question. If on one hand it is true that early ASCT improves PFS rates, on the other hand it is associated with a higher toxicity compared to a treatment with novel agents^[56]. It has to be also acknowledged that, whilst almost all randomized studies showed longer PFS for early ASCT, the benefit on OS was not uniformly reported^[25,56-58]. The lack of advantage observed in some cases in terms of OS is mainly due to the effective salvage therapy nowadays available, and to the possibility for patients to receive ASCT later in their disease history as a salvage treatment. For this reason some centres nowadays recommend ASCT only for those patients with high-risk features, whilst for standard risk patients a treatment option reserving ASCT for the time of relapse is considered acceptable^[59-61]. In this context it has to be emphasised, in patients for whom a delayed ASCT may be considered, the extreme importance of

early stem cell collection and cryopreservation; an early stem cell collection is particularly important in those patients receiving lenalidomide based treatments^[62,63].

Despite being a feasible option for carefully selected patients, delayed ASCT has some important caveats: Not only a significant percentage of patients might not be able to receive HDM at the time of relapse, due to the worsening of their clinical conditions^[56], but also a worst outcome could be expected due to the higher rate of adverse cytogenetic features in more advanced disease phases^[44]. Furthermore it has to be noted that reliable cost effectiveness data comparing early ASCT vs the continuation of a novel agent based therapy are currently not available^[64].

Based on the available data the recent guidelines from the American Society for Blood and Marrow Transplantation recommend performing ASCT early in disease history (within 12 mo)^[64], and there is a global consensus strongly in favour of upfront ASCT^[21,65]. Results of ongoing phase III studies are eagerly awaited to answer the burning question regarding the optimal timing of ASCT in young MM patients and whether, in the era of novel agents, HDM is still a need in order to treat MM.

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State of deceased donor transplantation in India: A model for developing countries around the world

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Abstract

Renal replacement therapy (RRT) resources are scarce in India, with wide urban-rural and interstate disparities. The burden of end-stage renal disease is expected to increase further due to increasing prevalence of risk factors like diabetes mellitus. Renal transplantation, the best RRT modality, is increasing in popularity, due to improvements made in public education, the deceased donor transplantation (DDT) programme and the availability of free and affordable transplant services in government hospitals and certain non-governmental philanthropic organizations. There are about 120000 haemodialysis patients and 10000 chronic peritoneal dialysis patients in India, the majority of them waiting for a donor kidney. Shortage of organs, lack of transplant facilities and high cost of transplant in private facilities are major barriers for renal transplantation in India. The DDT rate in India is now 0.34 per million population, among the lowest in the world. Infrastructural development in its infancy and road traffic rules not being strictly implemented by the authorities, have led to road traffic accidents being very common in urban and rural India. Many patients are declared brain dead on arrival and can serve as potential organ donors. The DDT programme in the state of Tamil Nadu has met with considerable success and has brought down the incidence of organ trade. Government hospitals in Tamil Nadu, with a population of 72 million, provide free transplantation facilities for the underprivileged. Public private partnership has played an important role in improving organ procurement rates, with the help of trained transplant coordinators in government hospitals. The DDT programmes in the southern states of India (Tamil Nadu, Kerala, Pondicherry) are advancing rapidly with mutual sharing due to public private partnership providing vital organs to needy patients. Various health insurance programmes rolled out by the governments in the southern states are effective in alleviating financial burden for the transplantation. Post-transplant immunological and pathological surveillance of recipients

remains a challenge due to the scarcity of infrastructure and other facilities.

Key words: Deceased donor transplantation; Kidney; India; Developing countries

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Core tip: Deceased donor transplantation (DDT) has been increasing in India, especially in the southern states due to proactive policies of the state governments and public private partnership. With the goal of achieving maximum organ harvesting from potential organ donors and universal access to transplant services, small steps of improvement have been made. The DDT program in India has to keep progressively expanding to cater to the end-stage renal disease affected population of India.

Abraham G, Vijayan M, Gopalakrishnan N, Shroff S, Amalorpavanathan J, Yuvaraj A, Nair S, Sundarajan S. State of deceased donor transplantation in India: A model for developing countries around the world. *World J Transplant* 2016; 6(2): 331-335 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/331.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.331>

INTRODUCTION

India is the third largest economy in the world, by purchasing power parity. However, the gross domestic product (GDP) spent on healthcare is only 4%, with merely 1.3% spent by the public sector^[1,2]. GDP per capita for India is United States \$1630.8^[3]. India faces tremendous challenges in providing basic healthcare facilities for its population, as the major percentage of the population lives below the poverty line. There has been a shift in disease epidemiology in India, with non-communicable diseases on the rise. Chronic kidney disease (CKD) is of increasing prevalence in India. CKD which is asymptomatic in early stages, puts greater financial burden on the stakeholders at the later stages. There are urban-rural and interstate disparities in the provision of renal replacement therapy (RRT), due to lack of skilled nephrologists, transplant surgeons and poor government support^[4]. The CKD registry of India found that diabetic nephropathy was the commonest cause (31%) of CKD^[5]. About 43% of the CKD patients had a monthly family income of < rupees 5000 (United States \$78.26) and RRT has an enormous financial impact on these patients. The majority of end-stage renal disease (ESRD) patients die within months of diagnosis as RRT is unaffordable to them. With risk factors like diabetes, hypertension and obesity showing an increasing trend, the financial strain of supporting RRT services is going to be enormous. Healthcare in India is provided by the public and private sector. In the southern states such as Tamil Nadu, Kerala, Puducherry,

Karnataka, Andhra Pradesh, government sponsored health insurance schemes have ensured that tertiary care hospitals provide maintenance haemodialysis, transplantation and follow-up either at very subsidized rate or free of cost and hence they cater to the lower socio economic sections of the society. RRT in private sector is unaffordable to the great majority of ESRD patients in India.

Renal transplantation is the best choice of RRT. India is a pluralistic country in terms of religion and no religion opposes saving a fellow human's life through organ donation. The first successful live renal transplant in India was done at Christian Medical College, Vellore in 1971. The Transplantation of Human Organs Act of 1994 was an initial step in promoting legal organ transplantation in India. Over the past 4 decades, the transplantation rate has shown a steady increase, though still much lower than in developed countries.

CURRENT STATE OF DDT IN INDIA

Deceased donor transplantation (DDT) is increasing in India, due to steps taken in both public and private sector, especially in the southern states. Healthcare spending is increasing partly because of revenue generation^[6]. Currently the deceased donor transplantation rate is 0.34 per million which was previously 0.08 per million population in 2013^[7]. As shown in Figure 1 and Table 1, a total of 1150 organs from 411 donors have been done harvested in India in 2014, comprising of 720 kidneys, 354 livers, 54 hearts, 16 lungs, 5 pancreas and 1 intestine, out of which 417 organs were harvested in Tamil Nadu. Figure 2 shows that the DDT program has steadily increased in 3 years. The generic immunosuppressive medications and induction molecules being manufactured in India have served as cost cutting measures to support multi-organ transplantation. In the government setup, transplantation services are offered at a free or subsidized cost. The cost of DDT could vary enormously in the private sector depending upon whether they are non-profit organizations or run for a profit. The DDT program in Tamil Nadu has brought down the incidence of organ trade^[8].

The main barrier to DDT in India is a shortage of harvested organ pool. In India, each year an estimated 137572 people die in road traffic accidents, and about 70% of them are declared brain dead, from whom organs can be harvested^[9]. Tamil Nadu state, with a population of 72 million, has developed a model approach to this problem which is being emulated by other states. Rounds of consultation have been held between the involved stakeholders and government officials to tackle the challenges of ensuring a transparent and efficient transplant program which provides practical guidelines for organ harvesting and transplantation. Government orders issued in this regard have greatly benefitted the DDT program in Tamil Nadu. There is a central coordinator for transplantation in the state of Tamil Nadu who is in charge of the donor list for

Table 1 Deceased organ donors in different states of India in 2014

State	Population	No. of donors	Organ donation rate
Tamil Nadu	72138958	136	1.9
Kerala	33387677	58	1.7
Maharashtra	112372972	52	0.5
Andhra Pradesh	84665533	52	0.6
Karnataka	61130704	39	0.6
Gujarat	60383626	28	0.5
Delhi-NCR	16753235	20	1.2
Puducherry	1244464	13	10.4
Uttar Pradesh	199581477	7	0.04
Chandigarh	1054686	6	5.7

deceased donor transplantation. This list has potential recipients from both public and private hospitals. As per the waiting list, organs are distributed whether it is private or public hospitals.

Public private partnership has been utilized to improve organ harvesting rates from potential organ donors. A total of 2028 transplantations from 677 donors have been done in Tamil Nadu between October 2008 and June 2015, comprising 1201 kidney transplants, 621 liver transplants, 135 heart transplants, 67 lung transplants and 4 pancreas transplants^[10]. In addition to the above, 1006 corneal transplants, 616 heart valves, 17 skin donations, 2 small bowel and 1 blood vessels transplant were done in this period. Female donors constituted only 1/5th of the donor pool. Donors comprised from 21 to 50 years of age.

FREE AND AFFORDABLE TRANSPLANTATION IN GOVERNMENT RUN HOSPITALS

In India, government hospitals provide healthcare free of cost or at a subsidized cost for the underprivileged sections of the society. In Government General Hospital (GGH), Chennai, renal transplantation has been regularly performed since 1987. Pre transplant dialysis, work-up of recipient and donor and transplant surgery are provided free of cost. Life-long post-transplant immunosuppression and follow up is provided free. Initially, only living related donor transplantation was done. Though the first deceased donor renal transplantation was done in 1996, the program picked up momentum only in 2008 when the Government of Tamil Nadu gave an impetus and made it one of its "flagship" programs. So far, 172 deceased donor renal transplantations have been done at GGH.

Successful deceased donor transplant program at GGH has achieved the following. Access to renal transplantation has improved. Economic and social barriers of transplantation have been broken. The problem of shortage of organs has been taken care of, at least to a small extent. Procurement and supply of less expensive generic immuno-suppressive by the government

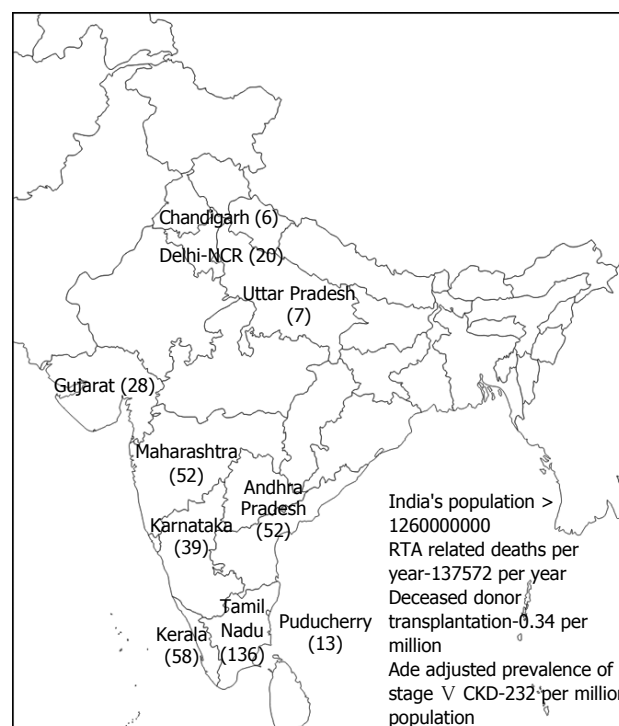


Figure 1 Map of India showing deceased organ donors in different states of India in 2014. CKD: Chronic kidney disease; RTA: Road traffic accidents.

curtails expenditure significantly. Continuous training of nephrologists, urologists, nursing staff and technicians in renal transplantation is creating a trained work force. This program has shown the benefits of "public - private" partnership. Grief counselors at Madras Medical college are provided by Multi Organ Harvesting and Networking (MOHAN) foundation. MOHAN foundation (<http://www.mohanfoundation.org/>) is a philanthropic non-governmental organization that aims to promote organ donation and transplantation. According to Indian law, a transplant coordinator must be present at every hospital where organ transplantation is being done. The role of the transplant coordinator is to grieve with the family and motivate the family members to get involved in the DDT program, thereby saving lives. MOHAN Foundation, through their educators, has trained 813 transplant coordinators. The MOHAN Foundation signed a memorandum of understanding with the Government General Hospital, Chennai, in 2010 to place its transplant coordinators there. Their presence has made a tremendous difference to the deceased organ donation program in the hospital. When a trained transplant coordinator counsels and spends time with grieving family, conversion figure in getting "yes for donation" is 65% in most hospitals.

THE ROAD AHEAD FOR DECEASED DONOR TRANSPLANTATION IN INDIA

In 2013, there were 137572 road traffic accidents in India. If we convert 50% of this figure into a prospective

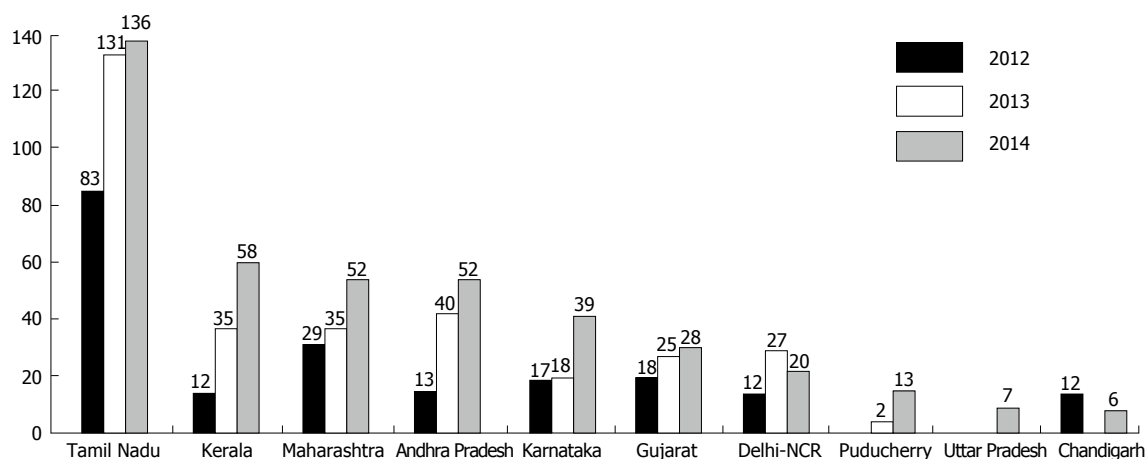


Figure 2 Number of renal transplants done in different states and union territories of India in 3 years (2012-2014).

organ donor pool, we will have more than 65000 donors supplying vital organs including 130000 kidneys yearly to be transplanted for the majority of prevalent end stage renal disease patients who require renal transplantation. The authors have also used poisoned organ donors from snake envenomation leading to brain death which also forms a donor pool of tens of thousands of organ donors in the country. With economic growth and increase in transplant centers to 166 in 2013, more centers undertake organ transplantation since last 2 years in India. It is believed that there are 120000 prevalent patients on maintenance haemodialysis and 10000 prevalent patients on chronic peritoneal dialysis in India, majority of them waiting for a deceased organ donation.

There are number of drawbacks in the DDT scenario in India. Ensuring optimal and prolonged function of the graft in the recipients is a great challenge. A complement dependent cytotoxicity cross matched technique is the predominant test to detect the compatibility between the donor and recipient. Human leukocyte antigen (HLA) matching is not done in DDT as the large majority of the patients waiting for the organ are not typed to look for HLA mismatch. Advanced immunological facilities for screening and matching are available only in the private sector and those available in a few flagship government hospitals charge a fee for the tests. Flow cytometry and luminex based platforms for testing for HLA based antibodies are not utilized in DDT, because of the lack of facilities. Hence immunological risk is not assessed regularly in recipients waiting for transplants and the long term outcome is unknown as there is a lack of dependable registries to capture the data of graft and patient survival. What we need is to find out the immunological risk to stratify the waiting patients into high, intermediate and low immunological categories. In order to augment the results of the transplantation, we require the following immunological platforms: Cell based assays, flow cytometry based assays, solid phase assays-enzyme linked immunosorbent assay, flowcytometry and microparticle based assays.

Scarcity of nephropathological services in many parts

of the country in evaluating graft dysfunction on a speedy basis is another limiting factor of the DDT program. There is a lack of knowledge of prior sensitization in the recipient as panel reactive antibodies are not evaluated in the great majority of recipients. Therapeutic drug monitoring of calcineurin inhibitors and mTOR inhibitors are fraught with inaccurate results due to lack of standardization. The prospective transplant programs should take this into consideration as a next step in promoting trouble free survival of the graft by allocation according to sensitization, avoiding HLA mismatches, careful monitoring and follow up with skilled transplant team. This can only be accomplished with robust support from the respective state governments who should set up a central 24 × 7 laboratory facility which can be cost beneficial to the stakeholders in the long run.

CONCLUSION

The DDT program in India is steadily increasing due to positive steps taken by some state governments. However, organ harvestation rates from potential donors can be further increased. Emphasis must be placed on road safety, less frequent road traffic accidents and higher organ harvestation rates from potential donors. Public awareness on this is on the increasing trend. Public private partnerships have had a positive impact on the DDT program. We foresee in the next 2 decades, India will emerge as the largest deceased donor transplantation in the world. This model of public private partnership in one of the largest developing economies can be emulated by other developing countries in South Asia and African continent.

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Basic Study

Role of cytomegalovirus on the maturation and function of monocyte derived dendritic cells of liver transplant patients

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Abstract

AIM: To study the impact of association between cytomegalovirus (CMV) pathogenesis with dendritic cell (DC) maturation and function was evaluated in CMV reactivated liver transplanted patients in comparing with non-reactivated ones, and healthy controls.

METHODS: Monocyte derived dendritic cells (MoDCs) was generated from collected ethylenediaminetetraacetic acid-treated blood samples from patient groups and controls. In these groups, expression rates and mean fluorescent intensity of DC markers were evaluated using flowcytometry technique. Secretion of cytokines including: interleukin (IL)-6, IL-12 and IL-23 were determined using enzyme-linked immunosorbent assay methods. The gene expression of toll-like receptor 2 (TLR2), TLR4 and IL-23 were analyzed using in-house real-time polymerase chain reaction protocols.

RESULTS: Results have been shown significant decreases in: Expression rates of MoDC markers including CD83, CD1a and human leukocyte antigen DR (HLA-DR), the mean fluorescence intensities for CD1a and HLA-DR, and secretion of IL-12 in CMV reactivated compared

with non-reactivated liver transplanted patients. On the other hand, significant increases have been shown in the secretions of IL-6 and IL-23 and gene expression levels of TLR2, TLR4 and IL-23 from MoDCs in CMV reactivated compared with non-reactivated liver transplanted recipients.

CONCLUSION: DC functional defects in CMV reactivated recipients, such as decrease in expression of DC maturation markers, increase in secretion of proinflammatory cytokines, and TLRs can emphasize on the importance of CMV infectivity in development of liver rejection in transplanted patients.

Key words: Cytomegalovirus; Dendritic cells; Liver transplantation

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Core tip: Cytomegalovirus (CMV) can interfere with maturation and antigen-presenting function of dendritic cell (DC). This interference with DC function could promote viral spread by paralyzing the adaptive immune system. CMV with DC infection induces inflammatory cytokines and activation of the interferon pathway in transplanted patients. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection.

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INTRODUCTION

Liver transplantation is the definitive treatment of choice for patients with end-stage liver disease^[1]. Graft rejection and infection remains major complications post liver transplantation^[2]. In liver recipients, cytomegalovirus (CMV) is the most determinative viral infectious pathogen cause of morbidity and mortality post-liver engraft and associate with diminished graft survival^[3]. This ubiquitous viral infection in immunocompromised transplant patients belongs to family *Herpesviridae*, subfamily *Betaherpesvirinae*, genus human herpes virus 5, species *Cytomegalovirus*^[4]. CMV primary infection results in life-long residence of the virus in the host, and reactivates in immunocompromised individuals frequently. Reactivation of CMV infection and development of related severe diseases and syndromes are common in solid organ recipients may lead to severe complications following transplant, such as acute rejection^[5,6]. CMV may lead also to higher

rates of bacterial and fungal infections in transplant recipients^[7]. In transplant patients, CMV infection causes both direct effects, reflecting cell destruction and indirect effects, such as acute or chronic rejection^[8]. Primary CMV infection induced immune related proinflammatory response that was maintained during latency. This continuous activation of the immune system may play a role in the acceleration of chronic diseases and pathogenesis of chronic allograft rejection^[9,10].

CD14⁺ monocytes and/or myeloid progenitor cells are site of CMV latency and are capable of harboring quiescent viral genomes^[11]. Monocyte represents a key cell type in the CMV pathogenesis, since mostly represent as an important cellular reservoir for latent virus^[12,13]. A number of studies have shown that CMV infection in monocytes is non-permissive and cellular differentiation is prerequisite for CMV replication^[11]. CMV replication can be reactivating in latently infected monocytes related to differentiation dependent manner^[11]. The dendritic cells (DCs) generated from CMV infected monocytes. CMV infected monocyte derived DCs (CMV-MoDCs) have an altered phenotype and functional defects^[14]. DCs are determinative initiators of cellular immunity against CMV infection^[1]. DCs also act with superiority over other antigen-presenting cells (APCs) in stimulating T-lymphocyte responses and maintaining protective antiviral immunity^[15].

CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity^[14,16]. This interference with DC function could promote viral spread by paralyzing the adaptive immune system^[17]. CMV with DC infection induces many hallmarks of innate immunity, such as the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients. This induction is rapid and can promote without requirement of CMV reactivation. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection^[8].

Therefore, in this study the impact of association between CMV pathogenesis with DC maturation and function was evaluated in CMV reactivated liver transplant patients in comparing with non-reactivated ones and healthy controls.

MATERIALS AND METHODS

Patients and samples

Ten liver transplanted patients who admitted at Transplant Center of Namazi Hospital, Shiraz, Iran were enrolled in this study between years 2012 and 2014. These patients divided to two groups including: 5 patients with CMV reactivation and rest of them without CMV reactivation. Therefore, CMV reactivation was confirmed in these transplanted patients using antigenemia protocol. The CMV antigen positive cells were counted and positive results are reported as one or more CMV pp65 antigen infected cell per 50000

Table 1 Underlying diseases in liver transplanted patients

Underlying diseases	Patients (n = 10)
PSC	2
Hypercholesterolemia	1
Cryptogenic cirrhosis	2
Hepatitis C virus infection	1
Hepatitis B virus infection	1
Wilson disease	1
NASH	1
Autoimmune hepatitis	1

PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steato-hepatitis.

white blood cells (WBCs). In all 5 patients with CMV reactivation 5 to 7 CMV pp65 antigen infected cell was found per 50000 WBCs, with a mean of 6 ± 1.01 cells/50000 WBCs. Underlying diseases for studied liver transplanted patients have been shown in Tables 1 and 2. The 20 mL ethylenediaminetetraacetic acid (EDTA)-treated blood samples were collected from each evaluated transplant recipients.

Also, 20 mL EDTA-treated blood samples were collected from 5 healthy volunteers as healthy controls. The age range (20-50 years old) and male to female ratio were similar in studied transplanted patient groups and healthy controls. This study was in accordance with the ethical standards of Shiraz University of Medical Sciences Committee (the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki). The informed consent was obtained from each studied transplanted patients. The immunosuppressive conditioning regimen used in liver transplanted patients was previously described^[18].

CMV antigenemia assay

CMV antigenemia protocol was performed on the EDTA-treated blood samples to determine viral reactivation by evaluation of the presence of lower matrix pp65 antigen in polymorph nuclear cells using the CMV Brite Turbo kit (IQ Products, Groningen, Netherlands) according manufacturer instruction as previously described^[19].

Generation of MoDCs

Leukocytes were isolated from 20 mL EDTA-treated blood samples collected from each liver transplanted patients with and without CMV reactivation using gradient centrifugation through Lymphodex (Inno-train, Germany), the cells from interphase were collected. CD14⁺ monocytes were isolated by positive selection using a MACS system (Miltenyi Biotech, Bergisch Gladbach, Germany), according to the manufacturer's protocol.

Monocytes were cultured in six-well cell culture plates in RPMI medium (Invitrogen, United States) supplemented with 4 mmol/L L-glutamine (Life technologies, United States), 100 IU/mL penicillin (Life technologies, United States), 10% heat-inactivated fetal bovine serum (Life technologies, United States), 1% sodium

Table 2 Average means fluorescence intensity for surface markers of monocyte derived dendritic cells in cytomegalovirus reactivated, non-reactivated liver transplanted recipients, and healthy individuals

Surface marker	CMV reactivated patients MFI \pm SE	CMV non-reactivated patients MFI \pm SE	Healthy controls of MoDCs MFI \pm SE
CD83	30.15 \pm 1.06	32.3 \pm 2.3	29.2 \pm 2.5
CD86	100.5 \pm 3.1	103 \pm 4.5	83 \pm 6.7
CD1a	47 \pm 1	80 \pm 3	53 \pm 13
HLA-DR	49.3 \pm 5.4	73.6 \pm 6.5	55.8 \pm 4.9

The data are the means \pm SE. MFI: Mean fluorescence intensity; HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

pyruvate (Bioidea, Iran), 1% non-essential amino acid (Life technologies, United States), 1000 IU/mL recombinant human granulocyte macrophage-colony stimulating factor (R and D Systems, United Kingdom) and 500 IU/mL recombinant human interleukin-4 (IL-4; R and D Systems, United Kingdom) in a 37 °C 5% CO₂ humidified incubator. Every 3 d 200 μ L of the medium was exchanged with fresh medium and cytokines. For mature cells, maturation was induced on day 5 by adding 1000 IU/mL recombinant human tumor necrosis factor- α (TNF- α ; R and D Systems, United Kingdom) and allowing maturation to proceed for 48 h.

Analysis of MoDC markers

Matured MoDCs were harvested and stained with fluorescently labeled monoclonal antibodies including: PE-anti-CD14, FITC-anti-CD83, FITC-anti-CD86, FITC-anti-CD1a and FITC-anti-HLA-DR (eBiosciences, United States). Cell suspension was mixed with antibody solution followed by incubation in the dark at 4 °C for 45 min. Cells were suspended in phosphate buffer saline and data acquisition for 10000 events was performed using flowcytometry (Becton Dickinson, San Jose, United States). FlowJo software (Flexera Company, United States) was used to analyze the expression rate and mean fluorescence intensity (MFI) of studied DC markers.

Measurement of cytokine levels

The IL-6, IL-12 and IL-23 cytokines released from MoDCs in culture supernatant were measured using the commercial human enzyme-linked immunosorbent assay Ready-SET-Go kits (eBioscience, United States) according to the manufacturer's protocols.

The gene expression of cytokines and toll-like receptors

Total RNA was extracted from MoDCs using RNX plus (CinnaGen, Iran). RNA samples were reverse transcribed using Reverse Transcriptase (Vivantis, Malaysia) and random hexamer as previously described^[20]. An amount of 1 μ g total RNA was used to produce cDNA. The primers that were used to analyze the gene transcripts including: TLR-2 (NM_003264.3), TLR-4 (NM_003266.3),

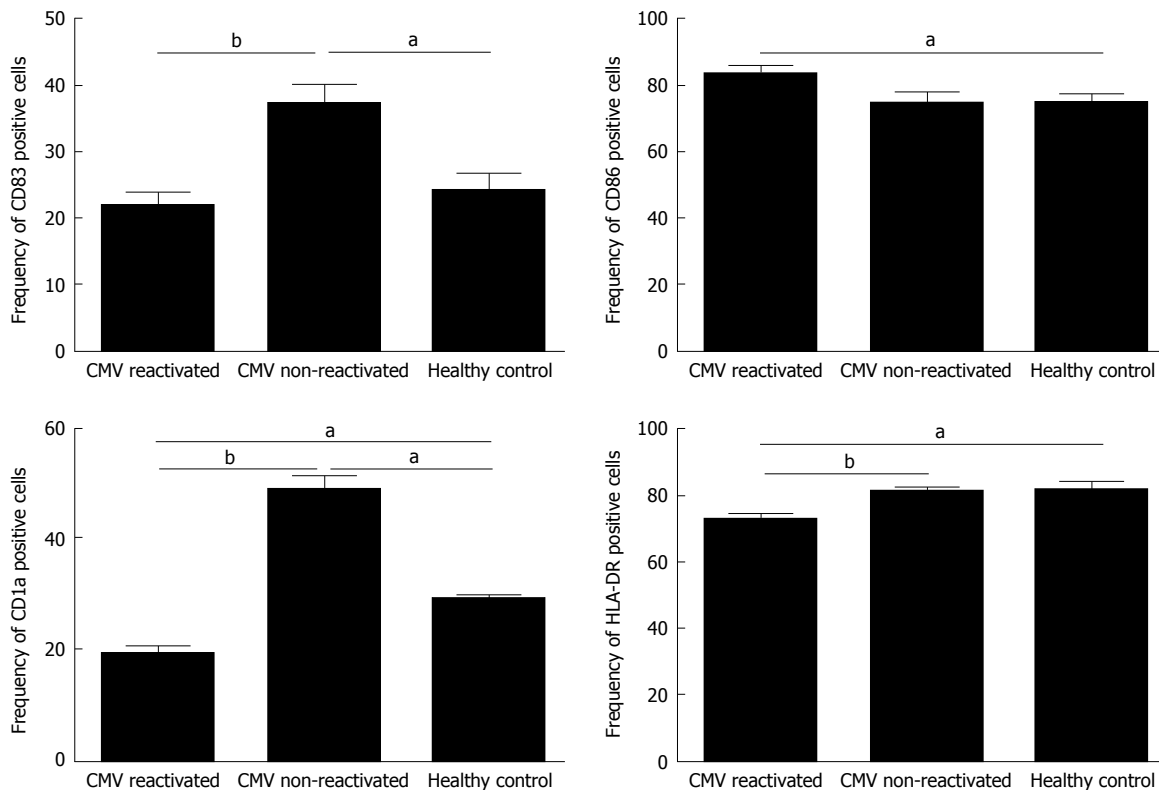


Figure 1 The expression rates for surface monocyte derived dendritic cell markers of CD83, CD86, CD1a and human leukocyte antigen DR in cytomegalovirus reactivated patients, cytomegalovirus non-reactivated recipients, and healthy control. The expression rates of CD83, CD1a and HLA-DR were significantly decreased in CMV reactivated patients vs non-reactivated recipients. Any significance is indicated ^a $P < 0.05$, ^b $P < 0.01$. The data are the means \pm SE. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus.

IL-23 (NM_016584.2) and β -actin (NM_001101.3)^[18]. The mRNA expression levels of the IL-23, TLR2 and TLR4 genes were finally determined in MoDCs of liver transplanted patients with and without CMV reactivation compared with healthy controls using in-house-real time polymerase chain reaction (PCR) protocols as previously described^[18].

The PCR reaction was carried out in a final volume of 20 μ L containing: 10 μ L SYBR green Premix by Ex taq (Takara, Japan), 0.4 μ L SYBR Green Dye, 0.8 μ L forward and 0.8 μ L reverse primers (8 pmol), 6 μ L H₂O and 2 μ L cDNA template. The thermal cycling profile was the same for each primer set and consisted of an initial denaturation at 95 $^{\circ}$ C for 2 min, followed by 40 amplification cycles of 95 $^{\circ}$ C for 30 s and 65 $^{\circ}$ C for 20 s using Step One Plus Real-Time instrument (ABI, Step One Plus, United States). The mean Ct value of target genes in each sample was normalized using β -actin gene Ct value to give a Δ Ct value. This was then normalized to healthy control ($\Delta\Delta$ Ct), and finally the $2^{-\Delta\Delta$ Ct}.

Statistical differences between studied groups were evaluated using non-parametric tests of version 15 of SPSS software (Chicago, United States). The sample analysis was also analyzed using version 5 of Graph Pad Prism software (United States). The $2^{-\Delta\Delta$ Ct value was calculated using Livak method for analysis of the expression level of studied genes. The P -value of < 0.05 was considered as significant.

RESULTS

Expression of MoDC markers in patient groups and controls

The expression rate ($P = 0.02$) and MFI ($P = 0.04$) of CD86 were both significantly increased in CMV reactivated patients in comparing with healthy controls with median value of 82% vs 75%, respectively (Figures 1 and 2). The expression rate of CD1a was significantly decreased in CMV reactivated patients in comparing with healthy controls with median value of 18% vs 30%, respectively ($P = 0.01$) (Figure 3). The expression rate of human leukocyte antigen DR (HLA-DR) was significantly decreased in CMV reactivated patients in comparing with healthy controls with median value of 72% vs 84%, respectively ($P = 0.01$) (Figure 4).

The expression rate of CD83 was significantly increased in CMV non-reactivated patients in comparing with healthy controls with median value of 40% vs 21%, respectively ($P = 0.02$) (Figure 1). The MFI of CD86 was significantly higher in CMV non-reactivated patients than that in healthy control ($P = 0.04$) (Figure 2). The expression rate of CD1a was significantly raised in CMV non-reactivated patients than that in healthy controls with median value of 50% vs 30%, respectively ($P = 0.01$) (Figure 1). The MFI of HLA-DR was significantly raised in CMV non-reactivated liver transplanted patients than that in healthy controls ($P =$

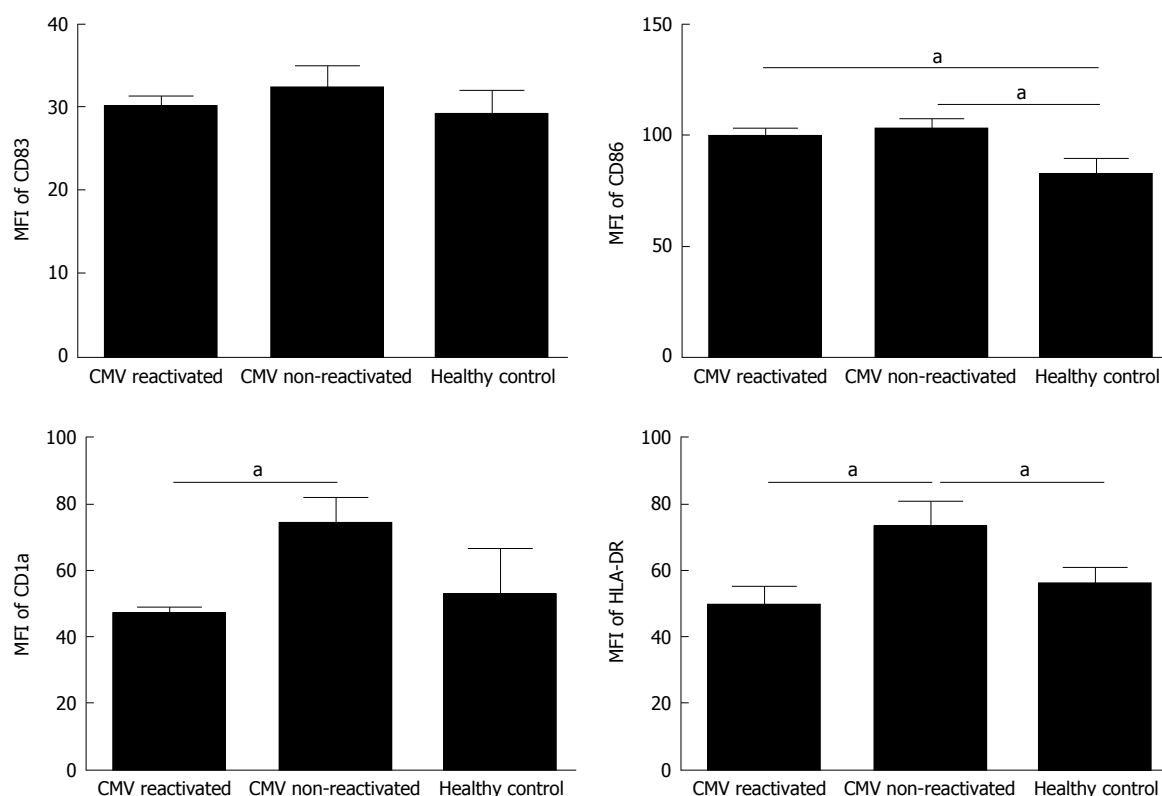


Figure 2 Mean fluorescence intensity for surface monocyte derived dendritic cell markers of CD83, CD86, CD1a and human leukocyte antigen DR in cytomegalovirus reactivated patients, cytomegalovirus non-reactivated recipients, and healthy control. The MFI of CD1a and HLA-DR were significantly decreased in CMV reactivated patients vs non-reactivated recipients. Any significance is indicated * $P < 0.05$. The data are the means \pm SE. MFI: Mean fluorescence intensity; HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus.

0.03) (Figure 5).

Expression rate of CD83 was significantly decreased in CMV reactivated compared with non-reactivated liver transplanted patients with median value of 22% vs 40%, respectively ($P = 0.007$) (Figure 1). The expression rate ($P = 0.007$) and MFI ($P = 0.02$) of CD1a was significantly lower in CMV reactivated compared with non-reactivated patients with median value of 18% vs 50%, respectively (Figures 1 and 2). The expression rate ($P = 0.007$) and MFI ($P = 0.03$) of HLA-DR was significantly decreased in CMV reactivated patients compared with non-reactivated recipients with median value 72% vs 80%, respectively (Figures 1 and 2).

Cytokine secretions by MoDCs in patient groups and controls

The secretion of IL-6 from MoDCs of CMV reactivated patients was significantly increased in comparing with healthy control with mean of 334.6 ± 2.2 pg/mL vs 312 ± 1.08 pg/mL ($P = 0.009$) (Figure 6A). The secretion of IL-12 from MoDCs of CMV reactivated patients was significantly decreased in comparing with healthy control with mean of 1.26 ± 0.04 pg/mL vs 1.54 ± 0.03 pg/mL ($P = 0.01$) (Figure 6B). The IL-23 secretion level from MoDCs of CMV reactivated patients was significantly increased in comparing with healthy control with mean of 13.53 ± 0.09 pg/mL vs 13.1 ± 0.1 pg/mL ($P = 0.02$) (Figure 6C).

The secretion of IL-6 from MoDCs of CMV non-reactivated patients was significantly decreased in comparing with healthy control with mean of 261.2 ± 3.72 pg/mL vs 312 ± 1.08 pg/mL ($P = 0.006$) (Figure 6A). Also, secretion of IL-12 from MoDCs of CMV non-reactivated patients was significantly increased in comparing with healthy control with mean of 1.98 ± 0.03 pg/mL vs 1.54 ± 0.03 pg/mL ($P = 0.009$) (Figure 6B). The secretion of IL-23 from MoDCs of CMV non-reactivated patients was significantly decreased in comparing with healthy control with mean of 8.77 ± 0.19 pg/mL vs 13.1 ± 0.1 pg/mL ($P = 0.008$) (Figure 6C).

The secretion of IL-6 from MoDCs was significantly higher in CMV reactivated patients than that in CMV non-reactivated ones with mean of 334.6 ± 2.2 pg/mL vs 261.2 ± 3.72 pg/mL ($P = 0.005$) (Figure 6A). The secretion of IL-12 from MoDCs was significantly lower in CMV reactivated patients compared with non-reactivated ones with mean of 1.26 ± 0.04 pg/mL vs 1.98 ± 0.03 pg/mL ($P = 0.007$) (Figure 6B). The secretion of IL-23 from MoDCs was significantly higher in CMV reactivated patients than that in CMV non-reactivated ones with mean of 13.53 ± 0.09 pg/mL vs 8.77 ± 0.19 pg/mL ($P = 0.007$) (Figure 6C).

Cytokine and TLR gene expression by MoDCs in patient groups and controls

The IL-23, TLR2, and TLR4 mRNAs was expressed 5.2

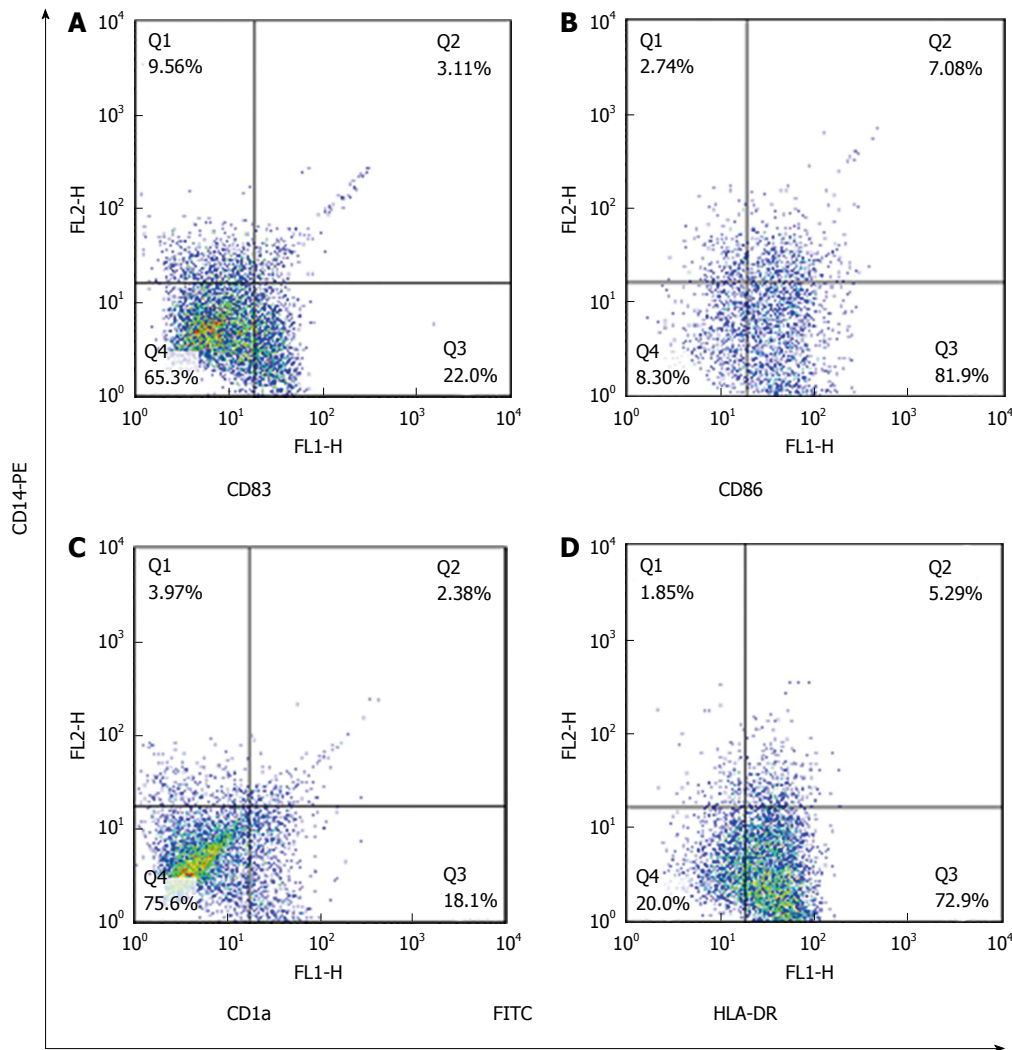


Figure 3 The expression rate of monocyte derived dendritic cell markers in cytomegalovirus reactivated patients was examined by dual-color cytometry. Expression of surface markers: CD83 (22%) (A), CD86 (82%) (B), CD1a (18%) (C) and HLA-DR (72.9%) (D) on MoDCs in CMV reactivated patients. CD14-PE, phycoerythrin-conjugated CD14, CD83-FITC, fluorescein isothiocyanate-conjugated CD83, CD86-FITC, fluorescein isothiocyanate-conjugated CD86, CD1a-FITC, fluorescein isothiocyanate-conjugated CD1a, HLADR-FITC, fluorescein isothiocyanate-conjugated HLA-DR. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

($P = 0.005$), 3.6 ($P = 0.007$), and 4.3 ($P = 0.009$) folds significantly more in CMV reactivated patients compared with healthy controls, respectively (Figure 7).

The gene expression level of IL-23 was the same in both CMV non-reactivated patients and healthy controls ($P = 0.6$) (Figure 7A). The TLR2 mRNA expression was significantly decreased in CMV non-reactivated patients in comparing with healthy controls ($P = 0.02$) (Figure 7B). The IL-23, TLR2, and TLR4 mRNAs was expressed 4.7 ($P = 0.007$), 5.4 ($P = 0.005$), and 2.8 ($P = 0.01$) folds significantly more in CMV reactivated compared with non-reactivated patients, respectively (Figure 7).

DISCUSSION

DCs have superiority over other APCs in viral infections to stimulate T-cell responses and maintaining protective antiviral immunity^[15]. DCs are critical initiators of cellular immunity against viruses especially CMV^[1]. CMV

as a determinative human pathogen can cause fatal complications and promote rejection in transplanted recipients^[21]. CMV infection can also increase the rate of immunosuppression with interfering the maturation and function of DCs post-transplantation^[16,22]. Therefore, in this report, the effects of CMV reactivation compared to non-reactivation were elucidated on maturation and function of DCs in liver transplanted recipients and healthy controls.

CMV and DC maturation was interested of related researchers in earlier studies^[16,22]. Down-regulation of major histocompatibility complex (MHC) class I and CD86 and CD83 costimulatory molecules on immature DCs and inhibition of DC maturation was indicated following *in vitro* CMV infection of DCs^[16]. CMV targets DCs and alters their functions by interfering with MHC-II biosynthesis and maturation, as well as with the expression and function of related endocytic proteases^[23]. CMV-infected DCs displayed abnormal

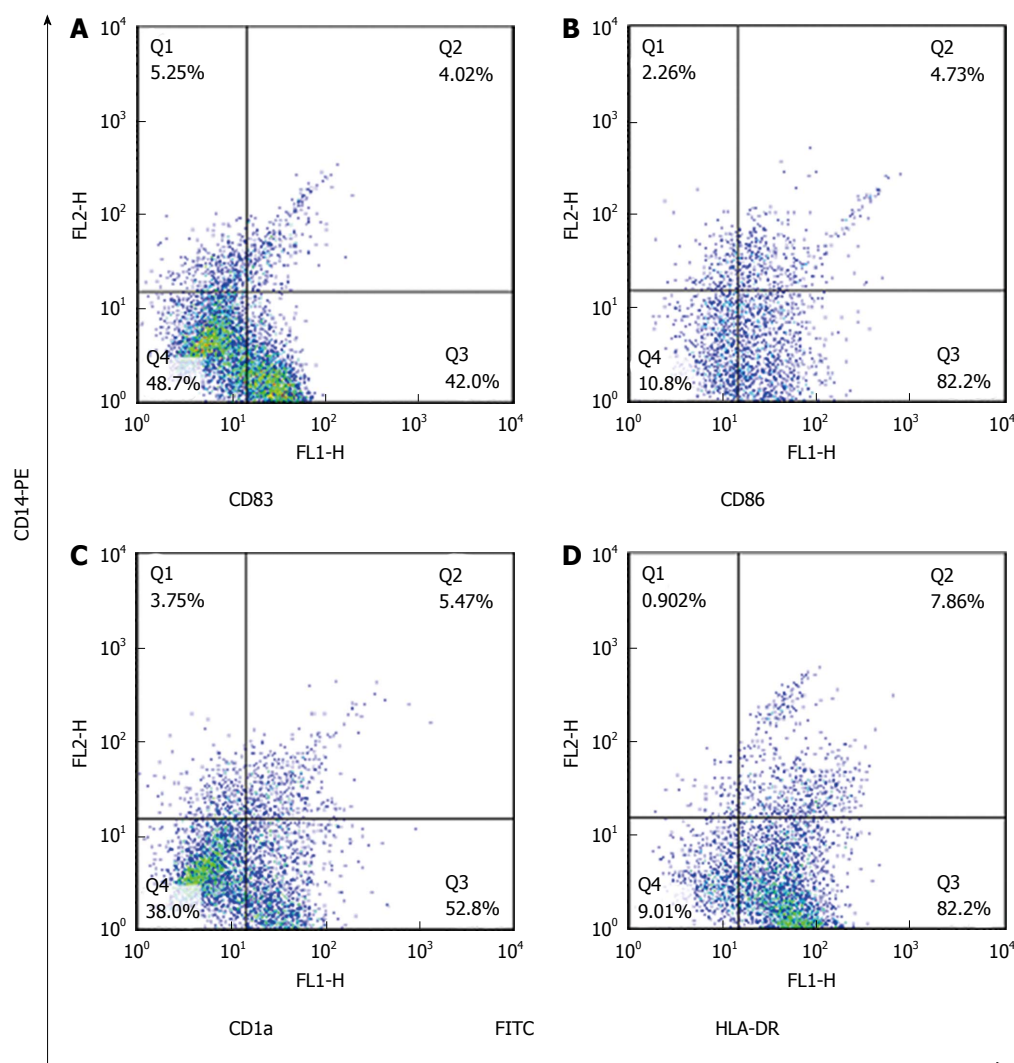


Figure 4 The expression rate of monocyte derived dendritic cells markers in cytomegalovirus non-reactivated patients was examined by dual-color cytometry. Expression of surface markers: CD83 (42%) (A), CD86 (82%) (B), CD1a (52.8%) (C) and HLA-DR (82%) (D) on MoDCs in CMV non-reactivated patients. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

phenotypic characteristic and stable expression of CD83 as a maturation marker^[24,25]. Infectivity of CMV in MoDCs resulted in maturation of the surviving cells, up-regulation of the CD86, and down-regulation of MHC-I and II^[21,26]. In this study, results also demonstrated the down-regulation of CD83, CD1a and HLA-DR molecules on MoDCs in CMV reactivated compared to non-reactivated liver transplanted patients. The MFIs of CD1a and HLA-DR were significantly down-regulated in CMV reactivated patients compared to non-reactivated ones. On the other hand CMV-mediated up-regulation of CD86 and down-regulation of CD1a and HLA-DR molecules were found in CMV reactivated patients compared to healthy controls. Therefore CMV interference with maturation of DCs promotes viral spread by paralyzing the adaptive immune system^[17]. Especially, in transplanted recipients, CMV-infected DCs are less capable of developing antiviral activated APCs, this may lead to impaired immune responses not only against CMV, but most likely also against other invading microorganisms.

Stimulation of toll-like receptors (TLRs) on DCs activates signal transduction pathways lead to induction of a range of antimicrobial genes and inflammatory cytokines^[11,27]. TLR signaling pathways trigger a series of interactions among specific intracellular mediators that ultimately result in the release of nuclear factor- κ B (NF- κ B) from its related endogenous inhibitors^[11]. Earlier reports emphasized that markedly up-regulation of TLR2 and TLR4 responsible for early activation of alloimmune T-cells favoring to acute renal and also liver allograft rejection^[28-30]. TLR2 was recently identified as a cell surface receptor activates secretion of inflammatory cytokine response to CMV infection^[4,11]. *In vitro* stimulation of TLR2 by CMV resulted in NF- κ B activation and cytokine secretion^[31,32]. CMV was also able to activate TLR4 and mediate cytokine secretion in human monocytic cells^[33]. Similarly, *TLR2* and *TLR4* gene expression by MoDCs was significantly increased in studied CMV reactivated liver transplanted patients compared to non-reactivated ones and healthy controls.

Pathological processes associated with CMV reac-

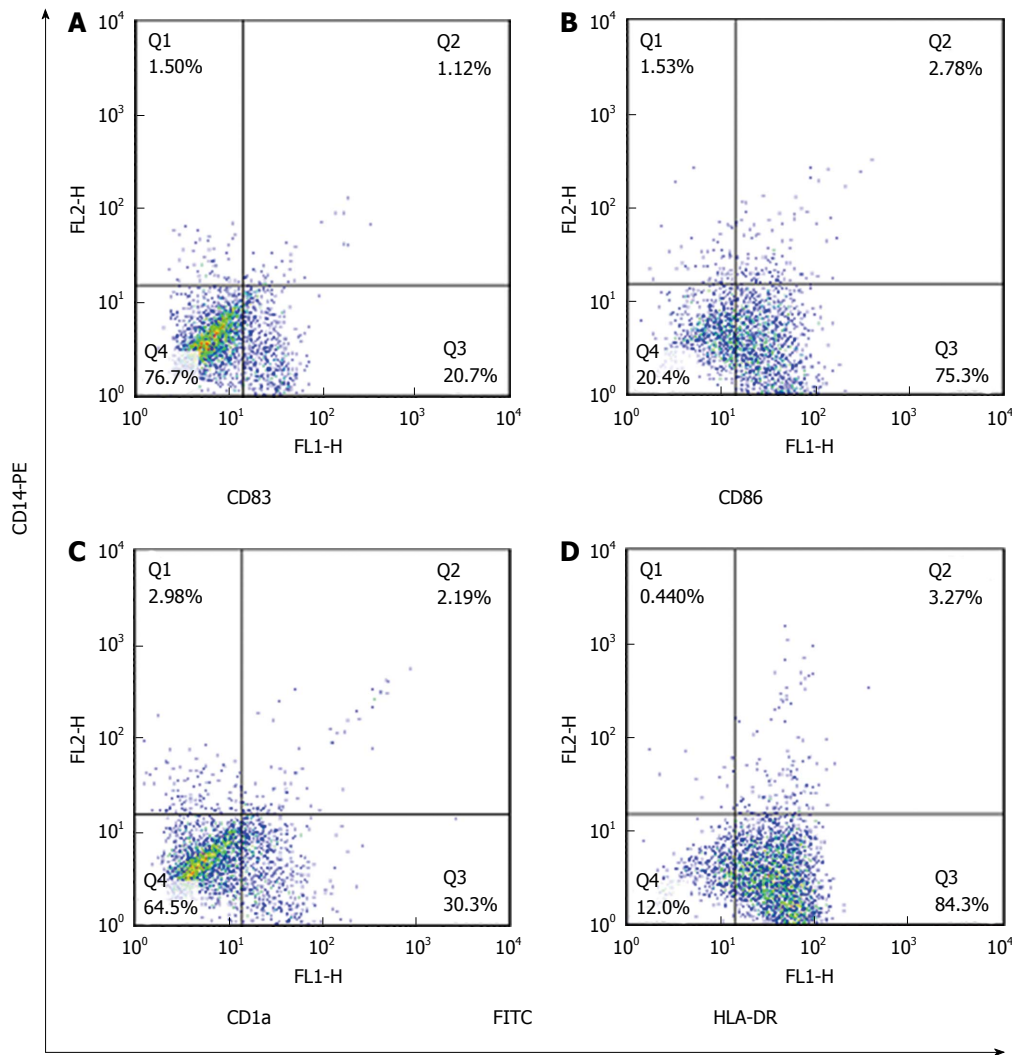


Figure 5 The expression rate of monocyte derived dendritic cell markers in healthy control was examined by dual-color cytometry. Expression of surface markers: CD83 (20.7%) (A), CD86 (75.3%) (B), CD1a (30%) (C) and HLA-DR (84%) (D) on MoDCs in healthy control. HLA-DR: Human leukocyte antigen DR; MoDC: Monocyte derived dendritic cell.

tivation appear to be mediated by the release of inflammatory cytokines^[11]. Following CMV infection DCs produce no IL-12 and only low levels of TNF- α ^[16]. Down-regulation of IL-12 production impairs the antiviral mechanisms of T cells and NK cells in patients with active CMV infection^[34]. Similar to previous reports, results of the present study revealed that secretion of IL-12 by MoDCs was significantly decreased in CMV reactivated liver transplanted patients compared to CMV non-reactivated ones and healthy controls. But, Th17 cell lineage and related cytokines like IL-17 and IL-23 have determinative role contribute to the mechanisms of allograft rejection^[35-39]. IL-6 is also essential for differentiation of IL-17-producing human Th cells^[40]. IL-6 and subsequent signaling pathways are important for activation and differentiating DCs. Activation and concomitant production of these cytokines also appear to be essential for reactivation and replication of CMV in infected patients such as transplant recipients^[21]. Similarly, IL-6 and IL-23 secretion and expression by

MoDCs are significantly higher in CMV reactivated in comparing with non-reactivated patients and healthy controls.

In conclusion, results of this study highlight the fact that, CMV and DCs contractions promote different pathways including: Interference with the maturation and expression of DC markers (CD83, CD1a and HLA-DR), IL-12 decreasing and IL-6 and IL-23 elevation from MoDCs and also increase of the mRNA expression levels of *TLR2*, *TLR4* and *IL-23* genes in MoDCs of CMV reactivated liver transplanted patients. These pathways can implicate in the development of acute or chronic allograft liver rejection.

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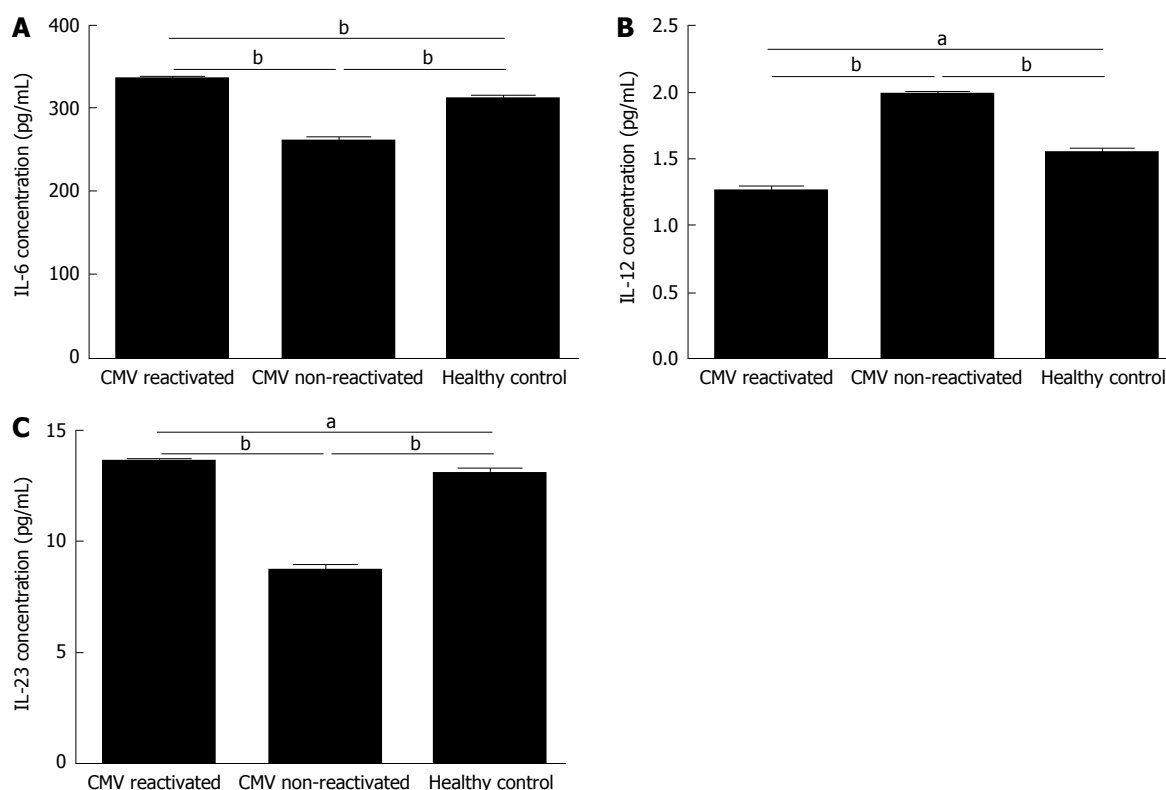


Figure 6 Levels of cytokines secreted by monocyte derived dendritic cells in cytomegalovirus reactivated patients, non-reactivated recipients, and healthy controls. The IL-6, IL-12 and IL-23 concentrations in the supernatants were measured by enzyme-linked immunosorbent assay. IL-6 and IL-23 secretions were significantly increased in CMV reactivated patients compared to non-reactivated recipients and healthy controls (A and C). IL-12 secretion was significantly decreased in CMV reactivated patients compared to non-reactivated ones and healthy controls (B). Any significance is indicated ^aP < 0.05, ^bP < 0.01. The data are the means \pm SE. CMV: Cytomegalovirus; IL: Interleukin.

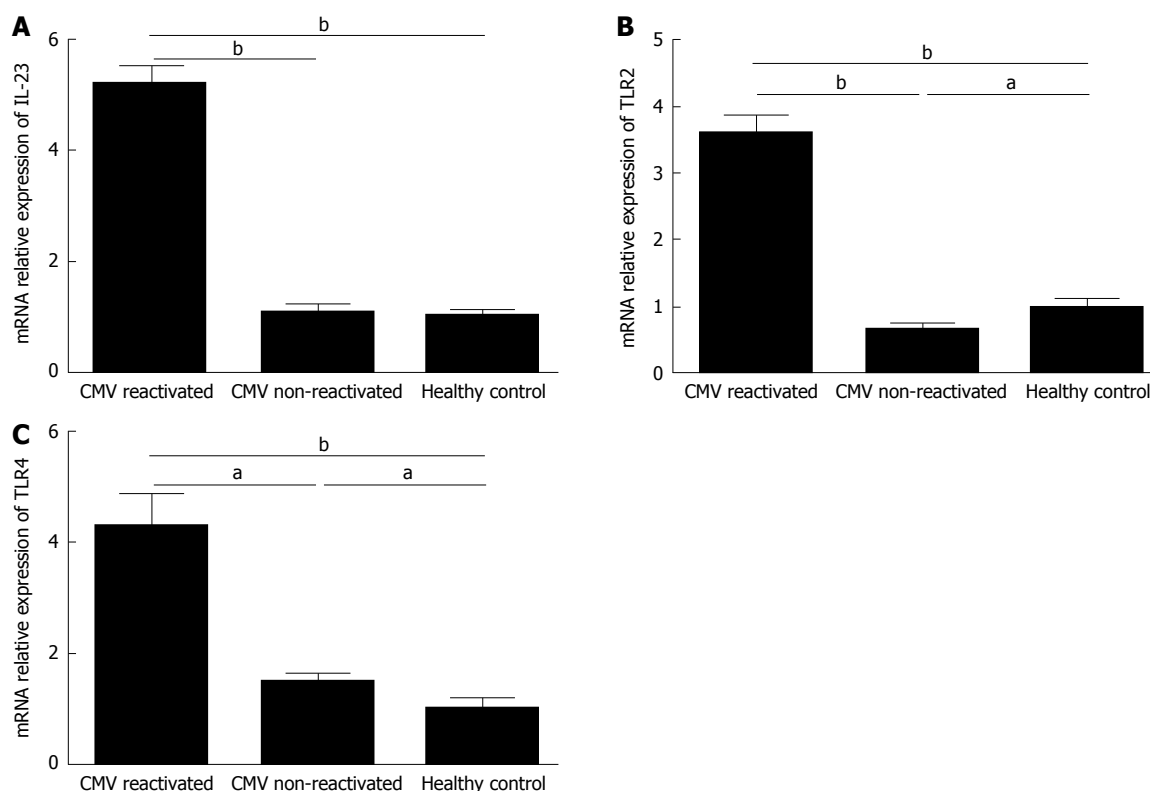


Figure 7 In cytomegalovirus reactivated patients, non-reactivated recipients, and healthy controls, mRNA relative expressions of interleukin-23 (A), toll-like receptor 2 (B) and toll-like receptor 4 (C) were determined by real-time polymerase chain reaction protocols. The gene expression levels of IL-23, TLR2 and TLR4 were significantly increased in CMV reactivated patients in comparing with non-reactivated ones and healthy controls. Any significance is indicated ^aP < 0.05, ^bP < 0.01. The data are the means \pm SE. CMV: Cytomegalovirus; IL: Interleukin; TLR: Toll-like receptor.

COMMENTS

Background

Cytomegalovirus (CMV) is the most determinative viral infectious pathogen cause of morbidity and mortality post-liver engraft and associate with diminished graft survival. CMV infected monocyte derived dendritic cells (CMV-MoDCs) have an altered phenotype and functional defects. DCs are determinative initiators of cellular immunity against CMV infection. CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection. However, association between CMV pathogenesis with DC maturation and function in CMV reactivated liver transplant patients was not yet evaluated.

Research frontiers

CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity. This interference can promote viral spread by paralyzing the adaptive immune system. CMV with DC infection induce the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients.

Innovations and breakthroughs

This is the first study evaluating the interference between CMV reactivation with maturation and antigen-presenting function of DCs in Iranian liver transplanted patients.

Applications

Interference of CMV and DCs can promote viral spread by paralyzing the adaptive immune system and induce the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients. Results of this study highlight the fact that, CMV and DCs contractions promote different pathways including: Interference with the maturation and expression of DC markers and cytokines in MoDCs of CMV reactivated liver transplanted patients. These pathways can implicate in the development of acute or chronic allograft liver rejection.

Terminology

CMV infected MoDCs have an altered phenotype and functional defects. DCs are determinative initiators of cellular immunity against CMV infection. DCs also act with superiority over other antigen-presenting cells in stimulating T-lymphocyte responses and maintaining protective anti-CMV immunity.

Peer-review

The manuscript entitled, "Role of cytomegalovirus on the maturation and function of monocyte derived dendritic cells of liver transplant patients", by Karimi *et al.*, demonstrated the functional defects of dendritic cells in CMV reactivated liver transplant recipients when compared to those without CMV reactivation or healthy norms demonstrating the differences in cytokine concentrations and expressions. This is very detailed investigation of cytokines of monocyte derived dendritic cells.

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Retrospective Cohort Study

Single-lung transplantation in emphysema: Retrospective study analyzing survival and waiting list mortality

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Abstract

AIM: To performed remains a subject of debate and is the principal aim of the study.

METHODS: This retrospective analysis included 73 patients with emphysema (2000-2012). The outcomes of patients undergoing single-lung transplantation (SL) ($n = 40$) or double-lung transplant (DL) ($n = 33$) were compared in a Cox multivariate analysis to study the impact of the technique, postoperative complications and acute and chronic rejection on survival rates. Patients were selected for inclusion in the waiting list according to the International Society of Heart Lung Transplantation criteria. Pre and postoperative rehabilitation and prophylaxis, surgical technique and immunosuppressive treatment were similar in every patients. Lung transplantation waiting list information on a national level and retrospective data on emphysema patient survival transplanted in Spain during the study period, was obtained from the lung transplantation registry managed by the National Transplant Organization (ONT).

RESULTS: Both groups were comparable in terms of gender and clinical characteristics. We found significant differences in the mean age between the groups, the DL patients being younger as expected from the inclusion criteria. Perioperative complications occurred in 27.6% SL vs 54% DL ($P = 0.032$). Excluding perioperative mortality, median survival was 65.3 mo for SL and 59.4 mo for DL ($P = 0.96$). Bronchiolitis obliterans and overall 5-year survival were similar in both groups. Bacterial

respiratory infection, cytomegalovirus and fungal infection rates were higher but not significant in SL. No differences were found between type of transplant and survival ($P = 0.48$). To support our results, national data on all patients with emphysema in waiting list were obtained ($n = 1001$). Mortality on the waiting list was 2.4% for SL vs 6.2% for DL. There was no difference in 5 year survival between 235 SL and 430 DL patients transplanted ($P = 0.875$).

CONCLUSION: Our results suggest that SL transplantation in emphysema produce similar survival than DL with less postoperative complication and significant lower mortality in waiting list.

Key words: Lung transplantation; Single-lung; Survival; Complications; Emphysema; Double-lung

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Core tip: This is a retrospective and comparative study of 2 cohort of patients with advanced-stage emphysema who were performed uni or bilateral lung transplant. The results of this study support the realization of single-lung transplantation in most of the cases of emphysema because it is technically simpler, it has less risk of surgical sutures, and finally it has less immediate postoperative complications. Single and double lung transplantation has a similar long-term survival. Moreover, if a second transplant is needed in the long-term, the contralateral transplantation has the same initial transplant survival if the patient remains in a similar clinical situation. Survival Spanish national register does not show difference between the two techniques too, supporting the results of our relatively small series. This strategy of performing single lung transplantation in most of the cases of emphysema would encourage and enhance the use of donors thanks to the twining procedure, and would decrease mortality in the waiting list as shown in the National Transplant Organization patients analysed. Proper pre and postoperative prophylaxis and postoperative early extubation protocol is essential to achieve good results.

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INTRODUCTION

The number of solid organ transplantations performed in Spain has steadily increased in the last 20 years from around 1300 in 1989 to over 4000 in 2009^[1]. This has been possible thanks to a greater number of donors, achieved as a consequence of a set of organizational measures, known as the "Spanish Model", directed by

the Spanish National Transplant Organization (ONT)^[1]. However, this increase in organ donation does not meet all lung transplantation requirements and, as in the rest of the world, organ availability remains the main limitation^[2]. The latest data available indicate that 238 lung transplantations, including combined transplantations, were performed in Spain in 2012. Only 48% of candidates on the waiting list were transplanted during this same year^[2]. The median time on the waiting list was 163 d in 2011 (interquartile range: 65-303). Waiting list mortality in the same period was 4.6%. Moreover, 2.7% were taken off the waiting list due to clinical worsening^[3].

Advanced-stage pulmonary emphysema is the most common indication for lung transplantation^[4]. In 2011, 29.5% of advanced emphysema patients were in need of this intervention, 26.2% of whom had chronic obstructive pulmonary disease (COPD) type 1, and 3.3% α 1-antitrypsin deficiency^[4]. The question remains whether single or double-lung transplantation should be performed^[5-9]. Single-lung transplantation (SL) has the advantage of making optimal use of available organs, but some studies indicate better outcomes and survival in patients with double-lung transplant (DL) compared to SL recipients^[8,10]. However, SL is often performed in older and retransplanted patients, who generally present with more comorbidities^[11]. One of the main problems in SL is native lung hyperinflation^[12,13], but minimally invasive techniques such as video-assisted thoracoscopic surgery (VATS)^[14] or bronchoscopic lung volume reduction^[15,16] are now available and have shown good results. An appropriate clinical protocol implementing these recent developments might help narrow the gap in survival rates of single and DL patients reported in the literature.

Our first goal was to evaluate our group's 10-year experience in lung transplantation in patients with emphysema, in order to assess and compare survival and outcomes of SL and DL recipients. We further compared and assessed our results against national data available in the Spanish Lung Transplant Registry (RETP), in order to explore the impact of SL or DL on patient survival and waiting list progress, and discuss the consequences of the different approaches.

MATERIALS AND METHODS

Local (single-center) data

We conducted a retrospective study of the records of patients transplanted in our center between 2000 and 2012. This study was approved by the Clinical Research Ethics Committee of the Galician Healthcare Authorities overseeing Complejo Hospitalario Universitario A Coruña (CHUAC). All procedures were performed in compliance with Spanish regulations and the Declaration of Helsinki.

Spanish national data

Further assessment of our data required comparison

Table 1 Demographic, clinical characteristics, and complications of patients in the single-center series *n* (%)

	Study cohort <i>n</i> = 73	Single-lung <i>n</i> = 40	Double-lung <i>n</i> = 33	<i>P</i> -value
Age in years (mean ± SD)	54.9 ± 7.1	57.3 ± 6.1	51.9 ± 7.3	0.001
Gender (male)	62 (85%)	33 (82.5%)	29 (87.9%)	0.520
Underlying disease				0.940
α1-antitrypsin deficiency	18 (24.6%)	10 (25%)	8 (24.2%)	
Chronic obstructive pulmonary disease	55 (75.3%)	30 (75%)	25 (75.2%)	

with Spanish national registry data. The 7 centers performing lung transplantation in Spain started around the same time; the economic and social status of the population attended is similar in all of them, and all use similar techniques and postoperative care protocols.

Lung transplantation waiting list information (1999 to 2012) on a national level was obtained from the ONT. All Spanish transplantation teams pool their data in the RETP that began its activity in 2001, the first year of complete data availability. Follow up information from patients transplanted between 2001 and 2012 was selected.

General care protocol for lung transplant recipients

Patients were selected for inclusion in the waiting list according to the International Society of Heart Lung Transplantation criteria^[17]. Between 2000 and 2003, DL was preferred in emphysema patients < 60 years of age, and SL was reserved for those > 60 years or with comorbidities. However, in view of the good clinical results with SL, we decided in 2003 that SL would be the preferred approach in all cases, including α1-antitrypsin deficiency. According to our protocol, the patient performs physical and respiratory exercises during the waiting period. Postoperative tracheostomy is used when necessary due to prolonged intubation. In addition, SL candidate receive antifungal prophylaxis with weekly amphotericin B lipid complex *via* aerosol. Patients with a history of recurrent infections also receive tobramycin before surgery^[18].

The surgical technique used by our group has not undergone substantial changes since our program began in 1999, and is similar to the recently described^[19]. Ventilation difficulties during the immediate postoperative period are critical in SL in emphysema^[20]. Patients are routinely extubated in the surgery room, or within the following 6 h whenever possible. This is possible in most cases, but patients requiring assisted ventilation after surgery also benefit from an optimized care protocol, including ventilation with 2 respiratory units.

Immunosuppressive treatment is described elsewhere^[18], and consisted of triple therapy including basiliximab for induction, oral or intravenous cyclosporine, azathioprine and decreasing doses of corticosteroids in all cases. Cyclosporine and/or azathioprine were switched

to tacrolimus and/or mycophenolate after repeated acute rejection or persistent rejection. All patients received antibacterial prophylaxis with amoxicillin and clavulanic acid, modified after transplantation according to the postoperative cultures of bronchial aspirate. In addition, all patients received antiviral prophylaxis with valganciclovir for 3-6 mo depending on their Cytomegalovirus (CMV) serology risk; antifungal prophylaxis with fluconazole, followed by amphotericin B lipid complex as described elsewhere^[21], and trimethoprim with sulfamethoxazole on alternate days were prescribed to prevent infection by *Pneumocystis carinii*.

Hyperinflation during the long-term postoperative period was treated with surgical or bronchoscopic volume reduction^[22].

Statistical analysis

Transplanted patients (in our center and on the national waiting list) were classified into 2 groups: SL or DL transplantation. Combined transplants were not included.

The total of 73 patients in this single-center analysis (see RESULTS) allows for the detection of a hazard ratio (HR) ≥ 2.6 with a confidence of 95%, and a statistical power of 80%, assuming 56% of censored measurements (percentage of patients alive after 5 years) and 54.7% of exposed patients (percentage of patients receiving DL transplantation). A retrospective analysis of the single-center data was performed comparing the demographic characteristics of the SL and DL groups, followed by univariate analysis to compare the qualitative variables, using the χ^2 test and student *t*-test for quantitative ones. Kaplan-Meier survival curves were compared using the log-rank test. The impact of the type of transplant, infections (cytomegalovirus or bacterial), and acute and chronic rejection on patient survival was determined in the local setting using a Cox multivariate analysis. Statistical analyses were performed with SPSS 16.0. The statistical analyses were review by Professor Salvador Pita, head of biostatistics department and paper coauthor.

RESULTS

A total of 280 patients were transplanted in CHUAC between 2000 and 2012, of whom 73 had a previous diagnosis of advanced-stage pulmonary emphysema: 40 underwent SL and 33 received DL. Both groups were comparable in terms of gender and clinical characteristics (Table 1). We found significant differences in the mean age between the groups, the DL patients being younger (Table 1), as expected from the inclusion criteria.

The average preoperative forced expiratory volume in 1 s (FEV₁) was 22.89% ± 6.95% (range 12%-49%). The median follow-up of the series was 67.4 mo (range: 0-156.5 mo; interquartile range: 22.4-96.4). Perioperative medical and surgical complications (hemothorax, lung edema, broncho-vascular sutures problems) were reported in 11 patients (27.6%) in the SL group,

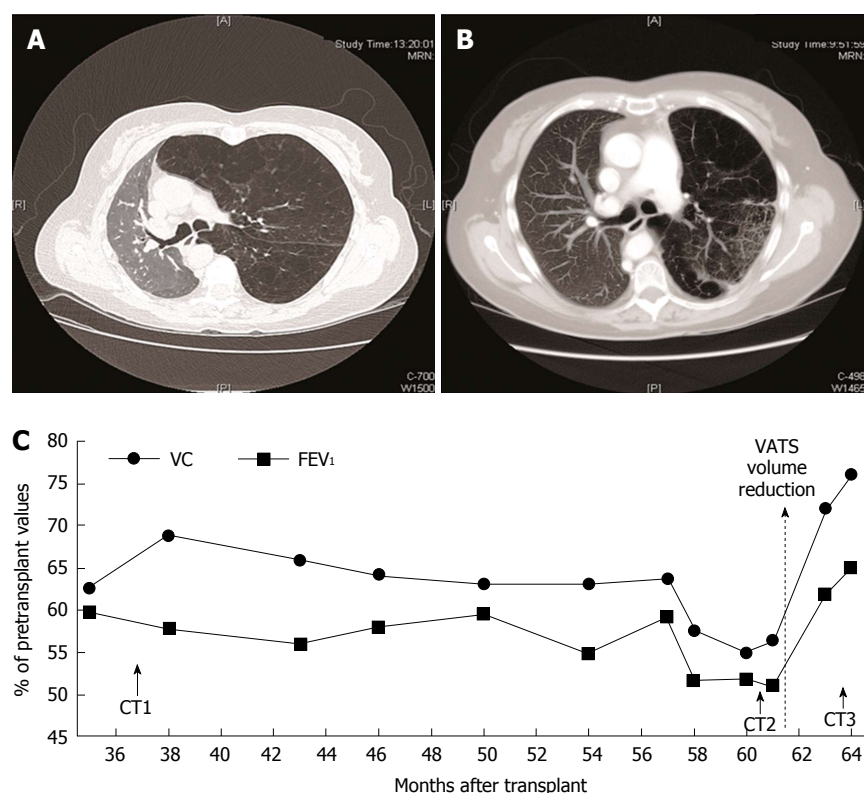


Figure 1 Results of patient undergoing volume reduction surgery by video-assisted thoracoscopic surgery. A: CT before surgery (CT2); B: CT after surgery (CT3); C: Spirometric values recovered after surgery. CT: Computed tomography; VATS: Video-assisted thoracoscopic surgery.

Table 2 Survival probability according to type of transplant

Time until transplantation	Single-lung		Double-lung	
	No. at risk	Survival probability	No. at risk	Survival probability
1 yr	34	85.0%	26	78.8%
2 yr	31	77.5%	24	72.7%
3 yr	29	72.5%	22	66.7%
4 yr	23	57.5%	20	60.6%
5 yr	20	52.4%	17	51.5%

Log-rank test = 0.001; $P = 0.976$.

compared to 18 subjects (54%) in the DL group, which was significantly higher ($P = 0.032$).

Complications reported during follow-up were similar in both groups (Table 1). Rejection episodes were treated with steroid boluses, combined with a modification of the patient's immunosuppressive treatment when deemed necessary.

Clinically significant hyperinflation of the native lung was detected in 3 SL patients during the follow-up period. This was diagnosed by worsening respiratory function, decreased %FEV₁ compared to initial post-transplant values, and confirmed by high-resolution computed tomography (CT). Transbronchial biopsy was performed to rule out other possible causes of functional deterioration, including bronchiolitis obliterative syndrome (BOS).

Volume reduction surgery by VATS was performed in 2 patients. The first case experienced a significant

improvement in functional capacity (Figure 1), recovering pretransplant spirometric values, leading to a clear improvement in the patient's quality of life. The second patient died 3 wk after the intervention due to sepsis caused by lung infection. The third patient underwent video-assisted bronchoscopic volume reduction. Endobronchial valves were placed in 3 segmental bronchi of the right upper lobe without incidents. Postoperative CT imaging showed atelectasis at this site^[22]; the patient experienced clinical improvement from stage 3 to 2 in the Medical Research Council dyspnea functional scale^[23], improved exercise tolerance and better quality of life, with no significant changes in spirometry. Lung volume reduction by bullae resection was performed in a fourth patient during the transplantation procedure without incidents.

Postoperative mortality (within 30 d after surgery), was significantly higher in DL: 4 patients (5.57%) died in the immediate postoperative period, compared to 2 (2.73%) in the SL group. Regarding survival, 85% of SL patients were alive one year after the intervention, 72.5% 3 years later, and 52.4% after 5 years, and in DL, 78.8%, 66.7% and 51.5% respectively (Figure 2). Survival probabilities are shown in Table 2. There were no significant differences between the 2 survival curves ($P = 0.976$). Multivariate regression analysis revealed that the type of transplant performed, single or double-lung, was not related to survival ($P = 0.802$), while age and having COPD as the underlying disease did not reach statistical significance (Table 3). Univariate and

Table 3 Cox regression analysis to predict mortality adjusting for different variables

	B	SE	Wald	P-value	HR	95%CI HR
Age in years	0.040	0.024	2.780	0.095	1.041	0.993-1.091
Gender (female)	0.475	0.391	1.481	0.224	1.609	0.748-3.459
Underlying disease (COPD)	0.737	0.407	3.278	0.070	2.088	0.941-4.651
Type of transplant (double-lung)	-0.086	0.345	0.063	0.802	0.917	0.466-1.804

COPD: Chronic obstructive pulmonary disease; B: Regression coefficient; SE: Standard error; HR: Hazard ratio.

Table 4 Univariate and multivariate analysis of postoperative complications in relation to type of transplant *n* (%)

	Single-lung <i>n</i> = 40	Double-lung <i>n</i> = 33	Univariate analysis	Multivariate analysis ¹	
			P-value	P-value	OR (95%CI)
Complications during follow-up					
Bronchiolitis obliterans syndrome	22 (55.0)	16 (48.5)	0.579	0.475	0.7 (0.2-1.9)
Acute rejection episodes	18 (45.0)	18 (54.5)	0.417	0.397	1.6 (0.6-4.4)
Bacterial respiratory infections	27 (67.5)	22 (68.8)	0.910	0.597	0.7 (0.2-2.3)
CMV infection/disease	19 (47.5)	9 (27.3)	0.077	0.104	0.4 (0.1-1.2)
Fungal infections	12 (30.0)	8 (24.2)	0.583	0.807	0.9 (0.3-2.7)

¹Multivariate logistic regression analysis adjusted for age, gender and underlying disease. CMV: Cytomegalovirus.

Table 5 Waiting list status of patients diagnosed with chronic obstructive pulmonary disease, emphysema or α 1-antitrypsin deficiency

		Final waiting list status				Total
		Active	Excluded	Deceased	Transplanted	
SL	No.	25	25	9	284	343
	Total SL %	7.3%	7.3%	2.6%	82.8%	100.0%
DL	No.	35	51	40	532	658
	Total DL %	5.3%	7.8%	6.1%	80.9%	100.0%
Total	No.	60	76	49	816	1001
	Total %	6.0%	7.6%	4.9%	81.5%	100.0%

Lung transplantation waiting list, Spain 1999-2012. SL: Single-lung; DL: Double-lung.

multivariate analyses of postoperative complications, presented in Table 4, show that the occurrence of complications was not related to the type of transplant, even after adjustment for age, gender and underlying disease.

The analyses of waiting-list national data included a total of 1001 patients with emphysema, COPD or α 1-antitrypsin deficiency: 343 were SL patients and 658 DL (Table 5).

Patient progress in the waiting list differed ($P = 0.068$) depending on the type of transplant awaited: 83% of those waiting for SL were transplanted, compared to 81% of DL waitlisted. In contrast, waiting list mortality was higher in DL group (6.1% vs 2.6%) (Table 5). In addition, time on the waiting list at national level was longer in the DL than in the SL group ($P < 0.001$), explained by the fact that patients in the SL group were transplanted earlier (Figure 3). Notably, no significant difference was found in survival-time curves between DL and SL in patients with COPD, emphysema or α 1-antitrypsin deficiency in the RETP ($P = 0.875$), shown in Figure 4.

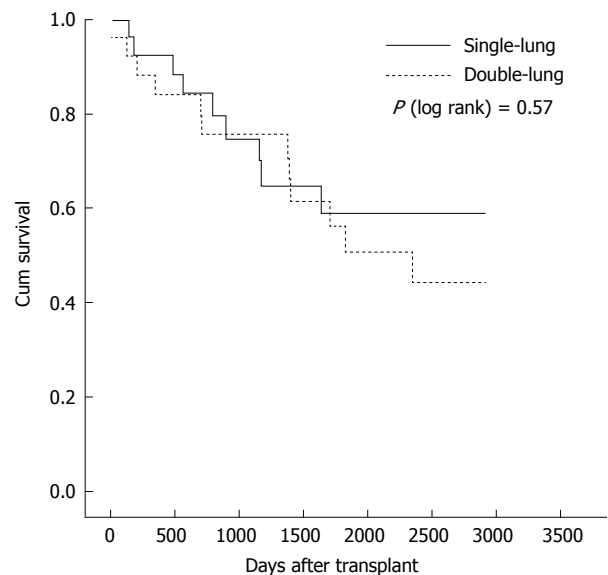


Figure 2 Survival curves of emphysema patients transplanted with 1 or 2 lungs (single-center series), transplants performed between 2000 and 2012.

DISCUSSION

Several previous studies have compared follow-up results of DL vs SL transplantation for emphysema, and the general conclusion has been that survival rates were better in the former case, at least in younger patients^[8,10,11]. However, comparison groups were not homogeneous and confounding factors were often present. Cassivi *et al*^[10] reported 5-year survival rates of 66.7% in DL recipients, vs 44.9% in SL transplanted patients, but most of the younger patients with α 1-antitrypsin deficiency had received 2 lungs. We considered the question of age, as Thabut *et al*^[8] concluded in their study that patients aged over 60 years may not have a

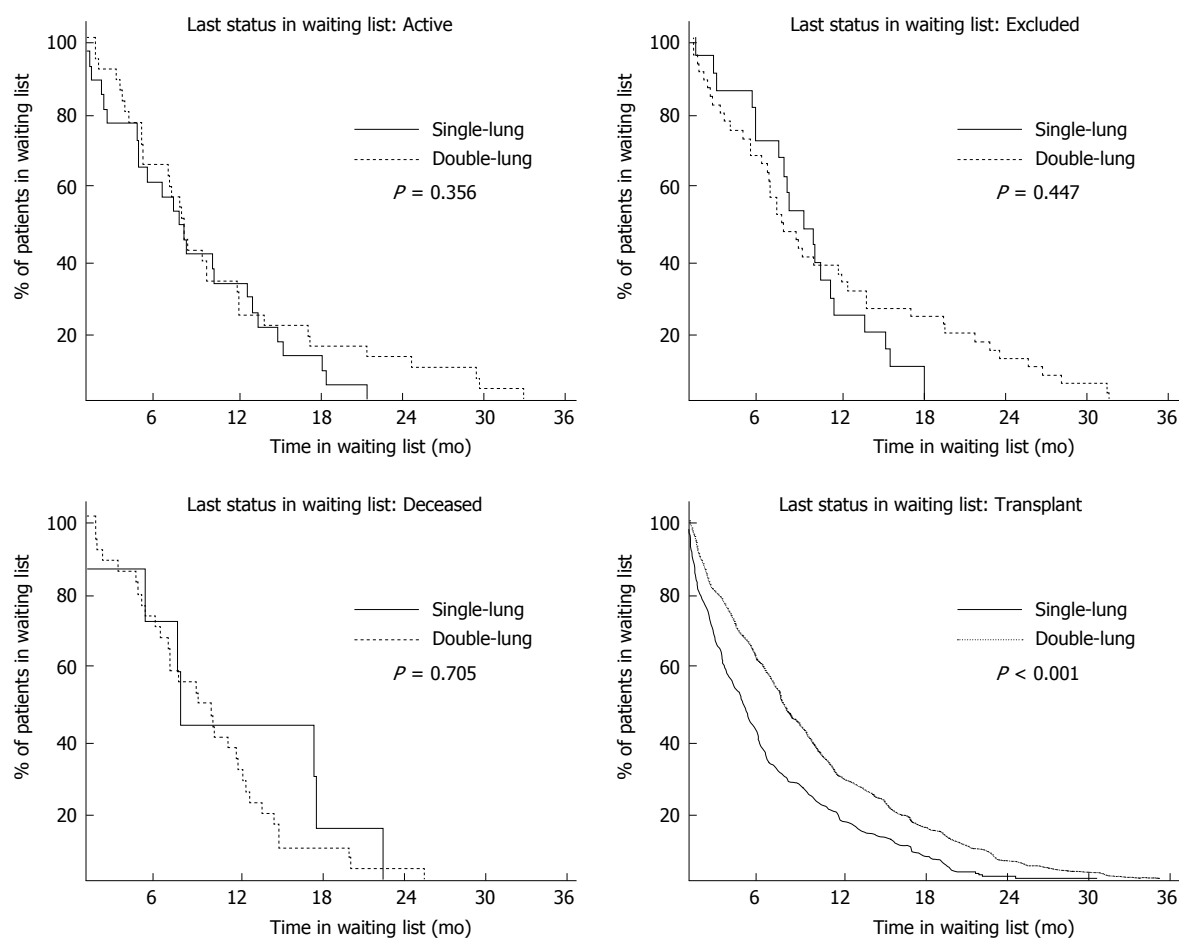


Figure 3 Time on lung transplantation waiting list according to final status. Patients diagnosed with COPD, emphysema or α 1-antitrypsin deficiency. National Registry of Donation and Transplantation, Spain 1999-2012. Log rank test used for survival curves comparison. COPD: Chronic obstructive pulmonary disease.

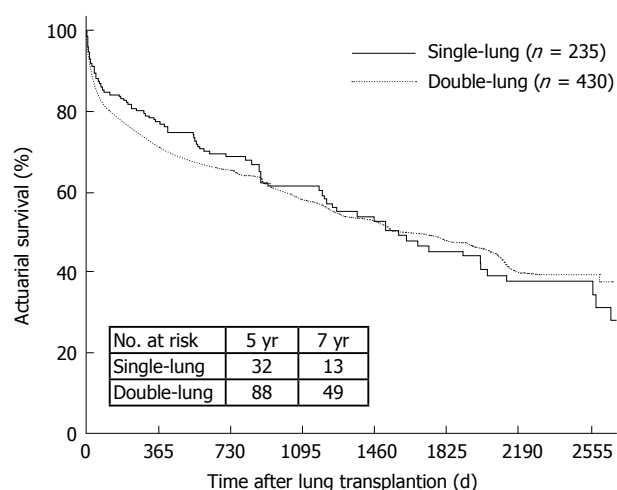


Figure 4 Post-lung transplantation survival of patients with chronic obstructive pulmonary disease, emphysema or α 1-antitrypsin deficiency. Data from the Spanish Lung Transplant Registry, transplants performed between 2001 and 2012.

survival benefit after receiving both lungs, but younger patients presented better survival rates after bilateral lung transplantation^[8]. Our single-center series is small compared to those of other reports exploring this topic, with the obvious limitations this brings, but on the other

hand, this means that our protocols and surgical techniques were totally homogenous. Multicenter studies are based on large databases that can be inaccurate or incomplete, and details that would have allowed to investigate the mechanisms responsible for greater survival after DL are often missing. For this reason, Thabut *et al.*^[8] were unable to differentiate causes of death. Our study shows no differences in terms of complications and survival in DL and SL patients. The benefits of a slightly better long-term survival reported in previous studies for DL recipients could be cancelled out by higher waiting list mortality, if DL is the preferred approach. Our results might be influenced by our preoperative prophylaxis protocol aimed at prevention of native lung colonization, early extubation (frequently extubated in the surgical room in SL), appropriate management of ventilation complications during the early postoperative period, and long-term management of hyperinflation. Our aim was to analyse fully comparable groups, and this was achieved in general terms, as can be observed in the demographic and clinical characterization of our series. Although the SL group was older than the DL group, our results showed no difference between the study groups in terms of long-term mortality, nor was morbidity higher in the SL group, as suggested in previous studies. Many authors

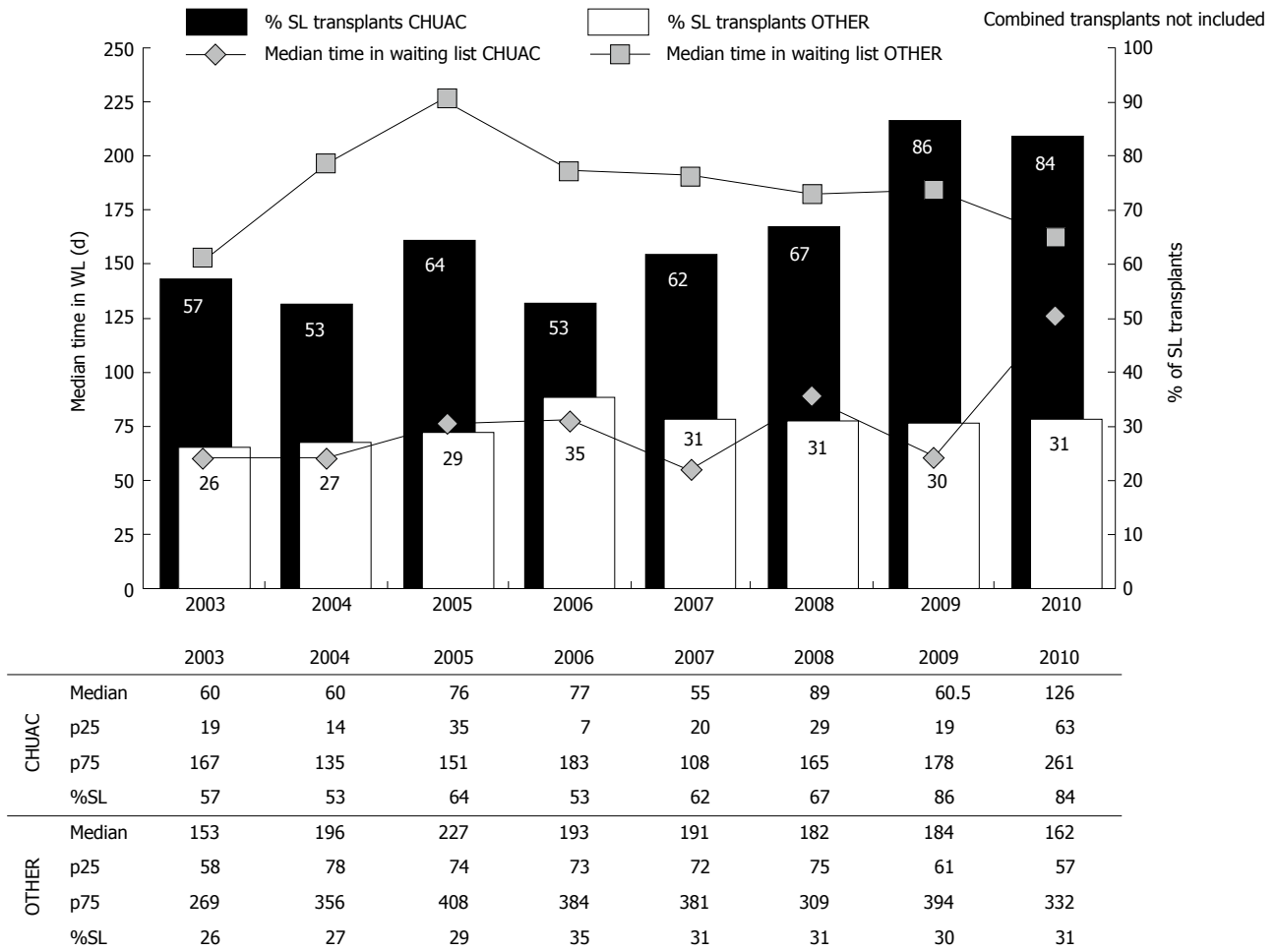


Figure 5 Median time on waiting list in our hospital (Complejo Hospitalario Universitario A Coruña) and other Spanish hospitals performing lung transplantation. (Spanish national data; OTHER) in the context of the percentage of single-lung transplants (SL) performed from 2003 to 2010. Preference for SL reduces the median number of days on the waiting list. CHUAC: Complejo Hospitalario Universitario A Coruña.

have advocated bilateral transplantation^[7,10-12,24], arguing that native lung hyperinflation may be responsible for poorer results after SL transplantation. In our series, lung volume reduction surgery was performed in 4 patients with generally good results, and increasing numbers of centers are implementing techniques such as VATS and endobronchial valves^[25-28]. In the present clinical setting, the possibility of appropriately controlling this complication is high, and, moreover, SL is technically simpler, anatomically less aggressive, and involves shorter total ischemia time^[5], which would explain the lower perioperative morbidity and mortality rates in our series. Furthermore, recent techniques such as normothermic *ex vivo* lung perfusion systems may allow the conservation and transplant of lungs in optimal conditions, likely improving the present results^[29,30]. Another possible benefit of SL is the treatment of BOS^[31], currently the main limiting factor for survival during follow-up. The therapeutic strategy in SL-transplanted patients is retransplantation in the contralateral side, which has shown lower morbidity and mortality in various series, compared to retransplantation in patients with a previous bilateral transplant^[32].

The results of our single-center series suggest that SL and DL transplantation have similar outcomes in terms of survival, but SL recipients presented fewer complications. Although this is a small series, our findings correlate with the data from the national registry, further showing lower mortality on the waiting list, we believe SL transplantation would be the preferable option in the context of organ shortages and waiting lists, as suggested elsewhere^[6,9,33,34]. Figure 5 shows how our preference for SL has clearly reduced the median number of waiting list days, compared to the rest of Spanish hospitals performing this procedure. In addition, our policy of performing preferably SL has led to the implementation of the "twinning procedure", 2 SL performed simultaneously from 1 donor^[35]. Twinning in the same hospital has been shown to be feasible with adequate planning, permitting better use of donors and reducing waiting list time and mortality. Our analysis of the data from the Spanish Registry indicates that patient progress on the waiting list is influenced by the type of transplant awaited. Patient survival is associated with the extra waiting time for DL, and advanced patients presenting would clearly benefit from shorter waiting

times. Munson *et al.*^[34] advocate shortening waiting list times with an optimized allocation program and, specifically, the performance of SL, arguing that bilateral transplantation maximizes the total number of life-years gained post-transplant only when waiting lists are short or if the local survival benefits of DL compared with SL are large^[34].

Our study, being small, retrospective and single-center, has several limitations and therefore conclusions drawn must be equally limited. However, it offers a comparison of outcomes of both transplantation possibilities in emphysema, while excluding the possible confounders occurring in multicenter studies, such as different care protocols used in the various hospitals, different surgical techniques, surgical teams or the lack of complete, validated data, among others. In these conditions, we have observed that the survival of both groups is similar. The postoperative complications in our group, with mortality rates twice those of DL transplant recipients, are consistent with longer and more complex interventions requiring double the number of sutures that increase the risk of complications.

In conclusion, our results suggest that SL could be the best option in the present clinical scenario, and we believe that this should be the treatment of choice in most of emphysema patients. This approach, in our experience, has no impact on patient survival or complications, may alleviate donor organ shortage, and contributes to decreasing morbidity and mortality on the national waiting list. In addition, this approach also allows contralateral transplantation, if needed to solve future complications^[36].

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COMMENTS

Background

Advanced-stage pulmonary emphysema is the most frequent indication for lung transplantation. However, whether single-lung transplantation (SL) or double-lung transplant (DL) transplantation should be performed remains a subject of debate.

Research frontiers

It is known that the quality of life in single lung transplantation is usually similar to double lung. Therefore, the shortage of organs and the consequent waiting list mortality encouraged to make unilateral transplantation is emphysema patients since it would reduce waiting list times, if they can provided that long-term survival were similar for both.

Innovations and breakthroughs

These study results show similar survival for uni and bilateral lung transplantation in patients with emphysema, with less immediate postoperative morbidity and mortality. Survival Spanish national register does not show difference between the two techniques too. Likewise, mortality in national waiting list is significantly higher in the group of both lungs. Only infections are more prevalent in the group-lung without statistical significance.

Applications

The results obtained reinforce their decision that single lung transplant is the transplantation of choice in most cases of emphysema without bacterial or fungal colonization.

Peer-review

This article is interesting and has a good potential.

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Retrospective Cohort Study

Impact of body mass index on outcomes of 48281 patients undergoing first time cadaveric liver transplantation

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Informed consent statement: Since this study was performed using data extracted from UNOS Standard Transplant Analysis and Research (STAR) Files, acquisition of patients consent statements was neither feasible nor required.

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Abstract

AIM: To investigate possible disparities in perioperative morbidity and mortality among different body mass index (BMI) groups and to simulate the impact that these differences might have had on the cohort of patients undergoing cadaveric liver transplantation (LT).

METHODS: All adult recipients undergoing first time LT for benign conditions and receiving a whole graft from brain-dead donors were selected from the united network of organ sharing registry. From January 1994 to June 2013, 48281 patients satisfied the inclusion criteria and were stratified by their BMI. The hypothesis that abnormal BMIs were independent predictors of inferior outcomes was tested with univariate and multivariate regression analyses.

RESULTS: In comparison to normal weight recipients, underweight and morbidly obese recipients had increased 90-d mortality (adjusted OR = 1.737; 95%CI: 1.185-2.548, $P = 0.005$) (adjusted OR = 1.956; 95%CI: 1.473-2.597, $P = 0.000$) respectively and inferior patients' survivals (adjusted HR = 1.265; 95%CI: 1.096-1.461, $P = 0.000$) (adjusted HR = 1.157; 95%CI: 1.031-1.299, $P = 0.013$) respectively. Overall, patients' 5-year survival were 73.9% for normal-weight, 71.1% for underweight, 74.0% for overweight, 74.4% for class I obese, 75.0% for class II obese and 71.5% for class III obese recipients. Analysis of hypothetical exclusion of underweight and morbidly obese patients from the pool of potential LT candidates would have improved the overall survival of the entire cohort by

2.7% (95%CI: 2.5%-3.6%).

CONCLUSION: Selected morbidly obese patients undergoing LT for benign conditions had 5-year survival rates clinically comparable to normal weight recipients. Impact analysis showed that exclusion of high-risk recipients (underweight and morbid obese patients) would not significantly improve the overall survival of the entire cohort of patients requiring LT.

Key words: Obesity; Impact analysis; Survival; Liver transplantation; Body mass index

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Core tip: Obesity has become a prevalent condition in many part of the world. Yet, evaluation of its impact on patients requiring liver transplantation is limited. Analysis of united network of organ sharing data of 48281 patients undergoing first time cadaveric liver transplantation has shown that, 5-year survival rates for selected underweight and morbidly obese patients were clinically comparable to normal weight recipients as 5-year survival for class III obese recipients was 71.5% vs 73.9% for normal weight patients. Impact analysis showed that exclusion of morbidly obese and underweight recipients would not significantly improve the overall survival of the entire cohort of patients undergoing liver transplant.

Ayloo S, Hurton S, Cwinn M, Molinari M. Impact of body mass index on outcomes of 48281 patients undergoing first time cadaveric liver transplantation. *World J Transplant* 2016; 6(2): 356-369 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/356.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.356>

INTRODUCTION

Since 1980, the incidence of obesity in the adult population has more than doubled in many countries^[1,2]. Obesity might cause a spectrum of disorders such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH) that can lead to cirrhosis and hepatocellular carcinoma^[3]. Data from the United States have shown that during the last decade, the indication for liver transplantation (LT) for NASH has risen from 1.2% to 9.7%. Currently it represents the third most common cause of liver failure but it is expected to be the leading indication by year 2025 if the current trends of obesity remain unchanged^[4].

Some studies have reported that obese recipients have worse outcomes than normal weight counterparts^[4-7]. However, some other investigators did not find any significant differences^[8,9]. One of the shortcomings of these studies is the lack of adjustment for known effect modifiers such as coexisting comorbidities. Therefore,

the controversy around the issue whether obesity itself is an independent predictor of poorer outcomes after LT still remains. In vision of these conflicting results, we reviewed the outcomes of a large cohort of adult patients who underwent LT in the United States with the intent of assessing if abnormal body mass index (BMI) was an independent predictor for patients' and grafts' survival after adjusting for clinical and demographic characteristics selected a priori. Secondary outcomes of this study were to investigate possible disparities in perioperative morbidity and mortality among different BMI groups and to simulate the impact that these differences might have had on the cohort of patients undergoing LT.

MATERIALS AND METHODS

Data of this study were extracted from United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research (STAR) Files that included socio-demographic and clinical variables of every donor and recipient of solid organ transplants performed in the United States during the period between January 1, 1994 and June 30, 2013. For each recipient, BMI was calculated using the formula: Weight (kg)/height (m)². The World Health Organization definitions were used to classify recipients in six categories: Underweight (BMI < 18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9), class I obese (BMI 30-34.9), class II obese (BMI 35-39.9) and class III obese patients (BMI ≥ 40)^[10]. Data for different BMI classes were not adjusted for ascites because the volume of peritoneal fluid drained during LT was not recorded in the STAR Files.

Every adult (age ≥ 18 years) undergoing a LT was considered a potential candidate without restriction of race, citizenship or UNOS region where surgery was performed. Recipients who underwent LT for known primary or secondary liver malignancies (*e.g.*, hepatocellular carcinoma, cholangiocarcinoma, neuroendocrine metastases, *etc.*) and recipients who had a malignancy found in their explanted livers were excluded to avoid confounders related to the neoplastic nature of their disease. Other exclusion criteria were transplants using grafts harvested from living or non-heart beating donors, split grafts, multivisceral or redo transplants and transplant performed across ABO incompatible blood groups as those recipients had increased risk of non-functioning grafts, perioperative morbidity and mortality. Additional exclusion criteria were missing data on recipients' weight or height, lack of records on short and long-term outcomes, or the presence of variables that were deemed implausible for an adult recipient^[11]. Cutoffs for these variables were: Recipient height ≤ 120 cm or ≥ 240 cm, recipient weight ≤ 30 kg or ≥ 250 kg, BMI ≤ 13 or ≥ 80, cold ischemia time ≤ 1 h or ≥ 24 h and warm ischemia time ≤ 10 min or ≥ 120 min. No imputations of missing data were performed and recipients who had more than 10% of omitted information were excluded.

For the purpose of this study, variables included in the final analysis were recipients' age at the time of transplant, sex, ethnicity, primary cause of liver disease, height and weight or BMI when available, presence of renal failure requiring hemodialysis before surgery, history of diabetes (type I or II), presence of chronic obstructive pulmonary disease (COPD), hypertension, model for end-stage liver disease score after its implementation in 2002 and beforehand when serum creatinine, bilirubin and INR were available for calculation, perioperative complications, perioperative mortality and overall patients' and grafts' survival. Donors' variables included age, gender, height and weight or BMI if available, primary cause of death and ethnicity. Intraoperative variables included warm ischemia time measured in minutes and cold ischemia times measured in hours.

Recipient overall survival was estimated by calculating the difference between the date of transplantation and the date of death from any cause. Censoring was used for recipients who were still alive at the end of the time interval of this study or who were alive at the time of their last available follow-up or at the time of retransplantation.

Graft survival was calculated by the difference between the date of transplantation and the date of recipient death or the first date that recorded graft failure or the date when the recipient underwent a redo LT. Perioperative adverse events leading to death were grouped in the following categories: Hemorrhagic (*e.g.*, intraoperative or postoperative bleeding), vascular (either arterial or venous thrombosis), biliary (anastomotic strictures or leaks), infections, acute cellular rejection, cerebrovascular complications (ischemic or hemorrhagic strokes) and primary graft non function defined as irreversible graft function requiring emergency liver replacement within the first 2 wk after LT. The remaining less common complications were categorized as others or unknown if the cause of death was not reported in the UNOS files.

Primary outcomes of this study included patient and graft survival, and secondary outcomes were perioperative causes of morbidity and mortality stratified by recipients' BMI groups.

Late causes of death (≥ 12 mo after LT) were grouped in the following categories: Infections, cardiopulmonary (*e.g.*, ischemic cardiomyopathy, embolism, insufficiency), renal failure, cerebrovascular events (ischemic or hemorrhagic strokes), malignancies (any type of cancer), graft failure (*e.g.*, recurrent disease or chronic rejection), and hemorrhagic (any cause). The remaining infrequent causes of death were grouped together under the category named "other", and if there was no recorded cause of death, patients were entered in the group named "unknown". This study was conducted and reported according to recommendations from the STROBE statement^[12] and did not require approval by the local ethic review board.

Statistical analysis

Sample size of this retrospective analysis was fixed. All variables of first time cadaveric LTs performed over a 19-year period in the United States had been captured in an electronic healthcare database prospectively maintained by UNOS and provided to the authors upon their request.

The cohort was described using estimates of central tendency (means, medians) and spread (standard deviation, interquartile range) for continuous data and frequency and percentages for categorical data.

Etiologies of end stage liver disease (ESLD) were grouped as follows: Hepatitis C virus (HCV), alcohol, alcohol and HCV, other viral hepatitis in combination with HCV, primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), congenital or metabolic diseases (*e.g.*, alpha-1-antitrypsin deficiency, Budd-Chiari syndrome, hemochromatosis, polycystic liver disease, *etc.*), NASH, hepatitis B virus (HBV), autoimmune, acute liver failure and other rare conditions.

The primary end points were overall patient and graft survivals stratified by recipients' BMI at time of transplant. Kaplan-Meier method was used to calculate survival estimates and analyzed with two-sided log-rank test, with the hazard ratio and two-sided 95%CIs. All hazard ratios (HR) and adjusted HR (AHR) involving patients' and grafts' survivals are reported with normal weight recipients as the reference group. The median follow-up time for both patients' and grafts' survival were estimated by means and medians of the reverse Kaplan-Meier method. Multiple clinically relevant two-way interactions were evaluated in the multivariable Cox model and included in the final model if significant at a *P*-value < 0.05. The proportional hazard assumption of the final adjusted model was tested visually by plotting the scaled Schoenfeld residuals of time and BMI, the main predictor of interest. Departure from linearity was assessed by plotting scatterplot smooth curves through residuals^[13,14]. Time-dependent covariates such as recipients' age, which allowed for a change in the hazard ratio over time, were considered and used in the model when appropriate.

To account for the cohort effects, all analyses were adjusted for year of transplantation. Univariate and multivariate Cox regression analyses were performed to test the null hypothesis that recipients' BMI was a predictor of patients' and graft survival. Only pre-transplant characteristics were used in the models and all confounders entered in the regression models were selected *a priori* as they had been shown to be correlated with patients' and grafts' survival in earlier studies: Year of transplantation, patients' and recipients' characteristics (age, gender, BMI), recipients' comorbidities (renal insufficiency, diabetes, COPD, hypertension), primary indication for LT, warm and cold ischemia times.

Secondary outcomes were perioperative morbidity and mortality. For these analyses, proportions were compared using the χ^2 test and continuous variables

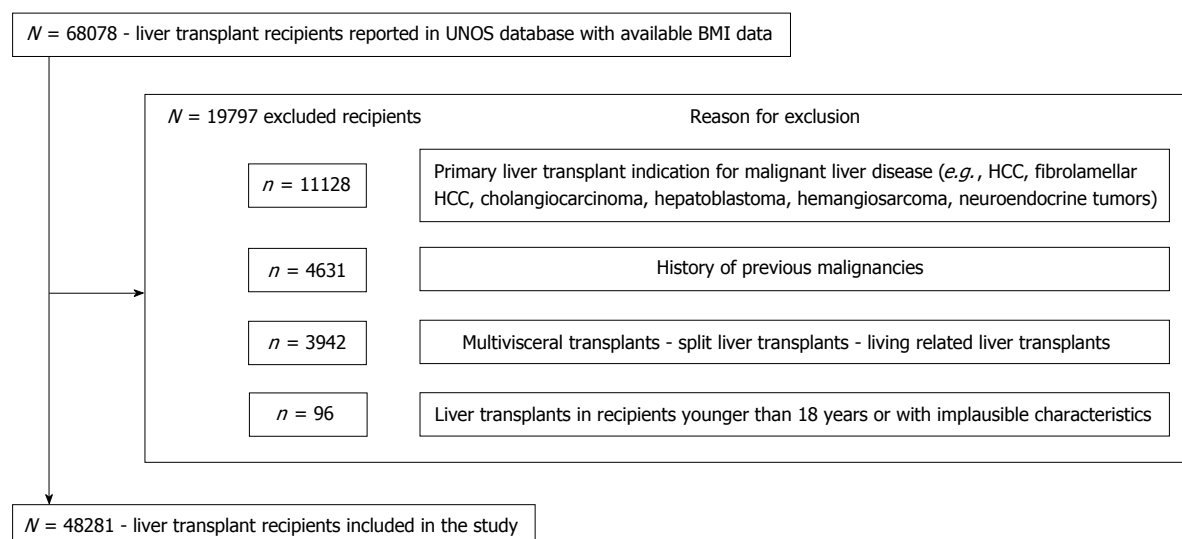


Figure 1 Flowchart of all the include and exclude patients in this study. BMI: Body mass index; UNOS: United Network for Organ Sharing; HCC: Hepatocellular carcinoma.

were compared using ANOVA test across multiple BMI groups. Perioperative mortality was calculated during the index admission, at 30, 60 and 90 d and at 1-year post LT. Unadjusted and adjusted risk estimates of perioperative mortality were calculated as odds ratios (OR) and adjusted OR (AOR) with 95%CI using logistic regression analysis. Risk estimates were adjusted for patients' and donors' BMI (six categories: Underweight, normal weight, overweight, class I obese, class II obese and class III obese), recipients' and donors' age (six categories: 18-45, 46-55, 56-65, 66-75, ≥ 76), recipients' and donors' sex, year of transplantation, recipients' comorbidities (four categories: Renal insufficiency requiring dialysis, diabetes type I and II, COPD, hypertension), warm and cold ischemia time, and primary indication for LT (twelve categories: HCV, Alcohol and HCV, HCV and other viral hepatitis, Alcohol, HBV, PSC, PBC, NASH, autoimmune, acute liver failure, congenital or metabolic disease, other).

All statistical analyses were performed using IBM SPSS Statistics version 20 (SPSS Inc. Chicago, IL, United States). Statistical significance was identified by two-tailed *P*-values of less than 0.05 and 95%CI.

Impact analysis of the potential benefit of allocating grafts to patients with BMIs that had the longest survival and lowest perioperative mortality risk was performed using estimates of central tendency and 95%CI. Microsoft® excel 2008 was used to calculate the overall number and 95%CI of preventable perioperative deaths and the number and 95%CI of life-years that could have been saved by allocation grafts to low-risk recipients.

RESULTS

Donors and recipients characteristics

Among 68078 LT recipients recorded in the UNOS registry, a total of 48281 (70.9%) met eligibility criteria

(Figure 1). Of these, 914 (1.89%) were underweight, 14529 (30%) had normal BMI, 16724 (34.6%) were overweight and 16114 were obese (33.3%). Within the group of obese recipients, 9944 (61.7%) were class I obese, 4438 (27.5%) class II and 1732 (10.3%) satisfied class III criteria (Table 1). Demographic and clinical characteristics of the donors are summarized in Table 2.

Primary outcomes

Overall survival: During the study period, 16689 patients (34.6%) died while 31539 were alive or censored. Median overall survival for the entire cohort was 12.7 years (95%CI: 12.5-12.9). Normal weight patients had the longest median survival (13.1 years; 95%CI: 12.6-13.6 years) while the shortest survival was observed in class III obese recipients (11.3 years; 95%CI: 10.3-12.3) and underweight patients (11.5 years; 95%CI: 10.4-12.7) (Table 3).

Kaplan-Meier functions, stratified by recipients' BMI, are reported in Figure 2. Logrank test showed a significant survival difference across BMI groups ($P = 0.004$) and pairwise comparisons showed that underweight ($P = 0.034$) and class III obese patients ($P = 0.001$) experienced significant lower survivals compared to normal weight counterparts.

At multivariate cox regression analysis, after adjusting for recipients' and donors' characteristics (age, gender, BMI, primary cause of end-stage liver disease, comorbidities), cold and warm ischemia times and year of transplantation, underweight status (AHR = 1.265; 95%CI: 1.096-1.461; $P = 0.001$) and class III obesity (AHR = 1.157; 95%CI: 1.031-1.299; $P = 0.013$) remained significant predictors for shorter survival in comparison to normal weight recipients (Table 4). On the other hand, being overweight appeared to have modest protective effect (AHR = 0.908; 95%CI: 0.864-0.954; $P = 0.000$).

Table 1 Demographic and clinical characteristics of the study population

Variable	Total number of patients (<i>n</i> = 48281) (100%)	Underweight (<i>n</i> = 914) (1.9%)	Normal weight (<i>n</i> = 14529) (30.0%)	WHO BMI classification			<i>P</i> value
				Overweight (<i>n</i> = 16724) (34.6%)	Class I (<i>n</i> = 9944) (20.5%)	Obese recipients Class II (<i>n</i> = 4438) (9.2%) Class III (<i>n</i> = 1732) (3.5%)	
Age in years, median (25 th , 75 th)	53 (46, 59)	50 (40, 57.2)	52 (44, 58)	53 (46, 58)	53 (46, 58)	52 (47, 58)	0.000
Gender, <i>n</i> (%)							
Male	30250 (62.7)	407 (44.5)	8573 (59.0)	11336 (67.8)	6489 (65.3)	2609 (58.8)	0.000
Female	18,031 (37.3)	507 (55.5)	5956 (41.0)	5388 (32.2)	3455 (34.7)	1829 (41.2)	
Recipient living status, <i>n</i> (%)							
Alive	27552 (57.1)	496 (54.3)	8040 (55.4)	9534 (57.1)	5825 (58.6)	2642 (59.6)	0.000
Dead	16689 (34.6)	346 (37.9)	5123 (35.3)	5804 (34.7)	3344 (33.7)	1471 (33.2)	
Lost at follow-up	3987 (8.3)	72 (7.9)	1348 (9.3)	1370 (8.2)	765 (7.7)	317 (7.2)	
Race, <i>n</i> (%)							
Non-hispanic white	36809 (76.2)	664 (72.6)	10850 (74.7)	12820 (76.7)	7687 (77.3)	3475 (78.3)	0.000
Non-hispanic black	3962 (8.2)	92 (10.1)	1237 (8.5)	1277 (7.6)	815 (8.2)	371 (8.4)	
Hispanic	5535 (11.5)	73 (8.0)	1534 (10.6)	2020 (12.1)	1191 (12.0)	500 (11.3)	
Asian	1446 (3.0)	78 (8.5)	782 (5.4)	415 (2.5)	131 (1.3)	30 (0.7)	
Other	529 (1.1)	7 (0.7)	126 (0.8)	192 (1.1)	120 (1.2)	62 (1.3)	
Recipient BMI, median (25 th , 75 th)	27.05 (23.8, 31.1)	17.63 (17.0, 18.1)	22.73 (21.2, 23.9)	27.26 (26.0, 28.5)	32.01 (30.3, 33.3)	36.85 (35.8, 38.1)	0.000
Primary indication for liver transplantation, <i>n</i> (%)							
HCV	13838 (28.7)	176 (19.3)	3538 (24.4)	5248 (31.4)	3101 (31.2)	1302 (29.3)	0.000
Alcohol	8111 (16.8)	163 (17.8)	2543 (17.5)	2909 (17.4)	1686 (17.0)	622 (14.0)	
Idiopathic	5073 (10.5)	77 (8.4)	1179 (8.1)	1656 (9.9)	1270 (12.8)	628 (14.2)	
Alcohol + HCV	3601 (7.5)	44 (4.8)	1033 (7.1)	1370 (8.2)	762 (7.7)	310 (7.0)	
PSC	2799 (5.8)	101 (11.1)	1396 (9.6)	903 (5.4)	282 (2.8)	92 (2.1)	
Congenital/metabolic disease	2567 (5.3)	80 (8.8)	870 (6.0)	826 (4.9)	455 (4.6)	215 (4.8)	
PBC	2485 (5.1)	92 (10.1)	1122 (7.7)	765 (4.6)	342 (3.4)	116 (2.6)	
NASH	2247 (4.7)	13 (1.4)	226 (1.6)	585 (3.5)	660 (6.6)	522 (11.8)	
HBV	1896 (3.9)	33 (3.6)	798 (5.5)	646 (3.9)	291 (2.9)	92 (2.1)	
Other	5664 (11.7)	135 (14.7)	1824 (12.5)	1816 (10.8)	1095 (11.0)	539 (12.1)	
MELD score, median (25 th , 75 th)	21 (16, 28)	22 (16, 28)	21 (16, 29)	20 (15, 28)	21 (16, 28)	22 (16, 31)	0.000
Cold ischemia time, hours, median (25 th , 75 th)	7.0 (5.4, 9.2)	7.1 (5.3, 9.0)	7.0 (5.3, 9.1)	7.0 (5.3, 9.1)	7.1 (5.4, 9.2)	7.2 (5.5, 9.3)	0.288
Warm ischemia time, minutes, median (25 th , 75 th)	44 (34, 55)	44 (35, 55)	43 (34, 55)	44 (35, 55)	44 (35, 56)	45 (35, 57)	0.000
Waiting time, days (including days on hold), median (25 th , 75 th)	120 (27, 335)	91 (21, 286)	106 (24, 308)	124 (28, 336)	131 (30, 358)	134 (31, 355.5)	0.000
Hospital stay after liver transplant, days, median (25 th , 75 th)	11 (8, 19)	13 (7, 23)	11 (8, 19)	11 (8, 18)	11 (8, 18)	12 (8, 19)	0.000
Preoperative comorbidities, <i>n</i> (%)							
Diabetes type I	536 (1.1)	9 (1.7)	108 (0.8)	181 (1.1)	155 (1.6)	60 (1.4)	0.000
Diabetes type II	8541 (18.0)	81 (9.1)	1827 (12.9)	2896 (17.6)	2179 (22.2)	1105 (25.2)	
Dialysis	3538 (7.3)	80 (8.8)	1059 (7.3)	1160 (6.9)	693 (7.0)	366 (8.2)	0.000
Hypertension	4124 (8.5)	54 (5.9)	989 (6.8)	1424 (8.5)	990 (10.0)	481 (10.8)	0.000
Chronic obstructive pulmonary disease	447 (0.9)	13 (1.4)	133 (0.9)	153 (0.9)	86 (0.9)	45 (1.0)	0.000

HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; BMI: Body mass index; WHO: World Health Organization; MELD: Model for End-Stage Liver Disease; PBC: Primary biliary cirrhosis.

Table 2 Donor demographic and clinical characteristics

Donor variable	Total number of donors (n = 48281) (100%)	WHO recipients' BMI				P value
		Underweight (n = 914) (1.9%)	Normal weight (n = 14529) (30.0%)	Overweight (n = 16724) (34.6%)	Obese recipients Class I (n = 9944) (20.5%) Class II (n = 4438) (9.2%) Class III (n = 1732) (3.5%)	
Age in years, median (25 th , 75 th)	40 (24, 53)	37 (20, 52)	39 (22, 53)	40 (24, 53)	41 (25, 53)	0.000
BMI, median (25 th , 75 th)	25.2 (22.3, 29.0)	23.6 (20.7, 27.1)	24.6 (21.7, 28.2)	25.3 (22.3, 29.0)	25.9 (23.0, 29.9)	0.000
Gender, n (%)						
Male	29034 (60.1)	486 (53.2)	8171 (56.2)	10219 (61.1)	2801 (63.1)	0.000
Female	19247 (39.8)	428 (46.8)	6358 (43.8)	6505 (38.9)	1637 (36.9)	
Primary cause of death, n (%)						
Anoxia	6895 (14.3)	163 (17.8)	2004 (13.8)	2337 (33.9)	675 (15.2)	0.040
Cerebrovascular	19840 (41.1)	355 (38.8)	5980 (41.2)	6869 (41.1)	1826 (41.1)	
Head trauma	20273 (42.0)	372 (40.7)	6161 (42.4)	7098 (42.5)	1831 (41.3)	
Central nervous system tumor	357 (0.7)	8 (0.9)	107 (0.7)	116 (0.7)	24 (0.5)	
Other	892 (1.8)	16 (1.8)	272 (1.9)	292 (1.7)	82 (1.8)	
Race, n (%)						
Non-hispanic white	34907 (72.3)	639 (69.9)	10488 (72.2)	12078 (72.2)	3264 (73.5)	0.000
Non-hispanic black	6758 (14.0)	127 (13.9)	1938 (13.3)	2334 (14.0)	640 (14.4)	
Hispanic	5118 (10.6)	109 (11.9)	1623 (11.2)	1790 (10.7)	427 (9.6)	
Asian	885 (1.8)	28 (3.1)	299 (2.1)	309 (1.8)	55 (1.2)	
Other	673 (1.3)	11 (1.2)	241 (1.6)	213 (1.2)	134 (1.3)	

BMI: Body mass index; WHO: World Health Organization.

Graft survival: During the study period, 20207 grafts failed (41.9%) and median graft survival was 11.8 years (95%CI: 11.6-12.0) (Table 5).

Figure 3 represents Kaplan-Meier probability functions for graft failure stratified by recipients' BMI. Underweight (11.1 years; 95%CI: 10.1-12.1, $P = 0.034$) and class III obese patients (10.7 years; 95%CI: 9.7-11.7, $P = 0.001$) had significant shorter median survivals when compared to normal weight recipients (12.0 years; 95%CI: 11.6-12.5).

The most frequent causes of graft failure were recipients' death (60.9%), recurrent disease (4.9%), primary graft non-function (3.5%), infections (3.5%), and unknown reasons (23.2%) (Table 6).

Cox-regression multivariate analysis showed that underweight status (AHR = 1.315; 95%CI: 1.129-1.531; $P = 0.000$) and class III obesity (AHR = 1.156; 95%CI: 1.021-1.309; $P = 0.022$) remained significant predictors for shorter graft survival in comparison to normal weight recipients after adjusting for both recipients' and donors' characteristics (age, gender, BMI, primary cause of end-stage liver disease, comorbidities), cold and warm ischemia times and year of transplantation. On the other hand, grafts transplanted in overweight recipients had lower risk of failure with AHR of 0.931 (95%CI: 0.882-0.981; $P = 0.008$) in comparison to normal weight recipients.

Secondary outcomes

Perioperative mortality: Statistical significant differences in perioperative mortality were identified between normal weight and class II and III obese patients at 30, 60, 90-d and at 1-year after LT (Figure 4).

Analysis of the most common causes of perioperative deaths during the index admission is summarized in Table 7. Overall, in hospital mortality was observed in 4.6% of the entire cohort and sepsis and multiorgan failure represented 31.9% of all causes of death. Comparison across BMI categories showed that only cardiovascular ischemic or embolic events were significantly higher in class II obese patients vs normal weight recipients (0.2% vs 0.05%).

Table 3 Mean and median overall survival by recipients' body mass index class

Recipient's BMI	Means and medians for survival time (yr)							
	Mean ¹				Median			
	Estimate	Std. error	95%CI		Estimate	Std. error	95%CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Underweight	10.8	0.3	10.2	11.4	11.5	0.6	10.4	12.7
Normal	11.6	0.1	11.4	11.7	13.1	0.2	12.6	13.6
Overweight	11.5	0.1	11.4	11.7	12.8	0.2	12.5	13.2
Obese-class I	11.3	0.1	11.2	11.5	12.4	0.2	11.9	12.8
Obese-class II	11.1	0.1	10.8	11.4	12.2	0.3	11.6	12.9
Obese-class III	10.7	0.2	10.2	11.1	11.3	0.5	10.3	12.3
Overall	11.5	0.0	11.4	11.5	12.7	0.1	12.5	12.9

¹Estimation is limited to the largest survival time if it is censored. BMI: Body mass index.

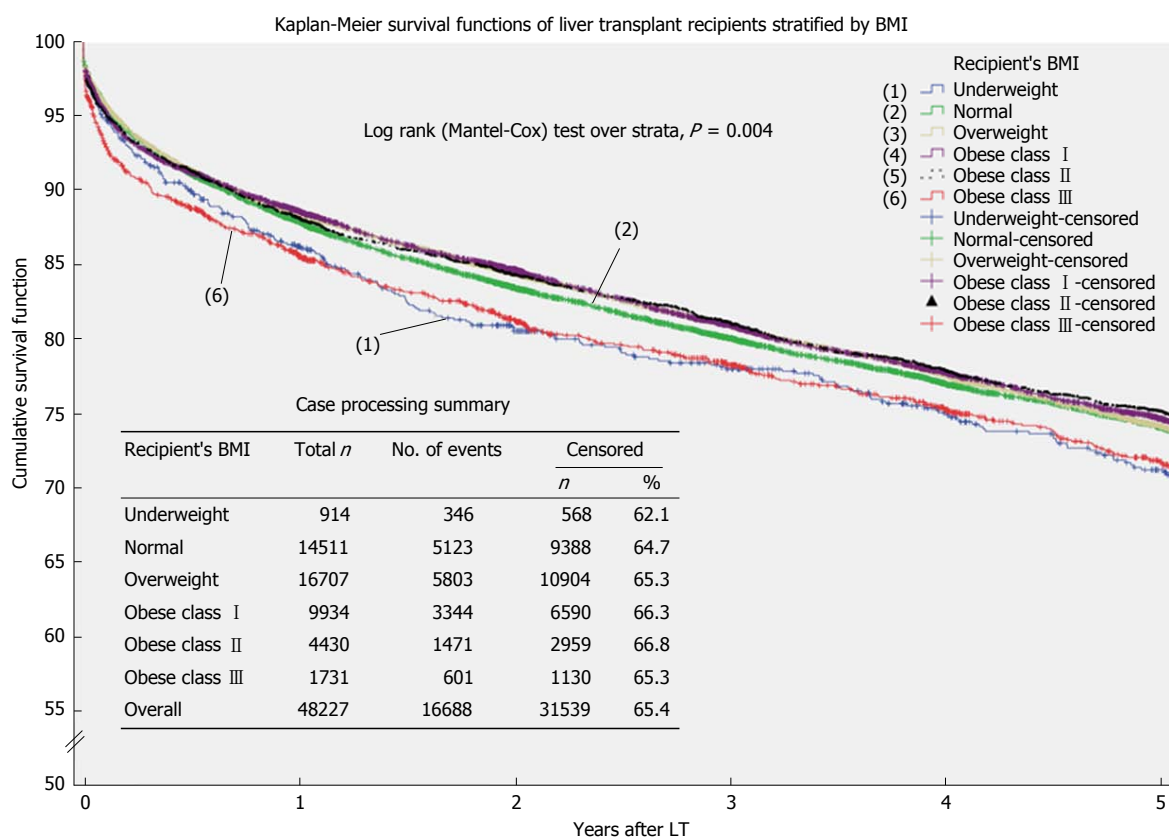


Figure 2 Kaplan-Meier overall survival functions of liver transplant recipients during the first 5 years stratified by their body mass index. Log rank (Mantel-Cox) test over strata showed significant survival difference ($P = 0.004$). In comparison to normal weight recipients, underweight recipients ($P = 0.034$) and class III obese patients ($P = 0.001$) experienced significant lower probability of overall survival. BMI: Body mass index; LT: Liver transplantation.

At multivariate logistic regression analysis, recipients' BMI category remained a significant predictor for in-hospital, 90 d and 1 year mortality after adjusting for cold and warm ischemia time, donors' characteristics, primary indication for LT and recipients' comorbidities (Table 8). Specifically, when compared to normal weight recipients, class III obesity was a predictor for in-hospital mortality (AOR = 1.749; 95%CI: 1.276-2.397; $P = 0.001$), 90 d mortality (AOR = 1.956; 95%CI: 1.473-2.597; $P = 0.000$) and 1 year mortality (AOR = 1.458; 95%CI: 1.154-1.842; $P = 0.002$). Also, being underweight was a risk factor for 90 d mortality (AOR = 1.737; 95%CI: 1.185-2.548; $P = 0.005$) and 1-year

mortality (AOR = 1.505; 95%CI: 1.105-2.048; $P = 0.009$) while being overweight was protective (AOR = 0.886 at 1-year post LT; 95%CI: 0.792-0.992; $P = 0.036$).

All causes of death: Analysis of all primary causes of mortality after LT is reported in Figure 5. Infections were responsible for 21.1% of all deaths, cardiopulmonary complications for 14.9%, and graft failure for 10.9%. Other main causes of mortality were malignant diseases (9.4%), unknown causes (8.3%) and other less common causes that represented 27.3% of all deaths when grouped together (Figure 5A).

Table 4 Univariate and multivariate Cox analysis of predictors of overall survival

Variable	Univariate analysis		Multivariate analysis	
	Hazard rate (95%CI)	P value	Hazard rate (95%CI)	P value
Recipient BMI		0.003		0.000
Normal weight (reference)	1		1	
Underweight	1.125 (1.009-1.255)	0.034	1.265 (1.096-1.461)	0.001
Overweight	1.005 (0.968-1.043)	0.807	0.908 (0.864-0.954)	0.000
Obese class I	1.024 (0.980-1.070)	0.284	0.947 (0.893-1.004)	0.067
Obese class II	1.042 (0.983-1.104)	0.169	0.971 (0.898-1.051)	0.470
Obese class III	1.163 (1.069-1.266)	0.000	1.157 (1.031-1.299)	0.013
Donor BMI		0.001		0.000
Normal weight (reference)	1		1	
Underweight	0.962 (0.897-1.033)	0.288	1.017 (0.928-1.114)	0.716
Overweight	1.060 (1.023-1.098)	0.001	1.009 (0.962-1.057)	0.717
Obese class I	1.049 (0.998-1.102)	0.059	0.986 (0.921-1.057)	0.695
Obese class II	1.129 (1.045-1.220)	0.002	1.020 (0.912-1.140)	0.729
Obese class III	0.988 (0.999-1.112)	0.988	0.889 (0.765-1.034)	0.128
Recipient age		0.000		0.000
18-45 (reference)	1		1	
46-55	1.264 (1.212-1.319)	0.000	1.207 (1.143-1.276)	0.000
56-65	1.536 (1.471-1.603)	0.000	1.490 (1.405-1.580)	0.000
66-75	2.005 (1.887-2.130)	0.000	2.069 (1.904-2.247)	0.000
≥ 76	3.224 (2.099-4.951)	0.000	2.476 (1.462-4.194)	0.001
Donor age		0.000		0.000
0-17 (reference)	1		1	
18-45	1.107 (1.050-1.166)	0.000	1.066 (0.996-1.141)	0.066
46-55	1.297 (1.223-1.376)	0.000	1.266 (1.170-1.370)	0.000
56-65	1.502 (1.411-1.598)	0.000	1.413 (1.300-1.537)	0.000
66-75	1.706 (1.583-1.840)	0.000	1.609 (1.453-1.782)	0.000
≥ 76	1.661 (1.448-1.883)	0.000	1.609 (1.340-1.932)	0.000
Recipient sex (male)	1.063 (1.030-1.097)	0.000	1.025 (0.979-1.073)	0.297
Donor sex (male)	0.951 (0.922-0.980)	0.001	0.967 (0.926-1.008)	0.967
Cold ischemia time (h)	1.010 (1.006-1.013)	0.000	1.008 (1.003-1.013)	0.001
Warm ischemia time (min)	1.002 (1.001-1.003)	0.000	1.002 (1.001-1.003)	0.000
Year of transplantation	0.996 (0.992-0.999)	0.017	0.987 (0.980-0.993)	0.000
Dialysis	1.507 (1.422-1.598)	0.000	1.492 (1.367-1.629)	0.000
Diabetes	1.406 (1.355-1.460)	0.000	1.314 (1.248-1.383)	0.000
COPD	1.384 (1.218-1.573)	0.000	1.250 (1.075-1.454)	0.004
Hypertension	1.207 (1.150-1.267)	0.000	1.057 (0.998-1.120)	0.059
Primary indication		0.000		0.000
HCV	1.356 (1.313-1.400)	0.000	1.429 (1.335-1.530)	0.000
Alcohol + HCV	1.214 (1.152-1.281)	0.000	1.477 (1.351-1.616)	0.000
HCV + other viral hepatitis	1.093 (0.932-1.283)	0.274	1.342 (1.098-1.638)	0.004
Other	1.020 (0.951-1.094)	0.583	1.111 (0.993-1.244)	0.067
Alcohol	1.060 (1.018-1.103)	0.005	1.188 (1.102-1.282)	0.000
HBV	0.669 (0.613-0.729)	0.000	0.782 (0.691-0.883)	0.000
PSC	0.597 (0.554-0.644)	0.000	0.709 (0.634-0.792)	0.000
PBC	0.670 (0.623-0.721)	0.000	0.715 (0.641-0.797)	0.000
NASH	0.906 (0.821-1.001)	0.051	0.953 (0.783-1.160)	0.630
Autoimmune	0.807 (0.742-0.878)	0.000	0.916 (0.810-1.036)	0.164
Acute liver failure	0.801 (0.689-0.931)	0.004	1.049 (0.822-1.339)	0.701
Congenital or metabolic disease	0.758 (0.703-0.817)	0.000	0.825 (0.736-0.926)	0.001

At multivariate analysis, after adjusting for patients' and donors' characteristics, primary indication for liver transplantation, comorbidities, year of transplantation and warm and cold ischemia times, class III obesity and underweight status remained independent factors associated with lower survival. HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; PBC: Primary biliary cirrhosis.

Compared to other BMI groups, class III obese patients died more frequently from infections and cardio-pulmonary complications. On the other hand, normal weight and overweight patients experienced a higher rate of malignant diseases (Figure 5B).

Impact analysis

Analysis of the hypothetical number of lives that could

have been saved within one-year post LT by allocating grafts only to low risk groups (normal weight, overweight and obese class I recipients) was performed using observed values and ranges of this study. If no transplants had been performed for class III obese patients, 55 deaths could have been avoided, 38 if no transplants had been done for class II obese and 18 if no transplants had been done for underweight

Table 5 Mean and median graft survival by recipients' body mass index class

Recipient's BMI	Means and medians for graft survival time (yr)							
	Mean ¹				Median			
	Estimate	Std. error	95%CI		Estimate	Std. error	95%CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Underweight	10.4	0.3	9.8	11	11.1	0.4	10.1	12.1
Normal	11.1	0.07	10.9	11.3	12	0.2	11.6	12.5
Overweight	11.1	0.07	11	11.3	12	0.1	11.6	12.4
Obese-class I	11	0.09	10.8	11.2	11.7	0.2	11.3	12.1
Obese-class II	10.8	0.14	10.5	11.1	11.7	0.3	11.1	12.3
Obese-class III	10.3	0.24	9.8	10.8	10.7	0.5	9.7	11.7
Overall	11	0.04	11	11.1	11.8	0.1	11.6	12

¹Estimation is limited to the largest survival time if it is censored. BMI: Body mass index.**Table 6** Summary of the primary causes of graft loss stratified by recipients' body mass index *n* (%)

Primary cause of graft failure	WHO recipients' BMI class							P value
	All recipients (n = 16715)	Underweight (n = 346)	Normal weight (n = 5129)	Overweight (n = 5813)	Obese recipients			
					Class I (n = 3351)	Class II (n = 1473)	Class III (n = 603)	
		(I)	(II)	(III)	(IV)	(V)	(VI)	
Primary graft non-function	603 (3.6)	11 (3.2)	178 (3.5)	221 (3.8)	128 (3.8)	48 (3.3)	17 (2.8)	≥ 0.05
Biliary complications	89 (0.5)	2 (0.6)	18 (0.4)	34 (0.6)	23 (0.7)	6 (0.4)	6 (1.0)	≥ 0.05
Vascular thrombosis	119 (0.7)	2 (0.6)	45 (0.9)	39 (0.7)	16 (0.5)	11 (0.7)	6 (1.0)	≥ 0.05
Recurrent disease	829 (4.9)	14 (4.0)	240 (4.6)	306 (5.2)	172 (5.1)	75 (5.0)	22 (3.6)	≥ 0.05
Acute rejection	158 (0.9)	2 (0.6)	57 (1.1)	55 (0.9)	27 (0.8)	10 (0.7)	7 (1.2)	≥ 0.05
Chronic rejection	270 (1.6)	7 (2.0)	104 (2.0)	82 (1.4)	50 (1.5)	17 (1.1)	10 (1.6)	≥ 0.05
Infection	589 (3.5)	11 (3.2)	163 (3.2)	216 (3.7)	119 (3.6)	53 (3.6)	27 (4.5)	≥ 0.05
Recipient death	10172 (60.9)	224 (64.7)	3107 (60.6)	3480 (59.9)	2037 (60.8)	945 (64.2)	379 (62.9)	≥ 0.05
Unknown	3886 (23.2)	73 (21.1)	1217 (23.7)	1380 (23.7)	779 (23.2)	308 (20.9)	129 (21.4)	≥ 0.05

WHO: World Health Organization; BMI: Body mass index.

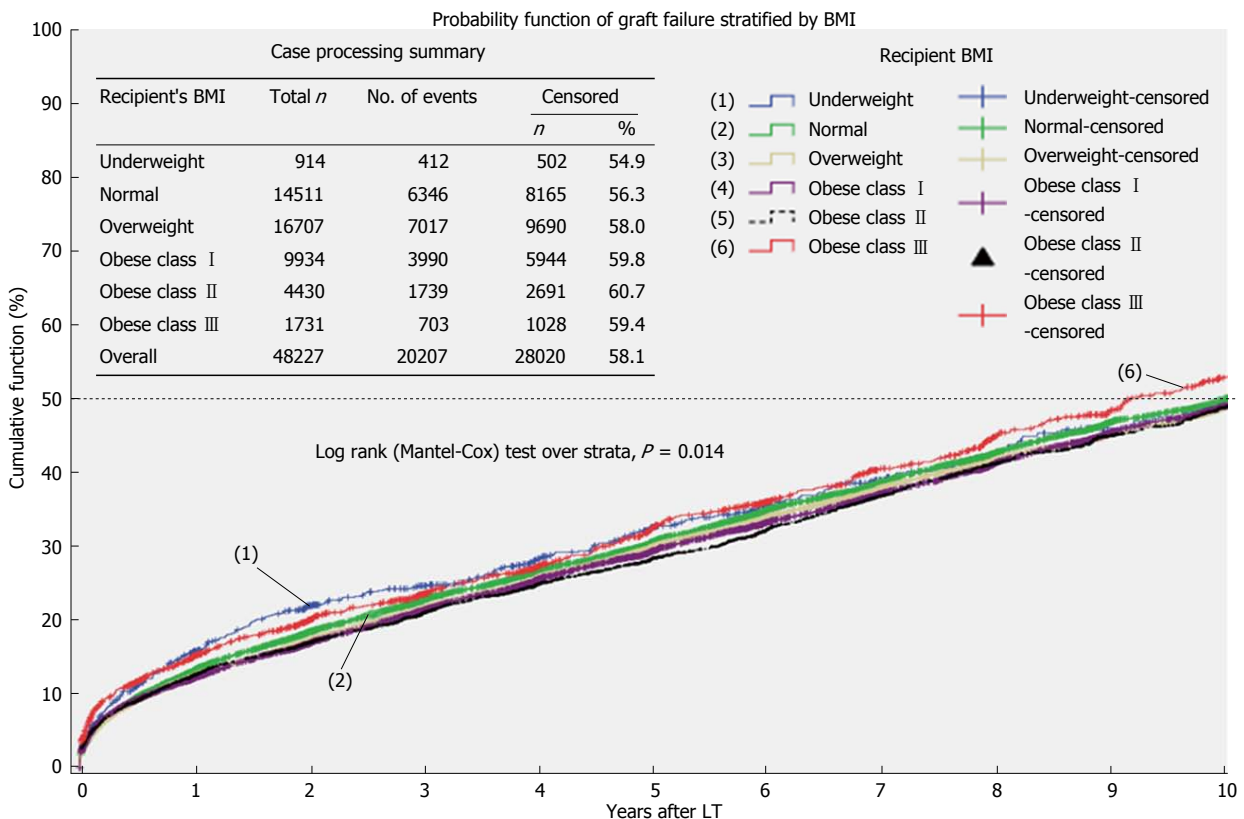
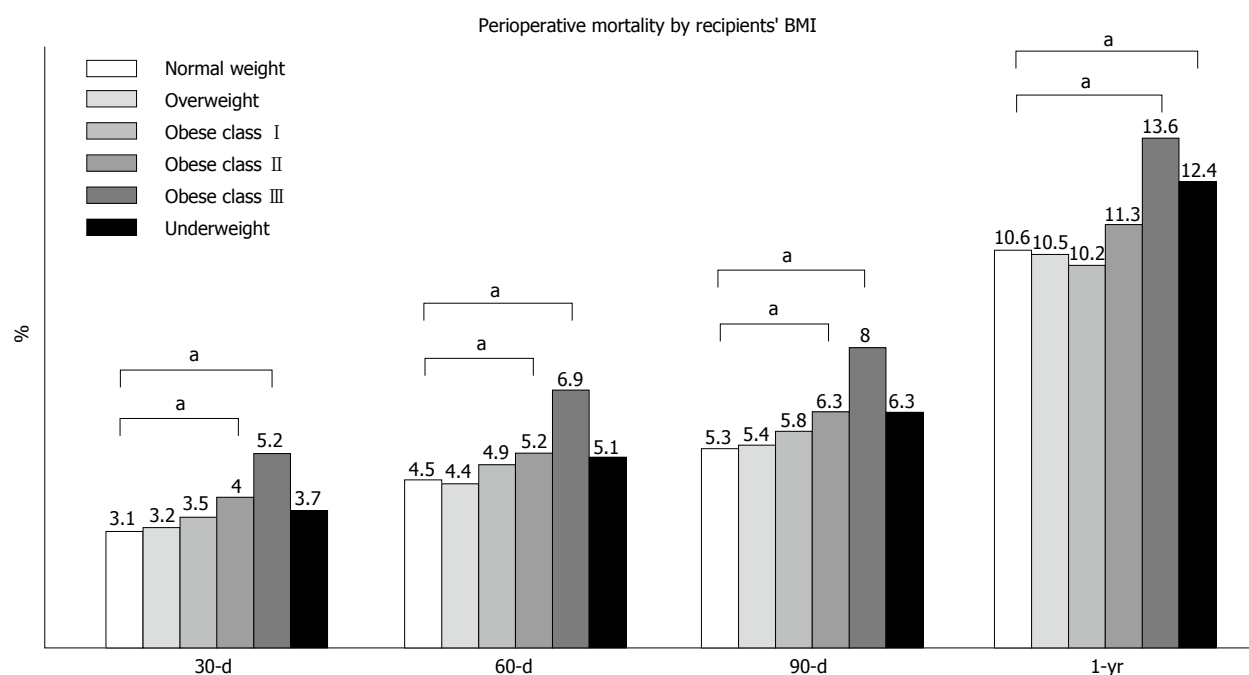
**Figure 3** Kaplan-Meier functions of graft survival stratified by recipients' body mass index. BMI: Body mass index; LT: Liver transplantation.

Table 7 Summary of the primary causes mortality during the index admission for liver transplantation stratified by recipients body mass index *n* (%)

Primary cause of perioperative mortality	All recipients (<i>n</i> = 48281)	WHO recipients' MBI class						Group comparisons	<i>P</i> value
		Underweight (<i>n</i> = 914)	Normal weight (<i>n</i> = 14529)	Overweight (<i>n</i> = 16724)	Obese recipients				
					Class I (<i>n</i> = 9944)	Class II (<i>n</i> = 4438)	Class III (<i>n</i> = 1732)		
Infections or multiorgan failure	718 (1.4)	22 (2.4)	202 (1.3)	229 (1.3)	155 (1.5)	67 (1.5)	43 (2.4)	(II) vs (V)	≥ 0.05
Cerebrovascular complication	155 (0.3)	4 (0.4)	47 (0.3)	60 (0.3)	25 (0.2)	14 (0.3)	5 (0.2)		≥ 0.05
Hemorrhagic	128 (0.2)	3 (0.3)	38 (0.2)	41 (0.2)	32 (0.3)	11 (0.2)	3 (0.1)		≥ 0.05
Single organ failure	102 (0.2)	1 (0.1)	25 (0.1)	35 (0.2)	21 (0.2)	13 (0.2)	7 (0.4)		≥ 0.05
Intraoperative complications	75 (0.1)	2 (0.2)	30 (0.2)	21 (0.1)	12 (0.1)	4 (0.09)	6 (0.3)		≥ 0.05
Cardiovascular or embolic event	53 (0.1)	1 (0.1)	8 (0.05)	16 (0.09)	13 (0.13)	12 (0.2)	3 (0.17)		≤ 0.05
Vascular thrombosis	22 (0.04)	0	5 (0.03)	9 (0.05)	2 (0.02)	4 (0.09)	2 (0.1)		≥ 0.05
Biliary complication	2 (0.004)	0	0	1 (0.005)	1 (0.01)	0	0		≥ 0.05
Primary graft non-function	62 (0.12)	1 (0.1)	11 (0.07)	27 (0.16)	12 (0.12)	5 (0.1)	6 (0.3)		≥ 0.05
Rejection	9 (0.01)	0	3 (0.02)	3 (0.01)	2 (0.02)	1 (0.02)	0		≥ 0.05
Other causes	751 (1.5)	12 (1.3)	203 (1.3)	235 (1.4)	177 (1.7)	91 (2.0)	33 (1.9)		≥ 0.05
Unknown	172 (0.3)	1 (0.1)	57 (0.3)	61 (0.3)	32 (0.3)	13 (0.2)	8 (0.4)		≥ 0.05
Total	2249 (4.6)	47 (5.1)	629 (4.3)	738 (4.4)	484 (4.8)	235 (5.2)	116 (6.6)		

WHO: World Health Organization; BMI: Body mass index.

**Figure 4** Graphical representation of perioperative and 1-year mortality stratified by recipients' body mass index. Statistical significant difference in perioperative mortality at 30 d, 60 d and 90 d post liver transplantation was found between normal weight recipients and class II and class III obese patients. At 1 year after surgery, a statistical significant difference in mortality was noted between normal weight recipient and class III obese patients. ^a*P* ≤ 0.05. BMI: Body mass index.

recipients. These results were equivalent to 2.38% of deaths for the entire cohort.

Analysis of the long-term impact of allocating grafts to underweight recipients showed a potential loss of 1009 life-years (95%CI: 390-1627 years), equivalent to 80 grafts (95%CI: 29-133 grafts). Allocation of grafts to obese class II recipients resulted in a potential loss of 2311 life-years (95%CI: 1690-2932 years) or equivalent to 183 grafts (95%CI: 129-240 grafts). Allocation of grafts to obese class III recipients resulted in a potential loss of 2056 life-years (95%CI: 1319-2793

years) or equivalent to 163 grafts (95%CI: 101-229 grafts). Overall, we estimated that avoiding LT for the two highest risk BMI groups (underweight and class III obese recipients) would have saved 3065 life-years (95%CI: 1710-4421 years) that were equivalent to 243 extra grafts (95%CI: 131-363 grafts).

In the best hypothetical scenario where all the extra-grafts were allocated to patients with the longest median survival (normal weight recipients), the net gain for the entire cohort was 15921 life-years (95%CI: 15375-22754 life-years) that corresponded to a 2.7%

Table 8 Multivariate logistic analysis of in hospital, 90-d and 1-year mortality stratified by recipients' body mass index at the time of liver transplantation

Variable	Multivariate analysis: In hospital mortality		Multivariate analysis: 90-d mortality		Multivariate analysis: 1-yr mortality	
	Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
Recipient BMI						
Normal weight	1		1		1	
Underweight	1.359 (0.865-2.135)	0.184	1.737 (1.185-2.548)	0.005	1.505 (1.105-2.048)	0.009
Overweight	0.942 (0.798-1.112)	0.481	0.995 (0.856-1.157)	0.950	0.886 (0.792-0.992)	0.036
Obese class I	1.171 (0.974-1.408)	0.094	1.185 (1.000-1.430)	0.050	0.900 (0.792-1.028)	0.120
Obese class II	1.135 (0.889-1.450)	0.309	1.197 (0.959-1.495)	0.112	1.004 (0.846-1.193)	0.960
Obese class III	1.749 (1.276-2.397)	0.001	1.956 (1.473-2.597)	0.000	1.458 (1.154-1.842)	0.002
Donor BMI						
Normal weight	1		1		1	
Underweight	1.581 (1.214-2.060)	0.001	1.449 (1.131-1.857)	0.003	1.200 (0.978-1.472)	0.080
Overweight	1.025 (0.881-1.194)	0.746	1.008 (0.878-1.158)	0.907	1.025 (0.922-1.140)	0.647
Obese class I	0.983 (0.788-1.226)	0.878	1.016 (0.832-1.239)	0.879	1.061 (0.913-1.233)	0.437
Obese class II	1.217 (0.878-1.686)	0.239	1.189 (0.880-1.606)	0.259	1.223 (0.973-1.536)	0.996
Obese class III	1.013 (0.647-1.585)	0.955	0.952 (0.626-1.448)	0.817	0.999 (0.731-1.365)	0.996
Recipient age						
18-45	1		1		1	
46-55	1.305 (1.089-1.563)	0.004	1.324 (1.122-1.583)	0.001	1.302 (1.146-1.479)	0.000
56-65	1.563 (1.292-1.891)	0.000	1.719 (1.447-2.041)	0.000	1.650 (1.443-1.888)	0.000
66-75	2.251 (1.737-2.917)	0.000	2.451 (1.941-3.094)	0.000	2.570 (2.146-3.078)	0.000
≥ 76	5.081 (1.410-18.316)	0.013	6.345 (2.060-20.105)	0.001	2.694 (0.871-8.328)	0.344
Donor age						
0-17	1		1		1	
18-45	1.207 (0.961-1.516)	0.106	1.216 (0.988-1.496)	0.065	1.138 (0.968-1.337)	0.118
46-55	1.492 (1.154-1.929)	0.002	1.411 (1.117-1.784)	0.004	1.464 (1.221-1.755)	0.000
56-65	1.357 (1.027-1.793)	0.032	1.388 (1.079-1.785)	0.011	1.365 (1.123-1.660)	0.002
66-75	1.308 (0.929-1.843)	0.124	1.379 (1.014-1.875)	0.041	1.550 (1.230-1.954)	0.000
≥ 76	0.639 (0.291-1.403)	0.265	0.864 (0.456-1.638)	0.654	1.527 (1.034-2.253)	0.033
Recipient sex (male)	0.909 (0.787-1.050)	0.194	0.966 (0.847-1.102)	0.608	1.051 (0.948-1.164)	0.344
Donor sex (male)	1.017 (0.888-1.164)	0.807	1.009 (0.892-1.142)	0.888	0.993 (0.904-1.092)	0.888
Cold ischemia time (h)	1.033 (1.019-1.047)	0.000	1.021 (1.007-1.035)	0.003	1.024 (1.013-1.035)	0.000
Warm ischemia time (min)	1.008 (1.005-1.011)	0.000	1.007 (1.005-1.010)	0.000	1.006 (1.004-1.008)	0.000
Year of transplantation	0.987 (0.968-1.006)	0.182	0.970 (0.953-0.987)	0.000	1.003 (0.989-1.016)	0.686
Dialysis	2.922 (2.378-3.590)	0.000	2.824 (2.326-3.429)	0.000	2.436 (2.071-2.865)	0.000
Diabetes	1.165 (0.990-1.371)	0.065	1.149 (0.992-1.331)	0.063	1.226 (1.095-1.374)	0.000
COPD	1.276 (0.803-2.027)	0.303	1.155 (0.996-1.341)	0.057	1.233 (0.883-1.720)	0.219
Hypertension	0.957 (0.796-1.151)	0.642	0.959 (0.810-1.135)	0.624	1.027 (0.905-1.166)	0.680
Primary indication for transplant						
HCV	1.369 (1.114-1.682)	0.003	1.215 (1.049-1.408)	0.009	0.980 (0.847-1.135)	0.791
Alcohol + HCV	1.307 (0.976-1.752)	0.073	1.038 (0.808-1.332)	0.772	0.961 (0.788-1.173)	0.698
HCV + Other viral hepatitis	1.307 (0.976-1.752)	0.073	0.895 (0.509-1.575)	0.701	0.876 (0.564-1.362)	0.558
Other	0.730 (0.549-0.970)	0.030	0.693 (0.544-0.883)	0.003	0.843 (0.677-1.050)	0.128
Alcohol	1.380 (1.095-1.740)	0.006	1.172 (0.988-1.391)	0.068	1.366 (1.153-1.618)	0.000
HBV	1.230 (0.871-1.737)	0.239	1.263 (0.912-1.747)	0.159	1.326 (1.023-1.719)	0.033
PSC	1.749 (1.229-2.489)	0.002	1.608 (1.190-2.172)	0.002	1.777 (1.377-2.293)	0.000
PBC	1.989 (1.394-2.837)	0.000	1.834 (1.352-2.487)	0.000	1.754 (1.365-2.252)	0.000
NASH	1.564 (0.899-2.722)	0.113	1.043 (0.660-1.649)	0.857	1.298 (0.902-1.869)	0.160
Autoimmune	1.179 (0.825-1.686)	0.365	0.879 (0.648-1.191)	0.405	1.027 (0.794-1.329)	0.840
Acute liver failure	0.839 (0.462-1.522)	0.839	0.797 (0.450-1.410)	0.435	1.183 (0.712-1.964)	0.517
Congenital or metabolic disease	1.139 (0.832-1.522)	0.563	1.094 (0.821-1.458)	0.540	1.162 (0.919-1.469)	0.211

HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; COPD: Chronic obstructive pulmonary disease; PBC: Primary biliary cirrhosis.

(95%CI: 2.5%-3.6%) improvement in overall survival for the entire cohort.

DISCUSSION

The main findings of this study were that class III obesity and underweight status were associated with higher perioperative mortality and inferior patient and graft survival in comparison to normal weight recipients.

To our knowledge, this is the largest multicentric retrospective observational study on the impact of BMI in LT recipients. One of its strengths is the fact that its sample size allowed us to adjust the analysis of primary and secondary outcomes for several confounders. Our study corroborated the results of several other investigators^[4,15-19] but it went against the findings of other groups^[8,9,18,20,21] including a recent meta-analysis^[9] of 13 studies involving 76620 LT recipients that found

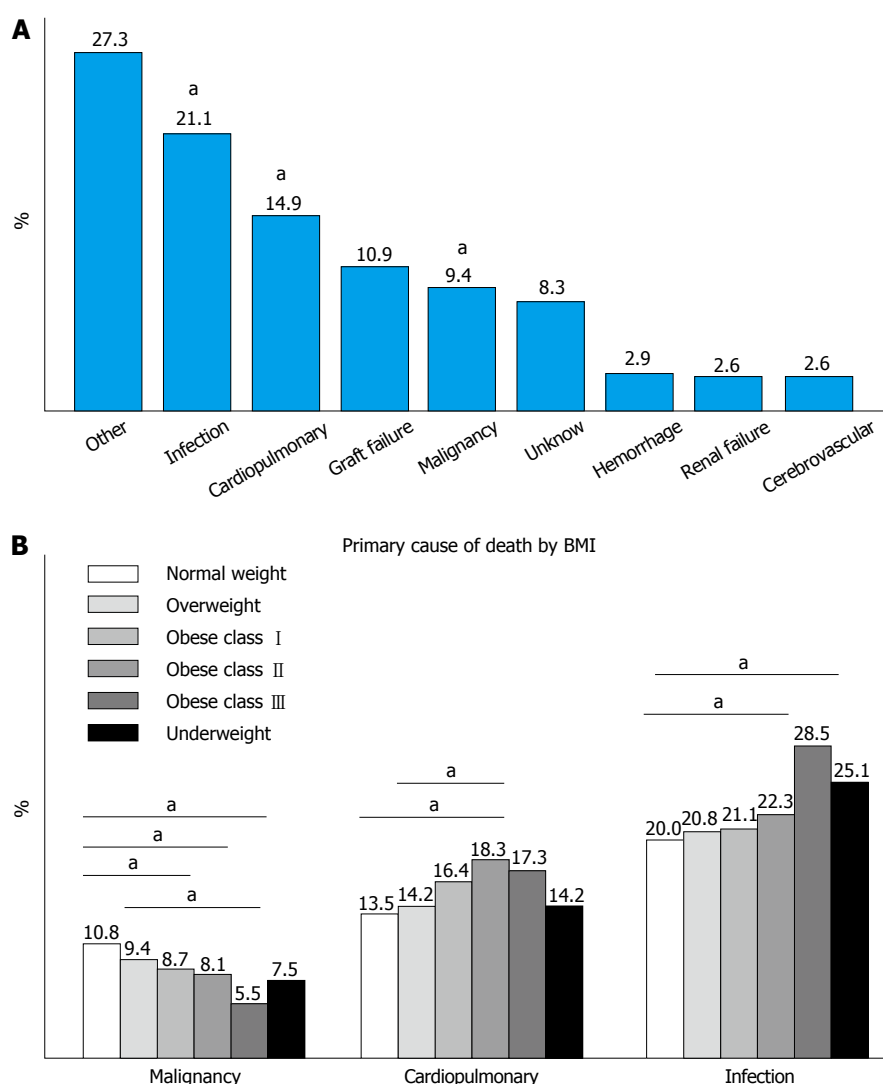


Figure 5 All causes of deaths after liver transplantation. A "a" represent statistical significant differences among recipients' BMI categories (A). Infections were more frequently observed in class II and class III obese recipients in comparison to normal weight patients. Similarly, deaths caused by cardiopulmonary complications were more frequent in class II obese patients vs normal weight or overweight recipients. On the other hand, deaths caused by malignancies were more frequent in normal weight and overweight recipients in comparison to other BMI categories (B). ^a $P \leq 0.05$. BMI: Body mass index.

that obesity did not impact survival of patients undergoing LT.

In 2008, Segev *et al.*^[22] found that in the United States, obese and morbidly obese patients were more likely turned down for a LT in comparison to normal weight candidates. A possible explanation for this is finding that LTs for obese patients can be challenging and require more resources in comparison to recipients with lower BMI indices^[6,7,23]. Yet, transplant centers are dealing with obese patients with increasing frequency because obesity is prevalent in many countries^[24] and in the context of insufficient number of grafts, this creates a unique ethical dilemma^[25,26]. One of the possible strategies is to deny LT to certain groups of high risk patients based on the utilitarian principle of maximizing results by transplanting only patients who have the best potential outcomes, and to accept the fact that patients who do not receive a LT would have significant shorter lives. In our study, 5-year survival for class III obese recipients was 71.5% vs 73.9% for normal

weight patients. Although statistically significant, the absolute difference was clinically irrelevant. Therefore, the exclusion of patients based only on their BMI might be unethical in vision of the fact that 5-year survival of obese and underweight LT recipients was higher than 50% conventionally considered the minimum survival benefit to justify allocation of liver grafts to patients with ESLD^[27,28].

One of the most pressing questions we wanted to address in this study was to quantify the impact of abnormal BMIs on the overall survival of the entire cohort of patients waiting for a LT. Therefore, we simulated clinical scenarios where different graft allocation policies were implemented. By excluding underweight and morbidly obese recipients (the two highest-risk categories for perioperative mortality but representing only 5.4% of the entire cohort), an extra 243 grafts (95%CI: 131-363) could have been used to transplant low risk patients. This strategy would result in an overall 5-year survival improvement of 0.5% (95%CI:

0.27%-0.75%) for the entire cohort. The main reasons for this marginal increase were the fact that underweight and class III obese patients represented only a very small percentage of the cohort, and the fact that the absolute difference in median survival between normal weight recipients and class III obese and underweight patients was only 1.8 and 1.6 years respectively. These relatively small differences are most likely due to the fact that LT recipients undergo rigorous cardiopulmonary testing prior to listing, and only the healthiest of the morbidly obese patients are cleared for transplantation with overall acceptable results.

Our study has several limitations. One of the most important is its retrospective design. Therefore, confounders like immunosuppression protocols, surgical skills and pre and postoperative care provided by so many transplant centers could not be controlled in the final analysis. Another main finding of this study was that the proportion of patients who died from malignant diseases was inversely correlated with their BMI. This phenomenon was observed also by Valentijn *et al.*^[29] in patients undergoing non-transplant related surgeries where 52% of underweight patients died of cancer-related deaths in comparison to 24% for class III obese. This might be due to different factors (*e.g.*, smoking habits), or to the fact that obese patients might have lower risk of developing cancer^[30]. Further investigations are needed to test if obesity is indeed a protective condition against malignancies after transplantation as one of the most important limitations of this study is its retrospective design.

Another limitation was our inability to adjust for the amount of ascites that often affects patients with ESLD. Therefore, the true incidence of obesity might have been overestimated. In addition, we intentionally included recipients transplanted over a long period of time to increase the study population. The advantage of having a large number of patients had to be weighed against the fact that over the study period, there have been significant changes such as immunosuppression protocols, perioperative care and patient selection with significant decrease in morbidity and mortality for obese patients undergoing LT during the last ten years. These improvements might have decreased our ability to detect any clinically significant differences in overall survival across different BMI categories.

Despite these limitations, our study has the strength of including a very large number of patients that allowed us to perform multivariate analyses to test if selected obese patients have significant worse outcomes than normal weight patients after LT. The results suggested that even for very selected class III obese and underweight recipients, perioperative morbidity and mortality are higher than normal-weight recipients. However, these differences are clinically inconsequential as these patients have good long-term outcomes and their exclusion has a minimal survival benefit for the entire cohort of patients waiting for LT. These findings might be of some help to clinicians and policy makers

who deal with the ethical dilemma of allocating liver grafts to recipients with abnormal BMI. The biggest challenge ahead of transplant programs remains the selection of those recipients who, despite their abnormal BMI, will have good outcomes and long-term benefit from LT.

COMMENTS

Background

Liver transplantation (LT) is the only treatment that can save patients' lives in the presence of irreversible liver failure. There has been a persistent discrepancy between the number of patients who are waiting for a liver transplant and the number of available livers. Several strategies have been used to increase the number of donors, but despite all the best efforts, a significant proportion of patients affected by end-stage liver disease still die while waiting for a suitable organ. Since organs are limited, the transplant community has used some criteria to prioritize the allocation of livers grafts to patients who are in urgent need of a transplant. The main reason for these criteria is to maximize the benefits and minimize the potential risks associated with such extensive surgeries. One of the emerging controversies in the field of transplantation is the allocation of livers to patients who are obese as they are considered at high risk of developing serious complications that can lead to death after LT. Therefore, there is evidence that obesity might be a negative factor that disadvantages some groups of patients who have lower probabilities of being selected for LT.

Research frontiers

The authors' group analyzed a very large database containing data prospectively collected from patients who underwent LT in the United States to assess if abnormal body mass index (BMI) was a negative predictor for survival after LT. Previous studies, using different databases, had conflicting results and controversy regarding LT, especially for obese patients, still persists.

Innovations and breakthroughs

This paper found that, although underweight and morbid obese patients had increased risks for perioperative complications and lower long term survival in comparison to normal weight recipients of liver transplants, the absolute differences were clinically negligible. In addition, impact analysis revealed that exclusion of high risk patients from undergoing LT did not improve the overall results for the entire group of patients who needed a LT.

Applications

Selected obese and underweight patients affected by end-stage liver disease should not be excluded from LT as their overall outcomes are clinically comparable to normal weight patients.

Terminology

BMI is the ratio between a person's stature and respective weight. In most cases, the higher is the BMI, the higher is the concentration of fat in the body. Persons with BMI higher than 30 are considered obese and individuals with BMI higher than 40 are considered morbidly obese. Obesity is associated with increased risks for metabolic derangements such as diabetes, hypertension, hypercholesterolemia and atherosclerotic diseases. Because of this association, obese patients are considered at higher risk of developing cardiopulmonary complications after LT and they absorb more resources when undergoing complex surgical interventions like LT.

Peer-review

This is a large retrospective study to attempt to answer if BMI affect outcomes of liver transplant patients. The study is well designed, performed, and written.

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Retrospective Cohort Study

Risk factors for fracture in adult kidney transplant recipients

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status means that health information custodians of all types can legally disclose personal health information to ICES without informed consent for purposes of analysis, evaluation and compiling statistical information about our health care system.

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Abstract

AIM: To determine the general and transplant-specific risk factors for fractures in kidney transplant recipients.

METHODS: We conducted a cohort study of all adults who received a kidney-only transplant ($n = 2723$) in Ontario, Canada between 2002 and 2009. We used multivariable Cox proportional hazards regression to determine general and transplant-specific risk factors for major fractures (proximal humerus, forearm, hip, and clinical vertebral). The final model was established using the backward elimination strategy, selecting risk factors with a P -value ≤ 0.2 and forcing recipient age and sex into the model. We also assessed risk factors for other fracture locations (excluding major fractures, and fractures involving the skull, hands or feet).

RESULTS: There were 132 major fractures in the follow-up (8.1 fractures per 1000 person-years). General risk factors associated with a greater risk of major fracture were older recipient age [adjusted hazard ratio (aHR) per 5-year increase 1.11, 95%CI: 1.03-1.19] and female sex (aHR = 1.81, 95%CI: 1.28-2.57). Transplant-specific risk factors associated with a greater risk of fracture included older donor age (5-year increase) (aHR = 1.09, 95%CI: 1.02-1.17) and end-stage renal disease (ESRD) caused by diabetes (aHR = 1.72, 95%CI: 1.09-2.72) or cystic kidney disease (aHR = 1.73, 95%CI: 1.08-2.78) (compared to glomerulonephritis as the reference cause). Risk factors across the two fracture locations were not consistent (major fracture locations *vs* other). Specifically, general risk factors associated with an increased risk of other fractures were diabetes and a fall with hospitalization prior to transplantation, while length of time on dialysis, and renal vascular disease and other causes of ESRD were the transplant-specific risk factors associated with a greater risk of other fractures.

CONCLUSION: Both general and transplant-specific risk factors were associated with a higher risk of fractures in kidney transplant recipients. Results can be used for clinical prognostication.

Key words: Fracture; Risk factors; Kidney transplant recipient; Prognostication; Cohort study

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Core tip: We examined risk factors for major and other fractures in adult kidney transplant recipients. Increasing age and female sex were associated with an increased major fracture risk, while diabetes or cystic kidney disease as the cause of end-stage renal disease and increasing age of the kidney donor were the transplant-specific risk factors associated with an increased major fracture risk. Risk factors were variable across fracture locations (major *vs* other fractures). General and transplant-specific risk factors for fracture

should be considered when assessing fracture risk in kidney transplant recipients. Different risk factors may need to be considered depending on the fracture location.

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INTRODUCTION

Kidney transplant recipients are at a higher risk of fracture compared to the general population^[1-4]. Reasons for the increased fracture risk are multifactorial, and may include perturbations in bone and mineral metabolism that occur in renal bone disease, and the administration of glucocorticoids after transplantation^[5]. However, the risk factors for fracture after transplant remain uncertain. In a recent systematic review many classical risk factors for fracture in the general population (*e.g.*, older age, female sex) were inconsistently associated with fractures in kidney transplant recipients^[6]. Unlike the transplant population, risk factors for fracture in the general population are well-established and are included in the World Health Organization's (WHO) Fracture Risk Assessment tool (FRAX). FRAX is used to guide treatment decisions in the general population by incorporating age, sex, clinical risk factors (body mass index, parental hip fracture, glucocorticoid use, rheumatoid arthritis, smoking, alcohol intake ≥ 3 units per day), and hip bone mineral density (optional) to predict the 10-year probability of hip fracture or major osteoporotic fracture (proximal humerus, forearm, hip, or clinical vertebral)^[7-9]. However, kidney transplant recipients may have different risk factors for fracture given the unique pathophysiology that underlies their bone disease^[10]. For example, in a recent cohort study the only classical risk factor for fracture that reached statistical significance in kidney transplant recipients was high alcohol use^[11]; however, this study had only 21 fracture events and may have had inadequate statistical power to identify other risk factors^[11]. The same study also found that FRAX may be a useful tool to predict fracture in kidney transplant recipients (area under the receiver operating curve 0.62); however, the authors hypothesized that incorporating transplant-specific risk factors for fracture may further improve the performance of FRAX^[11].

The WHO has called for a global strategy on fracture prevention and management^[12]. Such strategies require an understanding of well-validated fracture risk factors and prediction tools so populations at high risk can be targeted for diagnosis, treatment, and therapeutic trials.

Given that risk factors for fracture in kidney transplant recipients have not been well-established, we conducted this study to determine general risk factors (e.g., age, sex, previous fracture, previous fall) and transplant-specific risk factors (e.g., length of time on dialysis prior to transplant) associated with major fractures (proximal humerus, forearm, hip, and clinical vertebral) in kidney transplant recipients. In an additional analysis we assessed risk factors for other fracture locations (excluding major fractures, and fractures involving the skull, hands or feet).

MATERIALS AND METHODS

Design and setting

We performed a population-based cohort study using healthcare databases held at the Institute for Clinical Evaluative Sciences (ICES) in Ontario, Canada. Ontario residents have universal access to hospital and physician services. These datasets were linked using unique encoded identifiers and analyzed at ICES. This study was approved by the institutional review board at Sunnybrook Health Sciences Centre, Toronto, Canada.

Data sources

We used several databases to obtain our study cohort, characteristics, risk factors, and outcome data. Information on all kidney transplant recipients who received their transplant in Ontario was provided by the Canadian Organ Replacement Register. Information on provincial physicians' billing claims was provided by the Ontario Health Insurance Plan database. The Canadian Institute for Health Information Discharge Abstract Database provided information on diagnostic and procedural codes during Ontario hospitalizations and information on emergency room visits was provided by the National Ambulatory Care Reporting System. The Ontario Registered Persons Database provided information on vital status and demographics.

Cohort

We identified all first-time kidney-only transplants in Ontario from April 1st, 2002 to December 31st, 2009, restricting to individuals ≥ 18 years of age at the transplant date. We selected April 1st, 2002 as our cohort entry date as this was when Canada changed the International Classification of Disease (ICD) system from version 9 to 10. The cohort entry date (index date) was the date an individual received their kidney transplant.

Risk factors

We assessed several general risk factors for fracture (e.g., age, sex, and prior major fracture) which are incorporated in FRAX. We also assessed other general risk factors found to increase fracture risk in the non-transplant population, including: A fall with hospitalization in the year prior to transplantation, race/eth-

nicity, and diabetes (only type 1 diabetes is included in FRAX)^[13-15]. We assessed several transplant-specific risk factors including: Length of time on dialysis prior to transplant (years), type of donor (living vs deceased), cause of end-stage renal disease [ESRD, e.g., diabetes mellitus, glomerulonephritis, renal vascular disease, cystic kidney disease, or other (i.e., any cause of ESRD not included in the aforementioned categories such as pyelonephritis)], pre-transplant dialysis modality (peritoneal, hemodialysis, or pre-emptive), and donor characteristics (age and sex).

Outcomes

We followed kidney transplant recipients from the date of transplant until first fracture, death, or end of follow-up (March 31st, 2013). We did not censor kidney transplant recipients if they returned to chronic dialysis or if they had another transplant during follow-up. Our primary outcome was major fractures which were defined as a composite of hip, forearm, proximal humerus, and clinical vertebral fractures. We chose to assess risk factors for major fractures with hospital presentation (emergency room visit or hospital admission) as these fracture locations are associated with excess morbidity and mortality in the general population^[16-18]. We also assessed other fracture locations, defined as: Lower leg (ankle, tibia, fibula, patella), femoral shaft, rib/sternum/trunk, scapula, clavicle, and pelvis fractures. These fractures as a whole were considered the secondary outcome as they may be more common in kidney transplant recipients^[10]. For example, in prior studies ankle fractures were common in kidney transplant recipients^[1,19]. We included both high and low trauma fractures because, similar to low-trauma fractures, high-trauma fractures occur more commonly when an individual has compromised bone strength^[20]. We identified fracture events using the 10th version of the ICD system. To increase accuracy, diagnosis codes for hip, forearm, and femoral shaft fractures had to be accompanied by procedural codes identified from hospital encounters and physician billing codes^[21].

Statistical analysis

We compared differences in baseline characteristics of recipients with and without a fracture using the Mann Whitney *U* test for continuous variables and the chi-square test for categorical variables. We calculated the incidence rate of fracture (per 1000 person-years) censoring the observation period on the date of death, first fracture, or end of follow-up (March 31, 2013). We used the Cox proportional hazards model to assess effects of risk factors on the hazard of the first fracture. Prior to obtaining the adjusted hazard ratio (aHR) to quantify the effect of each risk factor, model assumptions such as the proportional hazards assumption and linearity of continuous factors (Martingale residuals) were assessed with a *P*-value < 0.05 used as criteria for a violation^[22-24]. We used the backward elimination

Table 1 Characteristics of kidney transplant recipients classified by major fracture status¹ *n* (%)

	No fracture (<i>n</i> = 2591)	Major Fracture (<i>n</i> = 132)	<i>P</i> -value
General risk factors			
Age (yr)	50.5 (41-61)	56.5 (45-63)	0.01
Women	928 (35.8)	66 (48.5)	0.004
Race/ethnicity			0.40
White	1845 (71.2)	103 (78)	
Asian	208 (8.0)	8 (6.1)	
Black	198 (7.6)	7 (5.3)	
Other ²	340 (13.1)	14 (10.6)	
Diabetes	673 (25.6)	40 (30.3)	0.27
Fall with hospitalization in the year prior to the transplant date	92 (3.6)	8 (6.1)	0.15
Major fracture prior to the transplant date ³			
Transplant specific risk factors			
Length of time on dialysis prior to transplant (measured in years) ⁴	2.8 (1.2-5.4)	2.7 (0.92-5.1)	0.56
Type of donor			0.47
Deceased (<i>vs</i> living)	1458 (56.3)	70 (53.0)	
Cause of end-stage renal disease ⁵			0.004
Glomerulonephritis	951 (36.7)	39 (29.6)	
Cystic kidney disease	385 (14.9)	31 (23.5)	
Diabetes	560 (21.6)	37 (28.0)	
Other	695 (26.8)	25 (18.9)	
Pre-transplant dialysis modality ⁶			0.99
Peritoneal dialysis	701 (27.1)	35 (26.5)	
Hemodialysis	1622 (62.6)	83 (62.9)	
Pre-emptive	268 (10.3)	14 (11.6)	
Donor characteristics			
Type of donor			0.47
Deceased (<i>vs</i> living)	1458 (56.3)	70 (53.0)	
Donor age (yr)	46 (36-54)	48 (41-55)	0.16
Donor sex			0.73
Women	1295 (50.0)	68 (51.5)	

Data are median (interquartile range) or *n* (%). ¹Major fracture events were comprised of forearm (*n* = 81), hip (*n* = 22), proximal humerus (*n* = 18), and clinical vertebral fractures (*n* = 13); ²Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; ³Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed; ⁴Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; ⁵Due to the small number of recipients with a major fracture who had renal vascular disease as the cause of their ESRD this category was combined into the other category; ⁶We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant. ESRD: End-stage renal disease.

strategy to select risk factors that would be entered into the final model, with recipient age and sex forced into the model. To decrease the possibility of missing important risk factors for fracture post-transplant, a priori we chose a *P*-value of ≤ 0.2 to select variables for inclusion in the final model^[25]. We assessed for multicollinearity among variables prior to entering variables into the backward elimination model. We found limited concern for multicollinearity, since all variance inflation factors were less than 2^[26]. There were missing data for the following variables: Donor age (2.2%), donor sex (< 1%), cause of ESRD (11.6%), race (10.7%), and donor type (< 1%). We handled missing data by assigning values randomly selected from observed values with the exception of donor age for which we supplemented missing values with the median age. In the final model we interpreted two-sided *P*-values < 0.05 as statistically significant. We performed all analyses using Statistical Analysis Software, version 9.4 (www.sas.com). The statistical methods of this study were reviewed by a biostatistician, Guangyong Zou, PhD.

RESULTS

Incidence of fracture

Of the 2723 kidney transplant recipients the total follow-up was 16274 person-years (average 6 years), during which 402 (14.8%) died and 132 (4.8%) sustained a major fracture (8.1 fractures per 1000 person-years, 95%CI: 6.8-9.6).

Baseline characteristics

Recipients who sustained a major fracture in follow-up compared to recipients with no major fracture had a significantly higher median age (56.5 years *vs* 50.5 years), were more likely to be women (48.5% *vs* 35.8%), and were less likely to have glomerulonephritis as their cause of ESRD (29.6% *vs* 36.7%) (Table 1).

Univariable analysis

We found older recipient age and female recipient sex were the general risk factors associated with an increased risk of major fracture (Table 2). For example,

Table 2 Univariable and multivariable analysis of risk factors for major fracture in kidney transplant recipients

Risk factors	Univariable analysis HR (95%CI)	Multivariable analysis HR (95%CI)
Age (per 5 yr increase)	1.13 (1.06-1.21)	1.11 (1.03-1.19)
Sex		
Men	Reference	
Women	1.65 (1.18-2.33)	1.81 (1.28-2.57)
Race/ethnicity		
White	Reference	
Asian	0.72 (0.35-1.47)	
Black	0.65 (0.30-1.39)	
Other ¹	0.78 (0.44-1.36)	
Diabetes (<i>vs</i> none)	1.40 (0.96-2.02)	
Fall with hospitalization in the year prior to the transplant date (<i>vs</i> none)	2.00 (0.98-4.09)	1.72 (0.84-3.50)
Major fracture prior to the transplant date ² (<i>vs</i> none)		
Length of time on dialysis prior to transplant (measured in years) ³	1.06 (0.61-1.84)	
Type of donor		
Living	0.99 (0.70-1.39)	
Deceased	Reference	
Cause of end-stage renal disease ⁴		
Glomerulonephritis	Reference	Reference
Cystic kidney disease	1.93 (1.20-3.08)	1.73 (1.08-2.78)
Diabetes	1.80 (1.15-2.82)	1.72 (1.09-2.72)
Other	0.92 (0.56-1.53)	0.88 (0.53-1.46)
Pre-transplant dialysis modality ⁵		
Hemodialysis	Reference	
Peritoneal dialysis	0.99 (0.67-1.47)	
Pre-emptive	0.96 (0.54-1.68)	
Type of donor		
Living	0.99 (0.70-1.39)	
Deceased	Reference	
Donor age (per 5 yr increase)	1.11 (1.04-1.18)	1.09 (1.02-1.17)
Donor sex		
Men	Reference	
Women	1.03 (0.73-1.44)	

¹Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; ²Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed;

³Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; ⁴Due to the small number of recipients with a major fracture who had renal vascular disease as the cause of their ESRD this category was combined into the other category; ⁵We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant. ESRD: End-stage renal disease; HR: Hazard ratio.

female recipients had almost a two-fold greater risk of major fracture (HR = 1.65, 95%CI: 1.18-2.33). Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed. Regarding transplant-specific risk factors, cystic kidney disease (HR = 1.93, 95%CI: 1.20-3.08) and diabetes (HR = 1.80, 95%CI: 1.15-2.82) as the cause of ESRD (compared to glomerulonephritis as the reference cause) were both associated with a higher risk of major fracture. Each 5-year increase in donor age was also associated with a greater risk of major fracture (HR = 1.11, 95%CI: 1.04-1.18).

Multivariable analysis

In the multivariable model, older recipient age (5-year increase) (aHR = 1.11, 95%CI: 1.03-1.19) and female recipient sex (aHR = 1.81, 95%CI: 1.28-2.57) were the general risk factors associated with a greater risk of major fracture (Table 2). Regarding transplant-specific risk factors diabetes (aHR = 1.72, 95%CI:

1.09-2.72) and cystic kidney disease (aHR = 1.73, 95%CI: 1.08-2.78) as the cause of ESRD (compared to glomerulonephritis as the reference cause), and older donor age (5-year increase) (aHR = 1.09, 95%CI: 1.02-1.17) were associated with a greater risk of major fracture.

Other fractures

When we assessed other fracture events (excluding the major fractures, and skull, hands, or feet) kidney transplant recipients had 141 fractures (8.7 fractures per 1000 person-years, 95%CI: 7.3-10.2). Recipients with *vs* without such fractures were significantly more likely to have diabetes (40.4% *vs* 25.4%) and were more likely to have had a fall with hospitalization in the year prior to transplant (7.1% *vs* 3.5%) (Table 3). In the multivariable model we found diabetes and a fall with hospitalization prior to transplantation were the general risk factors associated with an increased risk of fracture, while length of time on dialysis, and renal

Table 3 Characteristics of kidney transplant recipients classified by other fractures status³ *n* (%)

	No fracture (<i>n</i> = 2582)	Other fracture (<i>n</i> = 141)	<i>P</i> -value
General risk factors			
Age (yr)	52 (42-61)	54 (44-61)	0.18
Women	944 (36.6)	48 (34.0)	0.55
Race/ethnicity			0.33
White	1838 (71.2)	110 (78.0)	
Asian	208 (8.1)	8 (5.7)	
Black	198 (7.8)	7 (5.0)	
Other ¹	338 (13.1)	16 (11.4)	
Diabetes	656 (25.4)	57 (40.4)	< 0.001
Fall with hospitalization in the year prior to the transplant index	90 (3.5)	10 (7.1)	0.03
Major fracture prior to the transplant date ⁵	69 (2.7)	13 (9.2)	< 0.001
Transplant specific risk factors			
Length of time on dialysis prior to transplant (measured in years) ²	2.7 (1.1-5.4)	3.0 (1.7-5.3)	0.068
Type of donor			
Deceased	1439 (55.7)	89 (63.1)	0.09
Cause of end-stage renal disease			0.003
Glomerulonephritis	958 (37.1)	32 (22.7)	
Cystic kidney disease	397 (15.4)	19 (13.5)	
Diabetes	555 (21.5)	42 (29.8)	
Renal vascular disease	294 (11.4)	23 (16.3)	
Other	378 (14.6)	25 (17.7)	
Pre-transplant dialysis modality ⁴			0.09
Peritoneal dialysis	694 (26.7)	42 (29.8)	
Hemodialysis	1613 (62.5)	92 (65.3)	
Pre-emptive	275 (10.7)	7 (5.0)	
Donor characteristics			
Type of donor			
Deceased	1439 (55.7)	89 (63.1)	0.09
Donor age (yr)	46 (36-54)	48 (40-54)	0.13
Donor sex			
Women	1298 (50.3)	65 (46.1)	0.33

Data are median (interquartile range) or *n* (%). ¹Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; ²Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; ³Other fracture events were comprised of pelvis (*n* = 15), ankle (*n* = 37), patella (*n* = 8), tibia/fibula (*n* = 37), rib/sternum (*n* = 34), and other (femoral shaft, scapula, clavicle; *n* = 16); ⁴We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant; ⁵Prior major fracture had to occur from 1991 to cohort entry (date of transplant).

vascular disease and other causes of ESRD were the transplant-specific risk factors associated with a greater risk of other fractures (Table 4).

DISCUSSION

Similar to the general population, we found increasing recipient age and female sex were associated with an increased major fracture risk in kidney transplant recipients. Unique to the kidney transplant population, we also found diabetes or cystic kidney disease as the cause of ESRD and increasing age of the kidney donor were associated with a significantly increased major fracture risk. However, risk factors were not consistent across fracture locations with increasing age and female sex not associated with an increased other fracture risk. Our findings suggest that both general and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in kidney transplant recipients. However, different risk factors may need to be taken into account when considering different fracture locations.

We previously published a study of 321 kidney transplant recipients from Manitoba, Canada and found that FRAX was able to modestly predict fracture and may be a useful tool for clinicians to use to help guide treatment decisions; the area under the receiver operating curve value was 0.62 and there was concordance in the observed vs predicted 10-year major osteoporotic fracture probability (6.3% vs 5.6%, respectively)^[11]. However, the number of major osteoporotic fracture events was small (*n* = 21), with correspondingly wide 95% CIs^[11]. We hypothesized that a fracture prediction tool incorporating both general and transplant-specific risk factors may improve fracture prediction^[11]. However, model updating may not be needed as the absolute fracture rate found in the current study was lower than previously reported, similar to other recently conducted studies^[27,28]. Moreover, the strength of the transplant-specific risk factors was only moderate. Additionally, the large sample size needed to update a model and the reasonable performance of the original FRAX model in kidney transplant recipients further suggests model updating may not be needed. However,

Table 4 Univariable and multivariable analysis of risk factors for other fracture in kidney transplant recipients

Risk factor	Univariable analysis HR (95%CI)	Multivariable analysis HR (95%CI)
Age (per 5 yr increase)	1.09 (1.02-1.17)	1.03 (0.96-1.10)
Sex		
Men	Reference	
Women	0.99 (0.63-1.26)	0.97 (0.68-1.39)
Race/ethnicity		
White	Reference	
Asian	0.67 (0.33-1.37)	0.67 (0.32-1.39)
Black	0.59 (0.27-1.26)	0.47 (0.21-1.02)
Other ¹	0.82 (0.49-1.39)	0.73 (0.43-1.26)
Diabetes (<i>vs</i> none)	2.2 (1.57-3.08)	2.19 (1.38-3.49)
Fall with hospitalization in the year prior to the transplant date (<i>vs</i> none)	2.37 (1.25-4.52)	2.05 (1.07-3.93)
Length of time on dialysis prior to transplant (measured in years) ²	1.06 (1.00-1.12)	1.07 (1.01-1.14)
Type of donor		
Living	Reference	
Deceased	0.67 (0.47-0.92)	
Cause of end-stage renal disease		
Glomerulonephritis	Reference	Reference
Cystic kidney disease	1.4 (0.8-2.47)	1.35 (0.76-2.39)
Diabetes	2.47 (1.56-3.91)	1.40 (0.78-2.49)
Renal vascular disease	2.40 (1.41-4.10)	2.11 (1.22-3.65)
Other	2.04 (1.21-3.44)	2.03 (1.20-3.45)
Pre-transplant dialysis modality ³		
Hemodialysis	Reference	
Peritoneal dialysis	1.06 (0.74-1.53)	
Pre-emptive	0.43 (0.2-0.92)	
Donor age (per 5 yr increase)	1.07 (1.01-1.14)	1.06 (0.99-1.12)
Donor sex		
Men	Reference	
Women	0.83 (0.6-1.16)	

¹Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; ²Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; ³We defined hemodialysis and peritoneal dialysis based on the modality they first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant.

to gain a more complete understanding of fracture risk, it is likely important for clinicians to consider some transplant-specific risk factors (*e.g.*, cause of ESRD) in isolation, when assessing fracture risk. Future research should assess other potential transplant-specific risk factors (unavailable in our current analyses), including: Change in body mass index after transplantation (weight changes found to increase fracture risk in the general population) and fibroblast growth factor 23 (suppresses mineralization of the bone matrix)^[29,30].

We found that risk factors for fracture may vary across fracture locations. For example, there were different risk factors for fracture between our two fracture classifications (major fracture locations *vs* other fracture locations). A possible explanation for this finding is that in the kidney transplant population risk factors for fractures are site specific. For example, similar to what some studies have found in the general population, in our study increasing recipient age and female recipient sex were both associated with an increased major fracture risk^[31-33]. However, increasing recipient age and female sex were not associated with an increased risk of other fractures. This provides a potential explanation for the results of a previous systematic review which found risk factors for fracture in kidney transplant

recipients were inconsistent; studies in the review included different fracture locations^[6]. However, we cannot discount the possibility that the differences in risk factors across fracture locations found in this study were the result of a type II error. Future studies with larger sample sizes should assess site-specific risk factors for fractures (*e.g.*, ankle) in kidney transplant recipients.

Of concern, several of the risk factors for fracture identified in this study are becoming more common in recent eras of kidney transplant recipients. For example, we found diabetes as the cause of ESRD and older recipient age were significant risk factors for major fractures. The number of recipients with diabetes and the average recipient age has been increasing^[34,35]. Similar to results found in a previous study^[36], increasing donor age was also associated with an increased risk of major fracture. This is concerning as there has been an increase in the number of recipients receiving a kidney from older donors^[37,38]. It is important to note that donor age may only be a surrogate measure for recipient age, with kidneys from older donors often being allocated to older recipients. Nevertheless, the increase in the aforementioned risk factors may have important implications for fracture risk in future recipients.

Unfortunately, none of the risk factors for major

fractures found in this study are easily modifiable. However, a hospitalized fall in the year prior to transplant was a significant risk factor for other fractures; falls are potentially modifiable through the use of fall prevention programs^[39-41]. This is an important finding given the commonality of falls in kidney transplant recipients with over 10% of women recipients aged ≥ 50 years sustaining a fall with hospitalization in the first 3-years after transplant^[4]. The paucity of modifiable risk factors is concerning as one of the best ways to prevent fractures in the general population is to provide therapy (e.g., bisphosphonates); the efficacy of these therapies in kidney transplant recipients is unclear^[42]. However, given that not many recipients sustained a fracture the lack of modifiable risk factors may be less of a concern.

Limitations of the study are noted. First, we were unable to assess drug use (e.g., glucocorticoids) as a potential risk factor for fracture; drug information in our databases was only available for a minority of kidney transplant recipients; therefore, our sample size would have been decreased, limiting statistical power. It is important to note that a previous study found that kidney transplant recipients who received early corticosteroid withdrawal had a 1.6% reduction in fracture compared to recipients who received standard corticosteroid based immunosuppression^[43]. Future studies should explore this further, including measuring glucocorticoid use as a continuous variable and assessing the impact of reduced dose on fracture risk, with a consideration given to the impact this may have on long-term immunological outcomes (e.g., graft loss)^[44]. Second, we were unable to assess several risk factors, such as body mass index and estimated glomerular filtration rate, due to a high proportion of missingness ($> 50\%$). Third, the small number of fracture events may have limited statistical power and increased concerns about the validity of the model. However, we selected a liberal *P*-value in our backward elimination analysis to ensure we were not excluding potentially important variables. Additionally, it is unlikely there were type I errors given there were at least 10 events per variable^[45]. Finally, due to the small number of fracture events we were also not able to assess several of the other risk factors included in the FRAX algorithm (e.g., rheumatoid arthritis). Last, the generalizability of these results to other races/ethnic groups may be limited as the majority (72%) of our sample was White.

Both general and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in this unique patient population; however, risk factors may be variable across fracture locations. Future studies with larger sample sizes should assess the ability of other transplant-specific risk factors to predict fracture.

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COMMENTS

Background

Compared to the general population kidney transplant recipients are at an increased risk of fracture due to a multitude of factors, including: Chronic kidney disease-mineral and bone disorder and glucocorticoid administration post-transplant. However, risk factors for fracture are not well-established in the kidney transplant population. An understanding of risk factors for fracture is important to target high risk recipients for treatment and therapeutic trials.

Research frontiers

To determine the general and transplant-specific risk factors for fractures in kidney transplant recipients.

Innovations and breakthroughs

General and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in kidney transplant recipients. However, different risk factors may need to be taken into account when considering different fracture locations. Unfortunately, none of the risk factors for major fractures found in this study are easily modifiable. However, a hospitalized fall in the year prior to transplant was a significant risk factor for other fractures; falls are potentially modifiable through the use of fall prevention programs. This is an important finding given the commonality of falls in kidney transplant recipients.

Applications

The risk factors for fracture identified in this study are useful for clinical prognostication.

Terminology

Major fractures were defined as a composite of hip, forearm, proximal humerus, and clinical vertebral fractures. Other fracture locations were defined as a composite of lower leg (ankle, tibia, fibula, patella), femoral shaft, rib/sternum/trunk, scapula, clavicle, and pelvis fractures.

Peer-review

This is a well written study on an important topic in transplantation as the risk of

bone fractures after transplantation. The statistical analysis is well conducted.

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Retrospective Study

Long term outcomes of cardiac transplant for immunoglobulin light chain amyloidosis: The Mayo Clinic experience

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Abstract

AIM: To determine the outcome of orthotopic heart transplantation (OHT) in immunoglobulin light chain (AL) amyloidosis.

METHODS: The medical records of patients with AL who underwent orthotopic heart transplantation at the Mayo Clinic in Rochester Minnesota from 1992 to 2011 were reviewed. Patients met at least one of the following at: New York Heart Association class IV heart failure, ventricular thickness > 15 mm, ejection fraction < 40%. Selection guidelines for heart transplant included age < 60 years, absence of multiple myeloma and significant extra-cardiac organ involvement. Baseline characteristics including age, gender, organ

involvement, and New York Heart Association functional class were recorded. Laboratory data, waiting time until heart transplant, and type of treatment of the underlying plasma cell disorder were recorded. Survival from the time of OHT was calculated using Kaplan-Meier survival curves. Survival of patients undergoing OHT for AL was compared to that of non-amyloid patients undergoing OHT during the same time period.

RESULTS: Twenty-three patients (median age 53 years) with AL received OHT. There were no deaths in the immediate perioperative period. Twenty patients have died post OHT. For the entire cohort, the median overall survival was 3.5 years (95%CI: 1.2, 8.2 years). The 1-year survival post OHT was 77%, the 2-year survival 65%, and the 5-year survival 43%. The 5-year survival for non-amyloid patients undergoing OHT during the same era was 85%. Progressive amyloidosis contributed to death in twelve patients. Of those without evidence of progressive amyloidosis, the cause of death included complications of autologous hematopoietic stem cell transplantation for 3 patients, post-transplant lymphoproliferative disorder for 2 patients; and for the remaining one death was related to each of the following causes: acute rejection; cardiac vasculopathy; metastatic melanoma; myelodysplastic syndrome; and unknown. Eight patients had rejection at a median of 1.8 mo post OHT (range 0.4 to 4.9 mo); only one patient died of rejection. Median survival of seven patients who achieved a complete hematologic response to either chemotherapy or autologous hematopoietic stem cell transplantation was 10.8 years.

CONCLUSION: Our data demonstrate that long term survival after heart transplant is feasible in AL patients with limited extra-cardiac involvement who achieve complete hematologic response.

Key words: Heart transplantation; Autologous stem cell transplantation; Amyloidosis; Chemotherapy; Heart failure

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Core tip: Heart failure due to immunoglobulin light chain (AL) amyloidosis is a devastating disease with poor prognosis. Orthotopic heart transplantation (OHT) is controversial. Twenty-three patients with AL amyloid underwent OHT at our institution over a twenty-year period. Median survival was 3.5 years following OHT. Median survival of seven patients who achieved a complete hematologic response to treatment for AL was almost 11 years. This study demonstrates that long term survival after heart transplant is feasible in AL patients with limited extra-cardiac involvement who achieve complete hematologic response.

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INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is a systemic plasma cell disorder, characterized by the production of a kappa or lambda monoclonal light chain by a clonal population of bone marrow plasma cells^[1]. The monoclonal light chain misfolds into an insoluble beta-pleated sheet conformation. The aberrant protein subsequently accumulates in tissues, leading to organ dysfunction.

Cardiac involvement occurs in 50% of patients with systemic AL amyloidosis and is the most important risk factor for adverse prognosis and death^[2,3]. Amyloid fibrils infiltrate the extracellular space of the valves, atria and ventricles, as well as the perivascular spaces, resulting in biventricular wall thickening without ventricular dilation^[4]. As a result, atrial pressure increases, and atrial dilation occurs despite amyloid infiltration. Clinical features of cardiac amyloidosis include restrictive physiology, heart failure, dysrhythmias, and sudden cardiac death^[1,4,5]. In addition, there is evidence suggesting that the immunoglobulin free light chains are toxic to the myocardium^[6].

Patients with advanced heart failure due to AL have an extremely poor prognosis and often do not survive long enough to benefit from therapy for amyloidosis. Orthotopic heart transplantation (OHT) in AL has been limited due to the risk of disease progression in other organs and recurrence of amyloid deposition in the transplanted heart^[7,8]. Although earlier studies suggested inferior outcomes of OHT for compared with non-amyloid indications^[8,9], more recent reports have found survival similar to other forms of heart failure^[10]. Controversy regarding the role of OHT in AL remains^[11,12] and many centers consider amyloidosis to be a contraindication to OHT. The purpose of the current study was to determine the long term outcome and predictors of survival in a large single-center cohort of patients undergoing OHT for AL.

MATERIALS AND METHODS

Study population

Patients were identified from our institutional database of cardiac transplantation recipients. The diagnosis of amyloidosis was confirmed by demonstrating Congo red positivity in tissue samples. AL amyloid was confirmed by laser dissection mass spectrometry in all but two patients, who had typing performed by immunohistochemistry. During the period from May 31, 1992 to December 31, 2011, 3117 patients with

AL amyloidosis were seen at the Mayo Clinic. Twenty-one percent (668 patients) had overt congestive heart failure. One hundred and thirty-seven were referred for OHT evaluation, and 77 patients completed their evaluation for OHT. Of those completing the evaluation, 33 were deemed ineligible for OHT. Reasons for ineligibility included extensive amyloid in 29 and one instance of each of the following: Coexisting myeloma; coexisting lymphoma; improving cardiac status due to chemotherapy; and lack of financial approval. Forty-four patients (7% of patients with overt heart failure) completed evaluation and were listed for OHT, but only 23 were transplanted. Twenty-one were removed from the listing for the following reasons: Death ($n = 12$); further medical decline ($n = 5$); patient refusal ($n = 2$); myeloma ($n = 1$); and transplant elsewhere ($n = 1$). The median time to de-listing was 48 d (interquartile range 14, 111 d; range 0-341 d).

Throughout the 20-year period, all patients met at least one of the following at time of listing: New York Heart Association class IV heart failure, ventricular thickness > 15 mm, ejection fraction $< 40\%$. In 1998, additional selection guidelines were added: Age < 60 years; combination of the urine light chain, serum monoclonal protein and bone marrow plasmacytosis that does not infer the presence of multiple myeloma or related disorders including low bone marrow plasma cell labeling index; absence of renal involvement as defined by a 24-h urine total protein excretion of < 500 mg and creatinine clearance > 50 mL/min per square meter unless combined renal transplant planned; absence of liver involvement - if elevation of alkaline phosphatase was thought to be due to heart failure, liver biopsy was to be done to exclude interstitial amyloid deposits. The presence of vascular deposits in a biopsy of the rectum, fat or viscera was not an exclusionary criterion. Assignment of organ involvement was according to the consensus criteria from the 10th International Symposium on Amyloid and Amyloidosis^[13]. The modified body mass index (mBMI) was calculated as BMI multiplied by serum albumin level in gram per litre. For most patients the values used for listing and pre-operative BMI (and mBMI) were the same given the proximity of listing to OHT.

The autologous hematopoietic stem cell transplantation (AHSCT) protocol is as previously described, and 11 of the patients have been previously reported^[14]. Demographic, clinical and laboratory data were collected from the Mayo Clinic Transplant Center database, the Robert A Kyle Dysproteinemia database, and all medical records were reviewed. Because most of these patients were treated before era of the serum immunoglobulin free light chain assay, the ability to assign a hematologic response was limited. The determination of hematologic response was a hybrid of the two consensus guidelines. If patients had serum immunoglobulin free light chains measured ($n = 9$), then the 2012 consensus response criteria were applied^[15]; otherwise, the 10th consensus response criteria from the International Symposium

on Amyloid and Amyloidosis were applied^[13]. Two patients had measurable M-spikes, 8 had positive immunofixation of the serum or urine that could be followed, and 4 either had none of the aforementioned detected (or testing not performed prior to starting chemotherapy).

Immunosuppression

Post OHT, all patients received standard therapy for immunosuppression, according to our institutional protocol at the time of transplant. The first twenty-one patients received OKT3, cyclosporine, prednisone, and azathioprine or mycophenate mofetil. A gradual taper of cyclosporine was done over the first year to baseline immunosuppression. Surveillance endomyocardial biopsies to monitor for rejection were used to help guide prednisone taper. The last two patients received prednisone, mycophenate mofetil, and tacrolimus.

Statistical analysis

Medical records for the patients undergoing OHT for AL amyloidosis were reviewed. Survival from the time of OHT was calculated using Kaplan-Meier survival curves. Comparison of survival curves was done with the log-rank test. Baseline variables were tested for their impact on overall survival using Cox proportional modeling. The database was closed to follow up as of March 18, 2015. All statistics were calculated using JMP 10.0.0 (SAS, Carey, North Carolina).

RESULTS

Twenty-three patients with AL amyloidosis underwent OHT (Table 1). Fifty-two percent were female ($n = 12$), and all but two were Caucasian. Twenty-one patients had isolated cardiac involvement at baseline clinical evaluation; one patient (OHT#14) had mild peripheral nerve and gastrointestinal involvement, and one (OHT #15) had peripheral nerve involvement. Twenty-two patients had had a clonal lambda plasma cell disorder; one had a kappa clone. Three patients had renal transplantation, one simultaneous with the OHT and the others at 23 and 53 mo post OHT.

Twenty patients have died post OHT (Table 1 and Figure 1A). The baseline disease burden is outlined in Table 2. For the entire cohort, the median overall survival was 3.5 years (95%CI: 1.2, 8.2 years). The 1-year survival post OHT was 77%, the 2-year survival 65%, and the 5-year survival 43% (Figure 1A). Progressive amyloidosis contributed to death in twelve patients. Of those without evidence of progressive amyloidosis, the cause of death included post-AHSCT complications for 3 patients, post-transplant lymphoproliferative disorder for 2 patients; and for the remaining there was one death related to each of the following causes: Acute rejection; cardiac vasculopathy; metastatic melanoma; myelodysplastic syndrome; and unknown. Eight patients had rejection at a median of 1.8 mo post OHT (range 0.4 to 4.9 mo); only one patient died of rejection).

Table 1 Demographics and orthotopic heart transplantation outcomes

AL-OHT	M/F	Age at OHT	List to OHT (d)	Year OHT	PO FU (yr)	Major outcomes
8	F	52	62	1997	16.5	Alive, doing well
22	F	53	1160	2011	3.9	Alive, PTLD in remission, VGPR on bortezomib
23	M	58	13	2011	3.1	Alive, doing well
1	M	45	86	1992	14.1	Died, progressive amyloid; renal transplantation 53 mo post OHT
17	F	51	94	2003	10.8	Died, renal failure, debility; hematologic relapse and renal amyloid
9	M	56	16	1998	8.6	Died, metastatic melanoma
2	M	44	126	1993	8.4	Died, PTLD, sepsis, progressive amyloidosis
12	F	57	44	1999	8.2	Died, cardiogenic shock secondary to cardiac amyloid, required dialysis for renal amyloid post ASHCT#2
3	M	56	14	1994	7.5	Died, progressive amyloid
14	M	33	30	1999	6.3	Died, cardiac allograft vasculopathy
16	F	53	33	2000	5.4	Died, progressive GI amyloid and stroke; renal transplant 23 mo post-op
7	M	61	415	1997	3.5	Died, progressive amyloid autonomic and peripheral neuropathy
18	M	56	33	2004	3.1	Died, complications of myelodysplastic syndrome
5	F	47	68	1995	2.6	Died, PTLD, progressive multifocal leukoencephalopathy
10	F	54	5	1998	2.2	Died, progressive amyloid
19	M	62	18	2005	2.1	Died, progressive amyloid peripheral neuropathy and GI involvement, recurrent pneumonia
4	M	49	86	1994	1.2	Died, progressive amyloid
21	F	51	29	2007	1.2	Died, progressive GI amyloid, right heart failure, renal failure, steroid myopathy vs amyloid neuropathy
13	F	51	103	1999	0.9	Died, sepsis, multiorgan failure after AHSCT
11	F	48	31	1999	0.7	Died, disseminated fungal infection after AHSCT
15	F	60	33	1999	0.6	Died, progressive amyloid peripheral and autonomic neuropathy
20	F	52	33	2006	0.6	Died, progressive amyloid and overwhelming infection
6	M	56	99	1996	0.04	Died, refractory rejection; had combined renal and cardiac transplant
Median (IQR)		53 (33, 62)	33 (29, 94)			

The first three rows are alive. M: Male; F: Female; PO FU: Post-operative follow-up; PTLD: Post-transplant lymphoproliferative disorder; GI: Gastrointestinal; VGPR: Very good partial response; IQR: Interquartile range.

Table 2 Baseline disease burden

AL-OHT	IFE positive	g/dL	dFLC (mg/L)	Tx mBMI, kg g/L m ²	Creatinine (mg/ dL)	Alkaline phosphatase (U/L)	IVS (mm)	EF (%)	BM PC (%)
8	Yes			688	0.9	115	15	33	12
22	Yes	1.6	298	977	0.8	60	14	55	18
23	NA	0	1059	951	1.5	150	15	50	10
1	Yes			1120	1.4	112	16	40	8
17	Yes	0	70	913	0.9	302	16	64	12
9	Yes	0	271	822	1.7	334	12	78	8
2	Yes			1191	1.1	130	15	60	5
12	Yes	0		742	1.2	312	17	30	13
3	NA			729	1.2		18	56	20
14	Yes			620	0.9	207	16	20	12
16	No	0		402	1.1	145	12	53	15
7	No				1.2	132	15	57	6
18	Yes	0	245	935	1.2	90	18	56	17
5	No			599	0.9	428	22	40	4
10	Yes	0		629	0.7	110	16	44	5
19	Yes	0	304	744	1.1	90	16	24	12
4	NA			693	1.1		14	20	NA
21	Yes	1.9	138	632	0.9	70	13	43	13
13	Yes	0.14		779	1.0	312	16	40	5
11	Yes	0	279	513	0.7	156	21	30	12
15	Yes			788	1.2	118	17	50	9
20	Yes	0	87	865	1.5	112	14	40	7
6	Yes			894	1.1	229	13	35	19
Median (IQR)			27 (11-30)	761 (631, 919)	1.1 (0.9, 1.2)	132 (111, 267)	15 (14, 16)	40 (34, 56)	12 (7, 13)

The first three rows are alive. IFE: Immunofixation of serum and/or urine; dFLC: Difference between involved and uninvolved immunoglobulin free light chains; Tx: Transplant; mBMI: Modified body mass index [albumin × weight/(height)²]; IVS: Interventricular septum; EF: Ejection fraction; BM PC: Bone marrow plasma cells; NA: Not available; IQR: Interquartile range.

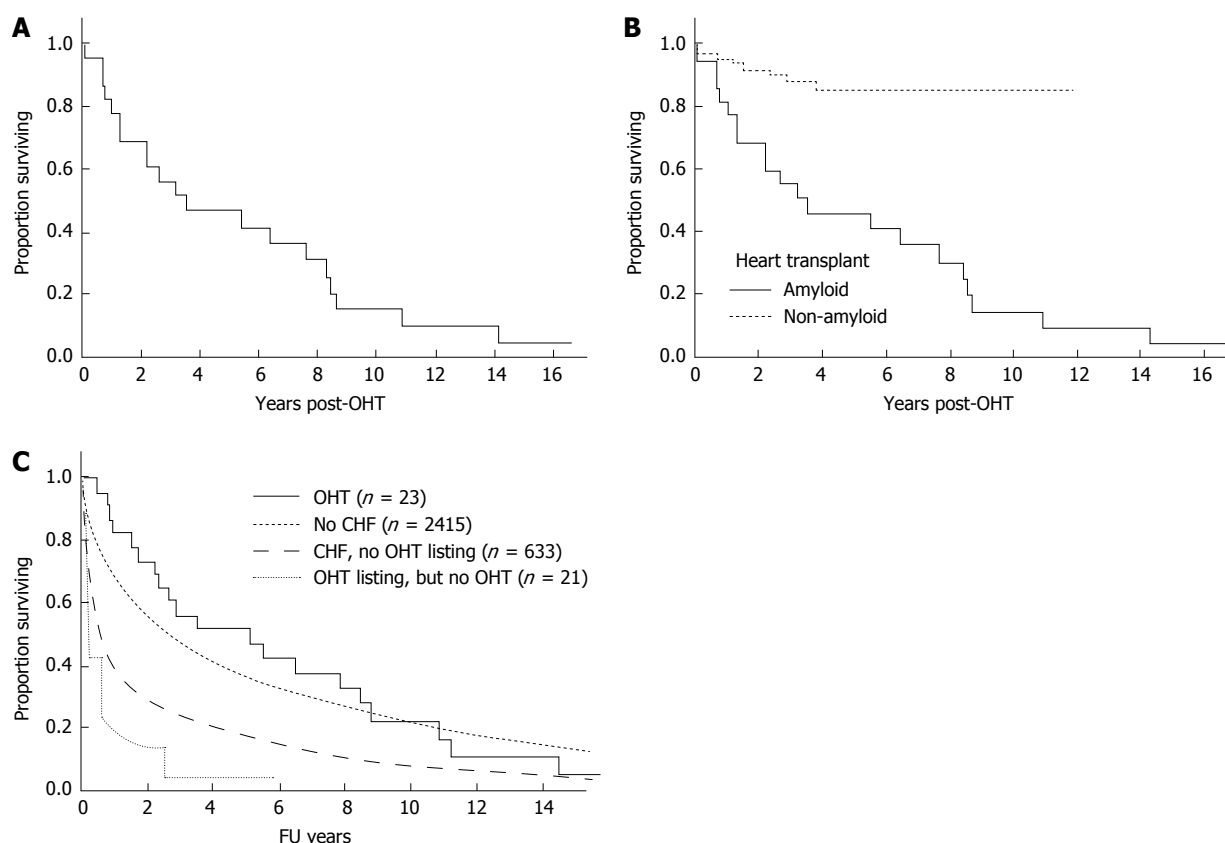


Figure 1 Overall survival. A: Overall survival from orthotopic heart transplant; B: Overall survival comparing OHT for AL amyloidosis to OHT from 1992 to 2011 for non-amyloid indications; C: Comparison of survival with non OHT subgroups. OHT: Orthotopic heart transplantation; CHF: Congestive heart failure; AL: Immunoglobulin light chain.

Figure 1B shows a comparison of this cohort to patients undergoing isolated OHT at our center for non-amyloid indications, where 1 year overall survival is $94.8\% \pm 2.1\%$ and 5-year survival $85.2\% \pm 4.4\%$. Given the small sample size, it is difficult to assess baseline factors that might predict for early death. Notably, no patient died within the immediate OHT peri-operative period. On univariate analysis none of the following factors were significant risk factors for poor overall survival: Age, gender, BMI or mBMI (at listing or at transplant), time from listing to OHT, serum creatinine, or bone marrow plasmacytosis.

Figure 1C shows the comparison of patients with AL who underwent OHT with AL patients without and with overt heart failure who did not undergo OHT, and with those who were listed but did undergo OHT.

Three patients had no therapy (chemotherapy or AHSCT) for amyloidosis and only four received treatment prior to OHT (Table 3). Reasons for no chemotherapy/AHSCT were: Inability to harvest stem cells for planned AHSCT; rejection two weeks after OHT; and death 7 mo after OHT. One patient received chemotherapy only prior to OHT, 4 received chemotherapy pre- and post-OHT, and 15 only post-OHT. The non-AHSCT first line therapies are shown in Table 3. Therapies beyond first line therapies included bortezomib, AHSCT, melphalan with corticosteroids, or an IMiD with dexamethasone; one patient received doxorubicin.

After their first line therapy, seven patients achieved a complete hematologic response (CR), 3 a very good partial response, 2 a partial response, and 11 remained immunofixation positive or were not assessed before death (Table 3). As shown in Figure 2A, patients achieving a CR fared much better than those who did not, achieving a median survival of 10.8 years.

Thirteen patients underwent AHSCT, performed at a median of 8 mo (range 3-24 mo) post OHT with one patient having a second AHSCT 82 mo post OHT. In two patients AHSCT was planned but could not be performed due to inability to harvest stem cells. The median survival of those undergoing AHSCT was 6.3 years (95%CI: 1.2, 8.6 years). Figure 2B demonstrates survival outcomes of those who received AHSCT vs those who did not. Among the patients who underwent AHSCT, 8 received full dose melphalan conditioning (200 mg/m^2), and 5 received attenuated doses. Two of the eight patients receiving full dose melphalan conditioning died within three years post AHSCT, and one is alive 33 mo at last follow-up. In contrast 3 of the 5 receiving attenuated melphalan conditioning died within 3-year post AHSCT. For the 2 who died within 100 d of AHSCT, the cause of death was disseminated fungal infection in one and sepsis leading to multi-organ failure in the other. Four of the AHSCT patients achieved a CR, 5 a very good partial response (PR) or PR, and 4 no significant response or not assessable.

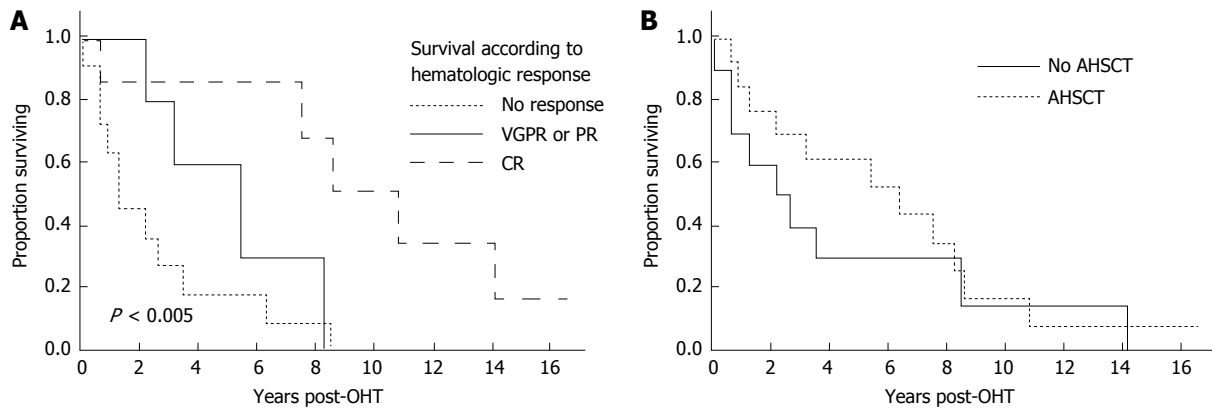


Figure 2 Influence of chemotherapy on overall survival. A: Overall survival (OS) based on hematologic response. The median OS for the 11 non-responders/non-evaluable patients was 1.2 years. The median OS for the 5 patients achieved VGPR or PR was 5.4 years. The median OS for the 7 patients achieving CR was 10.8 years; B: OS based on whether or not patient received AHSCT. The median OS for the 10 patients not undergoing AHSCT was 2.4 years; the median OS for the 13 patients undergoing AHSCT was 6.3 years. VGPR: Very good partial response; CR: Complete hematologic response; AHSCT: Autologous hematopoietic stem cell transplantation.

Table 3 Chemotherapy and response

AL-OHT	Rx relative to OHT	First amyloid directed therapy (Rx)	Response to 1 st Rx	Lines of Rx
8	Only Rx post-OHT	AHSCT ¹	CR	1
22	Rx pre- and post-OHT	Mel-Dex	CR	≥ 2
23	Rx pre- and post-OHT	Bortezomib-Dex ³	VGPR	≥ 2
1	Only Rx post-OHT	Mel-Pred	CR	≥ 2
17	Only Rx post-OHT	AHSCT ¹	CR	≥ 2
9	Only Rx post-OHT	AHSCT ¹	CR	≥ 2
2	Only Rx pre-OHT	Mel-Pred	IFE positive	1
12	Only Rx post-OHT	AHSCT ²	PR	≥ 2
3	Only Rx post-OHT	AHSCT ¹	CR	1
14	Only Rx post-OHT	AHSCT ²	IFE positive	1
16	Only Rx post-OHT	AHSCT ¹	VGPR	≥ 2
7	Rx pre- and post-OHT	VBMCP	IFE positive	≥ 2
18	Only Rx post-OHT	AHSCT ¹	VGPR	1
5	Only Rx post-OHT	Mel-Pred	NA	1
10	No treatment	None	NA	0
19	Only Rx post-OHT	AHSCT ²	PR	1
4	Rx pre- and post-OHT	Mel-Pred	IFE positive	≥ 2
21	Only Rx post-OHT	AHSCT ²	No response	≥ 2
13	Only Rx post-OHT	AHSCT ¹	IFE positive	1
11	Only Rx post-OHT	AHSCT ²	IFE positive	1
15	No treatment	None	NA	0
20	Only Rx post-OHT	Dex	CR	1
6	No treatment	None	NA	0

¹Melphalan conditioning 200 mg/m²; ²Melphalan conditioning 140 mg/m² in all but OHT #12 who got 150 mg/m²;

³Patient had AHSCT as second line and received Melphalan conditioning 200 mg/m². Amyloid directed therapy: AHSCT: Autologous hematopoietic stem cell transplant; Mel: Oral melphalan; Pred: Prednisone; Bortez: Bortezomib; Dex: Dexamethasone; VBMCP: Vincristine, BCNU, melphalan, cytoxan, prednisone; NA: Not available; CR: Complete hematologic response; PR: Partial response; VGPR: Very good partial response; IFE: Immunofixation.

DISCUSSION

Given the limited supply of donor hearts, OHT in AL amyloidosis remains controversial due to the risk of recurrent amyloidosis in the graft or progression of other organ involvement. This long term follow-up study reports the largest single center experience of OHT in AL. Our results support the use of OHT in AL amyloidosis patients with predominant cardiac involvement and no evidence of myeloma, especially if they have achieved (or are able to achieve) a complete hematologic response.

Although the median overall survival of our cohort was only 3.5 years, those patients who achieved a complete hematologic response had a remarkably good overall median survival of more than 10 years.

Superior survival is reported in patients with AL amyloidosis and cardiac involvement who undergo OHT compared to patients who do not^[14,16]. In a report of 14 patients from the United Kingdom, median overall survival was 7.5 years from OHT; in 8 patients who underwent AHSCT and OHT, survival was increased to 9.7 years^[17]. These data are confounded by selection

Table 4 Orthotopic heart transplantation in patients with amyloidosis

Ref.	n	AHSCT	Outcomes
Current series	23	13	1-yr OS 77% 5-yr OS 43%
MGH ^[10]	18	14	5-yr OS 60%
United Kingdom 2004 ^[19]	17 ¹	3	1-yr 59% 5-yr approximately 37%
United Kingdom 2010 ^[17]	14 ¹	8	1-yr OS 86% 5-yr OS 45%
Spanish registry ^[28]	13	3	1-yr OS 43% 5-yr OS 36%
German group ^[29]	12	5	1-yr OS 83% 3-yr OS 83%
ISHLT Registry ^[8,30]	10 ²	None	1-yr 88% 4-yr 38%
Maurer ^[16]	10	8	1-yr 90%
Stanford ^[20]	9	5	1-yr 100%
French registry ^[31]	8	3	1-yr 89%

¹Unclear how much overlap between these two groups. Intervals for Dubrey series was 1982-2002 and for Sattianayagam series, interval was 1984-2004, but there was no reference of which patients had been previously reported; ²At least 8 were AL; unclear what other 2 were. ISHLT: International Society for Heart Transplant; OS: Overall survival; AHSCT: Autologous hematopoietic stem cell transplantation.

biases, but the fact remains that 30%-40% of patients with AL amyloidosis die within the first 6 mo of their diagnosis due to cardiac causes^[18] making consideration of aggressive strategies imperative.

Most series of heart transplantation in AL have reasonable 1-year survival rates (Table 4). In our study there was no perioperative mortality. The major causes of death in our and other series are infection and progressive amyloidosis. If performed without chemotherapeutic support, 5-year survival is just 20%^[19]. Improved survival rates have been seen in patients who undergo AHSCT, with 1- and 5-year survival of 82% and 65% respectively in our earlier report^[14]. Recent reports suggest improved short term outcomes with advances in chemotherapy and AHSCT, with the Stanford series reporting 1 year survival of 100%^[20].

In the MGH series of 18 AL patients undergoing OHT approximately 60% of patients were alive at 6 years^[10], and, in contrast to earlier studies^[8,9,21], was similar to that of non-amyloid patients. Although overall survival in our study was reduced compared with patients transplanted for non-amyloid indications, our series includes many early era patients who did not receive the benefit of current therapy for amyloidosis. Nevertheless, the long term survival of the patients in our series who achieved complete hematologic response was remarkably good.

Selecting patients with primarily cardiac involvement in AL is challenging. Subclinical extra-cardiac organ involvement may progress post heart transplant to clinically important disease. Perivascular intestinal amyloid is common and not viewed as a barrier to cardiac transplantation. However, in our experience, patients with significant mucosal intestinal involvement

do poorly and are often not able to tolerate aggressive treatment for AL. Clearly not all patients with cardiac involvement will require heart transplant; there are patients with significant cardiac involvement who can have cardiac improvement with effective chemotherapy alone^[22-25]. Perhaps cardiac biomarkers like ST-2 may lend insight to those with irreparable damage despite effective chemotherapy^[26].

"Better selection" also means choosing those patients in whom the underlying plasma cell clone can be controlled, since in our study and others effective chemotherapy has resulted in the best outcomes post-OHT^[10,16,20]. Most of the patients in our series did not receive chemotherapy prior to OHT because the only chemotherapies available at the time of their diagnosis were oral melphalan and prednisone and high dose melphalan with AHSCT. Newer treatment options^[27], especially bortezomib containing regimens, are less myelosuppressive, making pre-OHT therapy a possibility. Furthermore, the improvement in chemotherapeutic regimens makes hematologic response more likely in the current era.

Achieving a hematologic response pre-OHT is not a simple matter. Time is of the essence in these patients. In our experience and others, approximately 40% of AL patients listed do not undergo OHT either due to death or deterioration^[10]. This seems to be related to both delayed diagnosis, as well as inability to support these patients with traditional heart failure therapy and devices. In the MGH series, patients with amyloidosis had a mortality hazard ratio of 4.7 (95%CI: 2.8, 11.8) as compared to non-amyloidosis patients while on the waiting list^[10]. The only predictive factor of survival to OHT in that study was BMI - patients with lower BMI fared better than those with higher BMI, although this was not confirmed in our study.

The number of AL amyloid patients transplanted at our institution in recent years has declined. This reduction is multifactorial, and reflects patients receiving earlier and more effective bone marrow directed treatment, more rigorous selection, the availability of OHT for AL at other medical centers, and our own reluctance to offer OHT after some discouraging outcomes. However, the excellent long term survival in this study of patients achieving CR, coupled with markedly improved short term survival recently reported^[20] have prompted renewed enthusiasm for OHT in AL in highly selected patients.

We recognize that our study is limited by being a small series of highly selected patients, lacking currently available cardiac biomarkers and modern markers of clonal burden and access to current treatment regimens. Despite these limitations, in carefully selected patients, long term survival can be achieved. Moving forward the challenge will continue to be the selection of the appropriate patients. The patients likely to derive the most benefit are those who: (1) have plasma cells that are responsive to chemotherapy; (2) have clinically significant involvement of the heart only; and (3) are

not demonstrating significant cardiac response despite effective chemotherapy. The current lack of effective short term cardiac support and the rapidly progressive nature of AL cardiac amyloidosis warrant consideration of revised guidelines for organ allocation in these patients.

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COMMENTS

Background

Cardiac involvement is present in approximately 50% of patients with immunoglobulin light chain (AL) amyloidosis and is associated with a dismal prognosis. Heart transplant for AL amyloid is controversial, due to concerns about amyloid deposition in the transplanted heart and the potential for increased morbidity and mortality from the underlying plasma cell disorder.

Research frontiers

The research goal was to review a single center experience with cardiac transplantation for AL amyloid and determine outcome.

Innovations and breakthroughs

This study demonstrates that long term survival is possible in highly selected patients with AL amyloid who undergo cardiac transplantation if the underlying plasma cell disorder can be controlled.

Applications

Patients with cardiac AL amyloid and limited extra cardiac involvement may be considered for cardiac transplantation. Long term survival is possible in those who achieve a complete hematologic response to chemotherapy or autologous stem cell transplantation.

Terminology

Immunoglobulin light chain AL is a plasma cell disorder which results in deposition of amyloid fibrils in the organs and tissues of the body. Autologous hematopoietic stem cell transplantation is a strategy to treat the underlying plasma cell disorder that causes AL amyloidosis.

Peer-review

The authors presented a good overview of patients with AL amyloidosis + advanced heart failure who received cardiac transplantation.

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Retrospective Study

Ventilator associated pneumonia following liver transplantation: Etiology, risk factors and outcome

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Abstract

AIM: To determine the incidence, etiology, risk factors and outcome of ventilator-associated pneumonia (VAP) in patients undergoing orthotopic liver transplantation (OLT).

METHODS: This retrospective study considered 242 patients undergoing deceased donor OLT. VAP was diagnosed according to clinical and microbiological criteria.

RESULTS: VAP occurred in 18 (7.4%) patients, with an incidence of 10 per 1000 d of mechanical ventilation (MV). Isolated bacterial etiologic agents were mainly *Enterobacteriaceae* (79%). Univariate logistic analysis showed that model for end-stage liver disease (MELD) score, pre-operative hospitalization, treatment with terlipressin, Child-Turcotte-Pugh score, days of MV and red cell transfusion were risk factors for VAP. Multivariate

analysis, considering significant risk factors in univariate analysis, demonstrated that pneumonia was strongly associated with terlipressin usage, pre-operative hospitalization, days of MV and red cell transfusion. Mortality rate was 22% in the VAP group *vs* 4% in the group without VAP.

CONCLUSION: Our data suggest that VAP is an important cause of nosocomial infection during post-operative period in OLT patients. MELD score was a significant risk factor in univariate analysis. Multiple transfusions, treatment with terlipressin, preoperative hospitalization rather than called to the hospital while at home and days of MV constitute important risk factors for VAP development.

Key words: Liver transplantation; Ventilator associated pneumonia; Perioperative period; Infection

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Core tip: Ventilator associated pneumonia (VAP) is a serious perioperative complication in liver transplant recipients, and its etiology and risk factors are still poorly understood. Therefore, we conducted this retrospective study in a big sample of patients to evaluate the incidence, risk factors, etiological agents and outcome of VAP considering 242 consecutive liver transplant recipients. VAP occurred with an incidence of 10 per 1000 d of mechanical ventilation (MV). Multivariate analysis demonstrated that VAP was strongly associated with terlipressin usage, pre-operative hospitalization, days of MV and red cell transfusion. Mortality rate was 22% in the VAP group *vs* 4% in the group without VAP.

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is the main hospital acquired infection in intensive care unit (ICU) and correlates with increased duration of mechanical ventilation (MV), length of ICU and hospital stay, and healthcare costs^[1]. The reported rates vary significantly depending on the population, the specific ICU, the preventive strategies and the definition^[2].

Liver recipients have high risk for prolonged post-operative MV due to multiple causes: Slow resolution of hepatic encephalopathy, muscle atrophy caused by pre-transplant poor nutrition and postoperative diaphragmatic dysfunction related to upper abdominal

surgery.

The risk of pneumonia may be increased because of the presence of alveolar oedema and pleural effusion, as a consequence of low serum protein concentration, large amount of blood product transfusions, immunosuppression and pre-existing risk factors like cardiac or renal failure.

The reperfusion damage has an important role in delaying extubation, which seems to be caused by the increased tumor necrosis factor (TNF) release from Kupffer cells. TNF leads to a histological damage in liver and lung tissue and could be a cause of alveolar oedema, haemorrhage and leukocyte invasion of the parenchyma.

Our study aimed to determine the incidence, etiology, risk factors and outcome of VAP in patients receiving orthotopic liver transplantation (OLT) from a deceased donor.

MATERIALS AND METHODS

Study design

After institutional review board approval, this retrospective study involved the patients who were admitted to our liver transplantation center from December 2006 to December 2010 and survived for at least 48 h. All patients had a diagnosis of end stage liver disease (ESLD) and underwent deceased donor OLT at the Transplantation Center of St. Orsola-Malpighi Policlinic in Bologna.

ESLD referred to the 4th stage or cirrhosis and was defined as the development of either a first major clinical complication of cirrhosis (variceal bleeding, ascites, jaundice, encephalopathy or spontaneous bacterial peritonitis) or hepatocellular carcinoma (HCC)^[3]. Clinical evaluation of those patients used the model for end-stage liver disease (MELD) score reporting the value of the day of the transplantation.

The exclusion criteria were acute liver failure, simultaneous kidney/liver or liver/heart transplantation.

We analyzed the incidence, etiology, risk factors and impact of VAP on clinical outcome. All patients were evaluated, at the moment of the admission, to confirm the absence of pneumonia. Patients were followed until hospital discharge or death.

Definitions

The suspicion of VAP was based on clinical criteria (new or progressive radiological pulmonary infiltrates plus two or more of the following: Temperature > 38.3 °C or < 36 °C, leukocyte count > 10 × 10⁹/L or < 4 × 10⁹/L and purulent respiratory secretions)^[4] appearing 48-72 h post intubation and initiation of MV.

A microbiologic strategy was then followed for diagnosis: Microbiologic lower respiratory tract samples were obtained with bronchoalveolar lavage (BAL) or endotracheal aspirate.

VAP diagnosis was defined in case of positive results

Table 1 Etiologic agents of ventilator-associated pneumonia

Microorganism	Total (n = 18)
<i>Klebsiella pneumoniae</i>	5/18 (28%)
<i>Escherichia coli</i>	5/18 (28%)
<i>Klebsiella oxytoca</i>	2/18 (11%)
<i>Enterobacter</i> spp.	1/18 (6%)
<i>Citrobacter</i> spp.	1/18 (6%)
<i>Pseudomonas aeruginosa</i>	8/18 (44%)
<i>Staphylococcus aureus</i>	4/18 (22%)
<i>Corynebacterium striatum</i>	4/18 (22%)
<i>Xantomonas</i> spp.	2/18 (11%)
<i>Acinetobacter</i> spp.	2/18 (11%)

The *Enterobacteriaceae* are written in bold. Note that in some patients more than one microorganism was found.

of quantitative culture of specimens from BAL or tracheoaspirate with protected brush (considering a threshold of 1×10^5 cfu/mL in a BAL fluid specimen, and 1×10^6 cfu/mL in an endotracheal aspirate specimen^[5].

Postoperative management

Immunosuppressive induction was achieved by administering 1 g of methylprednisolone at the time of reperfusion; the immunosuppressive regimen consisted of a combination of calcineurin-inhibitor and prednisone.

Postoperative interventions according to the European guidelines since 2002^[6] for VAP prevention consisted of semi-recumbent patient positioning, sedation resolution and use of a weaning protocol, strict hand hygiene, non-invasive ventilation, oral care with chlorhexidine, no ventilatory circuit tube changes unless specifically indicated, appropriately educated and trained staff, cuff pressure control every 24 h, enteral feeding, use of heat moisture exchangers and unit-specific microbiological surveillance.

Data collection

Pre-operative, intra-operative and post-operative data were recorded.

Preoperative data included age, weight, height, body mass index (kg/m²), body surface (m²), etiology of cirrhosis, presence of HCC at pre-operative investigation, MELD score at the transplantation day, Child-Turcotte-Pugh (CTP) score, serum bilirubin (mg/dL), serum creatinine (mg/dL), international normalized ratio, glycated haemoglobin (%), serum urea (mg/dL), serum glucose (mg/dL), serum albumin (g/dL), transjugular intrahepatic portosystemic shunt presence, ongoing therapy with diuretics, and terlipressin (instead of its indications as the clinical and laboratoristic parameters are included in other scores) at the time of transplantation, patient preoperative hospital stay rather than called to the hospital while at home.

Intra-operative data included length of surgery, anhepatic phase duration, number of packed red blood cells (RBC), fresh frozen plasma and platelets transfusions (units), duration of cold ischemia (h),

vasopressors usage in pre and post-reperfusion phase, donor age and gender. Quality of liver allograft was classified on the basis of Donor risk index (DRI)^[7] as low risk (DRI < 1.8) or high risk graft (DRI > 1.8)^[8].

Postoperative data considered: VAP incidence and etiology, duration of MV, time between intubation and VAP clinical manifestation, length of ICU stay and hospital mortality.

Statistical analysis

Statistical analyses were performed with SPSS 16.00. Continuous data are expressed as medians (25-75 interquartile range) while discrete data are represented by numerosity and relative frequencies. Patients were divided into two subgroups on the basis of presence or absence of VAP. Incidence of VAP is reported as episodes per 1000 d of MV. Differences between groups were assessed using χ^2 test or Fisher exact test for categorical variables and student's *t*-test or Mann-Whitney test for continuous variables. Variables which were significantly different between the two groups were individually analyzed with a univariate logistic regression model, considering VAP insurgence the dependent variable. Predictor variables found in univariate analysis were included into a multivariate logistic regression model using the Enter method, considering VAP insurgence the dependent variable. Results are expressed as hazard ratios, and *P* values with 95% CIs.

RESULTS

During the study period from 2006 to 2010, 284 patients underwent OLT at the Transplant Center of St. Orsola-Malpighi Hospital. Forty-two patients were not included in the analysis because they had: Combined liver/kidney or liver/heart transplantation (29 cases), transplantation for acute liver failure (6 cases) and other causes without concomitant cirrhosis (7 cases). The final analysis considered 242 patients with ESLD related to histologically proven liver cirrhosis.

Microbiologically confirmed VAP occurred in 18 (7.4%) patients, with an incidence of 10 episodes per 1000 d of MV, and none of these patients presented any criteria of pneumonia, from the in-hospital admission to the time of transplantation. The 18 patients received a diagnosis of VAP after positive BAL culture, and all of them were extubated within 48 h since pneumonia detection.

Isolated microbes belonged mainly to the group of *Enterobacteriaceae* (79%, 14 patients), including *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter* spp. and *Citrobacter* spp. The remaining bacterial etiologic agents were represented by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) (Table 1).

We observed that 25% of VAP episodes involved more than one microorganism.

Demographic data of the study population and

Table 2 Preoperative, intraoperative and postoperative variables

Variable	Patients in study (n = 242)	VAP-yes (n = 18)	VAP-no (n = 224)	P-value
Age (yr)	56 (19-69)	55 (37-66)	56 (19-69)	0.624
Weight (kg)	72 (39-106)	73 (47-93)	72 (39-106)	0.515
Height (cm)	170 (148-193)	169 (155-182)	170 (148-193)	0.495
BMI (kg/m ²)	25 (16-38)	24 (19-34)	25 (16-38)	0.452
BSA (m ²)	1.9 (1.3-2.3)	1.9 (1.4-2.1)	1.9 (1.3-2.3)	0.505
HCV ⁺	128 (53%)	8 (44%)	120 (54%)	0.455
HBV ⁺	49 (20%)	3 (17%)	46 (21%)	0.486
Alcohol abuse	37 (15%)	3 (17%)	34 (15%)	0.540
HCC	118 (49%)	4 (22%)	114 (51%)	0.019
MELD score	21 (6-48)	23 (14-48)	20 (6-45)	0.032
CTP score	11 (5-15)	11 (9-14)	11 (5-15)	0.060
Bilirubin (mg/dL)	5.9 (0.4-71.1)	8.0 (2.1-71.1)	5.6 (0.4-68.6)	0.054
Creatinine (mg/dL)	1.0 (0.0-5.2)	1.1 (0.5-5.2)	1.0 (0.0-4.9)	0.708
INR	1.6 (0.8-7.6)	1.9 (1.3-3.8)	1.6 (0.8-7.6)	0.020
HbA1c (%)	10.8 (4.5-17)	9.9 (8.1-14.9)	10.9 (4.5-17)	0.085
Urea (mg/dL)	0.3 (0.1-3.1)	0.3 (0.1-2.6)	0.3 (0.1-3.1)	0.554
Serum glucose (mg/dL)	105 (60-358)	102 (63-284)	105 (60-358)	0.369
Albumin (g/dL)	3.5 (2.0-5.3)	3.3 (2.6-4.5)	3.5 (2.0-5.3)	0.189
TIPS presence	15 (6%)	1 (6%)	14 (6%)	0.691
Furosemide therapy	144 (60%)	11 (61%)	133 (59%)	0.885
Canrenoate therapy	112 (46%)	5 (28%)	107 (48%)	0.102
Terlipressin therapy	20 (8.3%)	7 (39%)	13 (5.8%)	< 0.001
Preoperative hospital stay	82 (34%)	11 (61%)	71 (32%)	0.018
Intraoperative and postoperative variables				
Length of surgery (min)	560 (512-650)	570 (490-630)	580 (460-660)	0.067
Anhepatic phase duration (min)	120 (88-138)	118 (85-138)	140 (116-145)	0.067
RBC transfusions (units)	8 (0-65)	16 (6-48)	7 (0-65)	< 0.05
FFP transfusions (units)	9 (0-75)	10 (0-31)	9 (0-35)	0.122
Platelet transfusions (units)	2 (0-4)	2 (1-3)	2 (1-3)	0.587
CIT (h)	7 (6-9)	7 (7-9)	8 (7-9)	0.354
Pre-reperfusion VP infusion	68 (28%)	8 (22%)	60 (26%)	0.530
Post-reperfusion VP bolus	110 (45%)	8 (44%)	102 (45%)	0.520
Post-reperfusion VP infusion	118 (48%)	10 (55%)	108 (48%)	0.510
Duration of ICU stay (d)	5 (3-10)	16 (20-59)	5 (3-8)	< 0.05
Hospital mortality	14 (6%)	4 (22%)	10 (4%)	< 0.05
Median duration of MV (d)	0.42 (0.208-0.417)	1.125 (0.375-11.75)	0.38 (0.208-0.864)	< 0.05
Time between intubation and VAP insurgence (h)	-	72 (48-336)	-	-

Statistical analyses were performed using parametric tests and nonparametric tests (Wilcoxon's rank sum) when normality or variance assumptions were not met. Proportions were compared by Fisher's test. Statistical significance was defined as $P < 0.05$. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; BMI: Body mass index; BSA: Body surface; MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; INR: International normalized ratio; HbA1c: Glycated hemoglobin; TIPS: Trans-jugular intrahepatic porto-systemic shunt; RBC: Packed red blood cells; FFP: Fresh frozen plasma; CIT: Cold ischemia time; VP: Vasopressor; ICU: Intensive care unit; MV: Mechanical ventilation; VAP: Ventilator-associated pneumonia.

Table 3 Donor variables n (%)

Variable	Patients in study (n = 242)	VAP-yes (n = 18)	VAP-no (n = 224)	P-value
Donor age (yr)	56 (14-89)	58 (20-86)	56 (19-80)	0.624
Donor gender (male)	182 (75)	14 (77)	168 (69)	0.624
Donor risk index > 1.8	52 (21)	4 (22)	48 (21)	0.345

VAP: Ventilator-associated pneumonia.

general preoperative, intraoperative and postoperative characteristics are reported in Table 2 and the donor variables in Table 3.

Significant differences in MELD score were observed between the two groups; VAP patients had a mean MELD score of 23 vs 20 of control patients. Treatment with terlipressin was associated with a higher risk of pneumonia (39% of VAP episodes receiving terlipressin vs 5.8% in the control group).

Intraoperative data (Table 2) showed statistically significant differences between the two groups in red cell transfusion (red cell transfusion refers to the large amount of red cell transfusion): A median of 16 units per patient in the VAP group vs 7 in controls. Postoperative data (Table 2) showed that ICU stay of VAP patients was significantly longer (16 d vs 5 d) and was associated with a higher hospital mortality (22% of VAP patients died vs 4% of controls). VAP was

Table 4 Variables associated with ventilator-associated pneumonia in multivariate analysis

Variable	OR	95%CI	P-value
MELD score	0.98	0.8-10	0.670
CTP score	0.79	0.5-1.1	0.27
RBC transfusions (units)	1.1	1.04-1.1	< 0.001
MV (d)	1.10	1.03-1.15	< 0.001
Terlipressin therapy	31.49	4.7-49.2	< 0.001
Preoperative hospital stay (d)	1.8	1-1.9	< 0.05

Statistical significance was defined as $P < 0.05$. MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; RBC: Packed red blood cells; MV: Mechanical ventilation.

documented after a median of 72 h post intubation. Median intubation duration among all studied patients was 0.42 d, patients without VAP required a median of 0.38 (0.208-0.864) d of MV, while VAP patients required a median MV duration of 1.13 (0.375-11.75) d. This interval ran from the first intubation to the extubation or need for reintubation. The time from the second intubation to the extubation/exitus was not considered in the study.

Univariate logistic regression analysis found that MELD score, treatment with terlipressin, CTP score, days of MV, preoperative hospitalization and red cell transfusion were significantly associated with VAP (data not shown).

The multivariate logistic regression model constructed considering the variables which resulted significantly associated with VAP in univariate analysis resulted in a significantly increased risk of VAP for terlipressin use, red cell transfusion, duration of MV and preoperative hospitalization (Table 4).

DISCUSSION

It has been reported that the rate of VAP is usually 1 to 3% per day of intubation and MV and the rates of pneumonia are increased 6- to 21-fold for intubated patients and show a further rise with the duration of MV. It has been estimated that the overall rates are most commonly 10 to 15 cases per 1000 ventilator days for ICU patients, depending on the population studied. The National Nosocomial Infections Surveillance System reports a median occurrence of VAP of 4.6 -5.1 for 1000 ventilator days either in medical or surgical ICUs. Also, rates are generally higher in surgical ICU patients than in medical ICU patients^[9,10].

Data about the incidence of VAP in OLT patients are poor and highly variable, the incidence rates range from 5% to 48% and the rates of the VAP-related mortality from 36% to 53%^[11]. A recent monocentric Italian study was not able to detect increased frequency of VAP in a small population of OLT patients compared to a control group of non-OLT patients admitted to the same surgical ICU^[12]. Another study^[13] on the infections after OLT reported the occurrence of VAP in 17.5% of their

samples.

Our results show a higher incidence of VAP than previous results from similar patients. We have to underline that our patients presented a higher MELD score (mean values 20-23) than those considered in other studies (mean values 14-15), which could reflect worse general preoperative conditions predisposing to infections, although the mortality rate was comparable (22%).

As stated before, MELD score has already been associated with postoperative complications, and this association is concordant with the correlation between MELD score and the seriousness of the post-operative complications^[9].

The early identification of clinical predictors of severe prognosis, *i.e.*, the MELD score, could help to identify patients at major risk and to take appropriate measures, earlier intensive treatment and several strategies including the use of non-invasive ventilation when possible to reduce the rate of VAP^[14,15].

The quality of the liver graft, which has an important role in determining prognosis of transplanted organs, does not seem to play a role in early infectious complications like VAP. In fact, high risk grafts were equally distributed in the two groups, and this result has been corroborated in the literature^[11].

The microorganisms associated with VAP vary widely depending on the characteristics of the patients, the different ICUs and the length of in-hospital stay. Common pathogens include *Enterobacteriaceae*, *P. aeruginosa* and *S. aureus*^[16]. In our series, the microorganisms associated with VAP, after liver transplantation, are not different from those in non-OLT patients in ICU^[17]. The *Enterobacteriaceae* predominated over *P. aeruginosa* and *S. aureus*.

Our study confirmed previous finding that multiple blood transfusions were associated with VAP insurgence. This is because longer duration of significant bleeding during OLT may lead to more alveolo-capillary membrane damage and prolonged postoperative intubation.

Our study shows that patients receiving terlipressin for hepatorenal syndrome had an odds ratio of 31.49 times higher for VAP, in the multivariate analysis. Further studies may investigate if hepatorenal syndrome (HRS) or its treatment with terlipressin is the effective risk factor for VAP. That is a limitation of the current study. Terlipressin therapy for HRS requires hospital admission and this could influence the outcome, but it has a notorious detrimental effect on splanchnic microcirculation. We suppose that the vasoconstricting action could damage intestinal barrier and foster bacterial migration through haematic and lymphatic circulation to pulmonary parenchyma, and this mechanism could also explain the high incidence of *Enterobacteriaceae* among etiologic agents in our case series. Westphal *et al*^[17] showed in an animal study that terlipressin treatment induced important alterations in pulmonary circulation, decreased cardiac index, and

diminished systemic oxygen delivery and consumption.

Despite the mentioned results, this study presents some limitations. We reported a low number of pneumonia cases due to its globally low incidence and the limited sample size, since our data came from a single center.

In conclusion, this study was designed to investigate the incidence, the risk factors and the outcome of VAP after OLT. Incidence has been estimated to be 10 per 1000 d of MV. Our study confirms some of the risk factors for VAP found in other studies: RBC transfusion, duration of MV and preoperative hospitalization rather than direct admission from home. MELD score is higher in the VAP group and it represents a significant risk factor in univariate analysis, reflects worse general conditions and prospects higher postoperative complications. The adoption of MELD score could rationalize VAP prevention practice in patients at major risk, earlier intensive treatment to increase the ventilator-free days and several strategies including the use of non-invasive ventilation. Among the risk factors, we found the therapy with terlipressin, used for the treatment of hepatorenal syndrome. This drug exhibited, in animal models, some effects on pulmonary circulation and has a detrimental effect on splanchnic blood flow that could contribute to bacterial migration. Also hepatorenal syndrome could have contributed to this effect. Further studies are needed to clarify this correlation.

COMMENTS

Background

Patients undergoing orthotopic liver transplant (OLT) represent a special subpopulation at risk for nosocomial infections, in particular ventilator-associated pneumonia (VAP) is the main hospital acquired infection in intensive care unit and it is a serious perioperative complication in liver transplant recipients.

Research frontiers

VAP's etiology and risk factors are still poorly understood.

Innovations and breakthroughs

The authors conducted this retrospective study in a big (considering the peculiar population) sample of patients, 242 consecutive liver transplant recipients. Of course, none of the patients who developed VAP presented signs or symptoms of infection before liver transplantation. Model for end-stage liver disease score was a significant risk factor in univariate analysis, and probably it reflects worse general conditions. In multivariate analysis the authors found a statistically significant association with terlipressin therapy. Patients who were taking terlipressin received the last dose until the OLT to treat the hepatorenal syndrome that could be a risk factor by itself. The authors did not refer to clinical and laboratory parameters as they are included in other scores. Some patients received other vasopressors during the OLT, but there were no statistically significant differences between the two groups. As the authors remarked in the discussion, further studies are needed to clarify this finding.

Applications

The application of the authors' results aims to individualize patients at major risk, to apply earlier intensive treatment and several strategies to prevent VAP.

Peer-review

The authors performed a study on a very important infectious complication in

post-operative OLT setting. The paper is well written and the aim is clear.

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Retrospective Study

Liver transplantation for hepatocellular carcinoma in Ireland: Pre-operative alpha-fetoprotein predicts tumour recurrence in a 14-year single-centre national experience

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Abstract

AIM: To examine the results of orthotopic liver transplantation (OLT) for hepatocellular carcinoma (HCC) in Ireland over a 14-year period.

METHODS: Cases of HCC receiving OLT between January 1995 and September 2009 in the Irish Liver Transplant Unit were reviewed from a prospectively maintained database. Outcome measures included overall and recurrence free survival, alpha-fetoprotein (AFP) and tumour pathological features.

RESULTS: On explant pathology, 57 patients had HCC. The median follow-up time was 42.7 mo. The overall 1, 3 and 5 years survival was 87.7%, 72.1% and 72.4%. There was no difference in survival when compared

to patients undergoing OLT without malignancy. The tumour recurrence rate was 14%. The Milan criteria were exceeded in 32% of cases but this did not predict overall survival or recurrence. On multivariate analysis pre-operative AFP > 100 ng/mL was an independent risk factor for recurrence (RR = 5.2, CI: 1.1-24.3, $P = 0.036$).

CONCLUSION: Patients undergoing OLT for HCC had excellent survival even when conventional listing criteria were exceeded. Pre-operative AFP predicts recurrence independent of tumour size and its role in selection criteria should be investigated in larger studies.

Key words: Liver transplantation; Alpha-fetoprotein; Hepatocellular carcinoma; Transplantation selection criteria; Liver cirrhosis

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Core tip: We have shown good survival from a medium volume transplant centre in a small cohort of patients exceeding Milan criteria. We show an association between a pre-operative alpha-fetoprotein (AFP) > 100 and hepatocellular carcinoma (HCC) recurrence, independent of tumour size. Our study supports other single centre experience on survival after transplant for HCC with low AFP and indicates that AFP needs to be interrogated in large, multi-centre studies to see if it can be included in transplant listing criteria to augment the current radiology based dimensional criteria.

O'Connor DB, Burke JP, Hegarty J, McCormick AP, Nolan N, Hoti E, Maguire D, Geoghegan J, Traynor O. Liver transplantation for hepatocellular carcinoma in Ireland: Pre-operative alpha-fetoprotein predicts tumour recurrence in a 14-year single-centre national experience. *World J Transplant* 2016; 6(2): 396-402 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/396.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.396>

INTRODUCTION

Hepatocellular carcinoma (HCC) is the 5th most common cancer and 3rd leading cause of cancer-related death worldwide^[1]. The incidence and related mortality are increasing, particularly in Western countries^[2]. It is now well accepted that the optimal treatment for small HCC in the setting of cirrhosis is orthotopic liver transplantation (OLT)^[3]. Since the publication and adaptation of the Milan criteria^[4] the outcomes have improved dramatically compared to results from the era prior to established selection criteria^[5]. Patients undergoing OLT for HCC within the Milan criteria achieve outcomes comparable to non-malignant transplant cohorts. However, recurrence is the most important cause of post-transplant death^[6]. Appropriate patient selection is crucial as patients with large or biologically

unfavourable tumours have unacceptable recurrence and overall survival rates^[7]. It is essential that centres which provide OLT for HCC audit their results to ensure outcomes compare to international survival rates thus enabling appropriate patient prioritisation and organ allocation.

The aim of the current study was to determine the outcomes of OLT for HCC in a single, national institution over a 14-year period. Overall and recurrence free survival rates were compared to clinical and pathological factors using multivariate analysis to identify independent predictors of recurrence.

MATERIALS AND METHODS

All patients undergoing OLT with HCC proven on explant pathology between January 1995 and September 2009 were included in the study. All OLT in the Republic of Ireland are carried out in the Liver Unit of St Vincent's University Hospital. The Liver Unit maintains a prospective database containing patients' clinical details. Tumour characteristics are recorded on a computerised pathology database. A retrospective review of this data was performed. Patient characteristics recorded included age at OLT, sex, aetiology of underlying liver disease, pre-operative alpha-fetoprotein (AFP), survival, and recurrence status. Tumour data recorded included size and number of tumours, compliance with the Milan and University of California San Francisco (UCSF) criteria and microvascular invasion. The study was approved by the St Vincent's University Hospital ethics review board.

Patient selection and transplant protocol

All patients listed were classified clinically as cirrhotic. HCC diagnosis was based on a combination of ultrasound, computed tomography (CT) and double-contrast magnetic resonance imaging (MRI). After 1996 patients were listed for OLT if they met Milan criteria on pre-operative imaging. Patients in Ireland listed for OLT for HCC receive an adjusted Model for End-Stage Liver Disease (MELD) score^[8]. All OLT were from deceased donor transplants and organs were retrieved before cardiac death. Patients were followed up at a dedicated transplant clinic every three months. In general, post-transplant immunosuppression consisted of a reducing dose of corticosteroids and a calcineurin inhibitor (tacrolimus) or azathioprine. Follow up included annual abdominal ultrasound and CT where appropriate.

Survival and recurrence

Overall patient survival was determined from date of OLT until the most recently attended clinic. HCC recurrence free survival was determined by the date of the most recently available radiological imaging. Deaths from recurrence were prospectively recorded in the database. Patients without recurrence that died were documented free of recurrence only if the most recent available imaging or post-mortem report excluded recurrence. Two investigators (O'Connor DB

Table 1 Patient demographics

Male:female	44:13
Age, median (IQR)	59.1 (53.5-63.6)
Aetiology of HCC	
Alcoholic liver disease	17 (29.8%)
Hepatitis C	17 (29.8%)
Haemochromatosis	13 (22.8%)
α -1-antitrypsin deficiency	3 (5.3%)
Primary sclerosing cholangitis	2 (3.5%)
Primary biliary cirrhosis	2 (3.5%)
Autoimmune hepatitis	2 (3.5%)
Hepatitis B	2 (3.5%)
Cryptogenic	2 (3.5%)
Cystic fibrosis	1 (1.8%)
Sarcoidosis	1 (1.8%)
Nash	1 (1.8%)
Pre-operative α -fetoprotein, median (IQR)	8.8 (3.3-29.2)
Compliant with Milan criteria	41 (71.9%)
Compliant with UCSF criteria	49 (86.0%)
Largest lesion, median (IQR)	3 (2.5-4.5)
Cirrhosis	53 (93.0%)
Steatosis	2 (3.5%)
Multifocal lesions	24 (42.1%)
Micro-vascular invasion	24 (42.1%)
Tumour differentiation	
Well	24 (42.1%)
Moderate	28 (49.1%)
Poor	5 (8.8%)
Incidental lesions	5 (8.8%)

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco; IQR: Interquartile range.

and Cooney A) independently reviewed the database to ensure accuracy of the survival and recurrence data. Patients were censored in September 2009 to ensure a minimum of 5-year follow-up.

Tumour characteristics

All explants were examined by a histopathologist experienced in HCC pathology (Nolan N). Tumour size, number of lesions, presence of macro or microvascular invasion, and condition of the non-tumour bearing liver were recorded. Tumours were graded as well, moderate or poorly differentiated. Compliance with Milan or UCSF criteria was based on size and number of lesions and was determined by explant pathology rather than pre-operative imaging.

Statistical analysis

Patients were divided into groups based on meeting or exceeding listing criteria, presence or absence of vascular invasion, tumour grade, and pre-operative AFP levels to determine impact on overall survival and recurrence free survival. Data is presented as median (interquartile range). Factors affecting survival were determined by a Cox Proportional Hazard Model and significant factors were incorporated into a multivariate analysis. Kaplan-Meier analysis and the log-rank test were used to illustrate differences between recurrence free and overall survival according to clinical factors. Comparisons between the HCC and control cohort were

made using Fisher's Exact test. All calculations were done using SPSS version 12.0 (SPSS, Inc., Chicago, IL). $P < 0.050$ was set as the threshold for statistical significance.

RESULTS

During the 14-year study period 57 patients underwent OLT for HCC confirmed on explant pathology. One patient received OLT in 1995 and 56 patients were transplanted between 1998 and 2009. This represented 11.3% of the 504 patients undergoing OLT in the Liver Unit during that time. HCC was diagnosed radiologically in 52 cases pre-operatively and 5 cases were incidental findings in cirrhotic patients. HCC was absent on explant pathology in 4 additional patients transplanted for presumed HCC, representing false positives who were excluded from the analysis. Pre-operative AFP, tumour histopathology and clinical follow up data were available for all 57 patients. Median follow up was 42.7 (14.6-67.6) mo.

The median age at OLT was 59 years. The most common underlying causes of cirrhosis were alcoholic liver disease (30%), hepatitis C (30%) and Haemochromatosis (23%). The Milan criteria were exceeded in 16 (28%) and 8 patients (14%) exceeded UCSF criteria. Median largest tumour size was 3 (2.5-4.5) cm. Micro-vascular invasion was present in 24 (42%) tumours. The mean time to OLT following diagnosis was 3 mo. Bridging therapy was not routinely used. Only 4 patients underwent trans-arterial chemo-embolization and this was not included in statistical analysis. Patient and tumour characteristics are outlined in Table 1.

Survival

Overall survival at 1, 3 and 5 years was 87.7% (50/57), 72.1% (31/43) and 72.4% (21/29) respectively. The HCC transplant group were compared to a cohort of 313 patients undergoing OLT between 1998 and 2008 who underwent their primary, non-emergent, transplant during that period. There was no statistical difference between the HCC and control cohort in 1 (87.7% vs 89.1%, $P = 0.450$), 3 (72.1% vs 84.2%, $P = 0.050$) and 5 years (72.4% vs 80.9%, $P = 0.211$) overall survival rates. No clinical or pathological variable significantly affected overall survival in those undergoing OLT for HCC (Table 2). Overall survival was not affected by patients exceeding the Milan (Figure 1A) or UCSF (Figure 1B).

Recurrence

Recurrence free survival was 86%, 69.7% and 69.5% at 1, 3 and 5 years respectively. There were 8 recurrences in total (14%) and 5 patients died from recurrence. Recurrence occurred within 1 year in 3 patients, within 2 years in 3 and beyond 3 and 5 years in one patient each. The location of recurrent disease was hepatic in 3 (including 2 patients with additional

Table 2 Univariate analysis of factors affecting overall survival

	HR	CI	P-value
Male sex	0.786	0.301-2.055	0.623
Age	1.001	0.954-1.051	0.952
Aetiology of HCC			
Alcoholic liver disease	0.523	0.175-1.567	0.247
Hepatitis C	2.098	0.849-5.183	0.108
Haemochromatosis	0.715	0.239-2.143	0.549
Other	1.198	0.459-3.126	0.712
Pre-operative α -fetoprotein > 100 ng/mL	1.502	0.437-5.165	0.519
Compliant with Milan criteria	0.994	0.381-2.590	0.989
Compliant with UCSF criteria	0.871	0.290-2.618	0.805
Largest lesion	1.207	0.963-1.513	0.102
Cirrhosis	23.309	0.024-224.813	0.369
Steatosis	0.044	0.000-187.285	0.465
Multi-focal lesions	1.201	0.499-2.890	0.683
Micro-vascular invasion	1.489	0.619-3.578	0.374
Tumour differentiation			
Well	0.862	0.349-2.131	0.748
Moderate	1.100	0.448-2.698	0.835
Poor	1.159	0.268-5.022	0.843
Incidental lesions	0.450	0.060-3.391	0.438

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco.

extra-hepatic metastases), porta-hepatis lymph nodes in 2, and in one patient multiple recurrence occurred in lung, omentum and sacrum. Hepatic recurrences were diagnosed on CT and extra hepatic disease was confirmed by biopsy. Recurrence free survival was similar between patients meeting or exceeding the Milan (Figure 1C) and the UCSF criteria (Figure 1D). Underlying liver disease, tumour size or vascular invasion did not affect recurrence free survival. On univariate analysis only poorly differentiated tumours and AFP levels > 100 ng/mL were associated with reduced disease free survival (Table 3) and a shorter time to recurrence (Figure 2). On multivariate analysis, pre-operative AFP > 100 ng/mL remained an independent predictor of recurrence free survival (HR = 5.2, $P = 0.036$).

Patients exceeding Milan and UCSF criteria

Eight patients exceeded both Milan and UCSF criteria. Five were alive at 5 years and one patient with recurrence was alive after 3 years follow-up. Recurrence only occurred in 2 cases. One patient died from recurrence after 14 mo and one died from a separate malignancy at 2 years. Micro-vascular invasion was present in 4 cases. AFP exceeded 100 ng/mL in the patient who died from recurrence.

DISCUSSION

The current study confirms that OLT for HCC is an effective treatment modality and that survival rates are comparable to those undergoing OLT for non-malignant disease. Patients exceeding the Milan or UCSF criteria were not at increased risk of reduced overall survival or increased recurrence. Pre-operative serum AFP is an

Table 3 Univariate analysis of factors affecting recurrence free survival

	HR	CI	P-value
Male sex	0.681	0.156-2.971	0.609
Age	1.004	0.932-1.081	0.922
Aetiology of HCC			
Alcoholic liver disease	0.775	0.155-3.887	0.757
Hepatitis C	3.272	0.798-13.417	0.100
Haemochromatosis	1.210	0.243-6.024	0.816
Other	0.027	0.000-16.500	0.271
Pre-operative α -fetoprotein > 100 ng/mL	6.668	1.661-26.768	0.007
Compliant with Milan criteria	1.354	0.271-6.761	0.712
Compliant with UCSF criteria	0.739	0.148-3.692	0.712
Largest lesion	1.326	0.976-1.801	0.071
Cirrhosis	23.025	0.000-327.873	0.604
Steatosis	0.045	0.000-546.731	0.664
Multifocal lesions	2.100	0.494-8.930	0.315
Micro-vascular invasion	1.560	0.376-6.463	0.540
Tumour differentiation			
Well	0.249	0.046-1.340	0.105
Moderate	1.553	0.374-6.443	0.544
Poor	5.631	1.074-29.510	0.041
Incidental lesions	0.041	0.000-720.752	0.523

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco.

independent risk factor for recurrence.

The landmark Milan publication in 1996 established listing criteria based on a single HCC of less than 5 cm or up to 3 tumours, each less than 3 cm^[4]. This was validated in other single-centre studies and together demonstrated a 5-year survival of 70% and recurrence rates of less than 15% which became the gold standard outcome in OLT for HCC^[9-11]. These criteria continue to be used in Ireland and many centres worldwide. Our institution is a medium volume centre performing approximately 60-70 OLT per year. The outcomes of our patient cohort compare favourably to recently published series' from higher volume centres^[12,13]. Our patient demographic is different to most centres as in over 50% of patients the underlying liver pathology was alcoholic liver disease or haemochromatosis. Worldwide, the main causes of HCC are hepatitis B and C virus but in this study they only accounted for 33% of HCC^[14]. However aetiology of HCC did not significantly impact on overall or disease free survival.

The majority of patients with HCC present with disease beyond the Milan criteria^[15]. Acceptable 5-year survival and recurrence rates observed in a subgroup of patients with larger tumours led to the publication of the UCSF criteria which proposes listing patients with a single tumour up to 6.5 cm or up to 3 lesions, none larger than 4.5 cm and total tumour burden not exceeding 8 cm^[16]. This has been reproduced in single-centre studies with short follow up but never in multi-centre or nationwide population studies but in recent years several units have called for an extension of the criteria. Patients beyond the Milan criteria did not experience inferior survival in our centre but our numbers are too small to support calls for extension of the criteria based simply

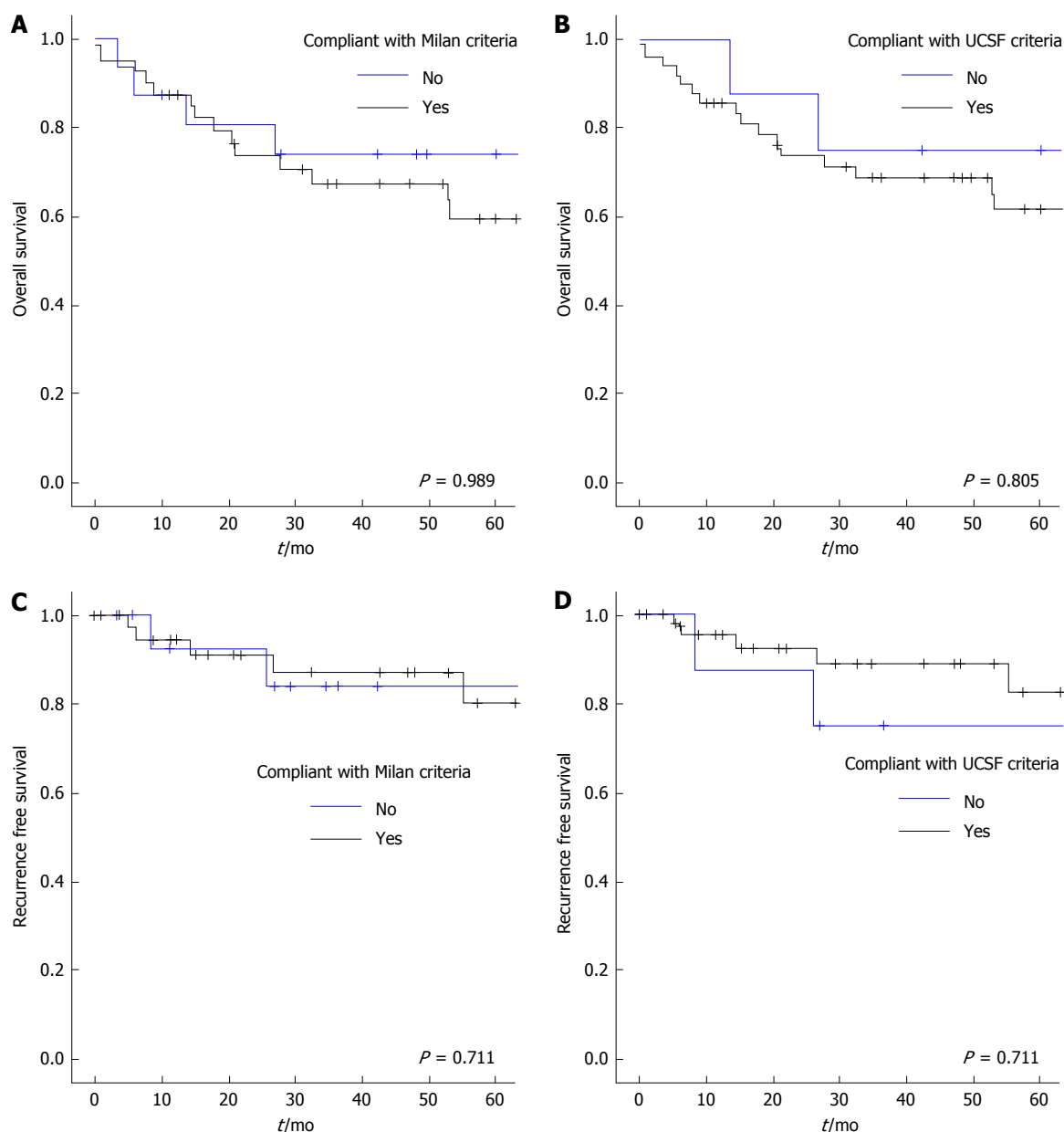


Figure 1 Kaplan-Meier estimates of overall survival (A and B) and recurrence free survival (C and D) in relation to compliance with the Milan and University of California San Francisco criteria. UCSF: University of California San Francisco.

on size and number of tumours. The limitations of pre-operative imaging for staging in the setting of cirrhosis also impede raising the threshold. One large study showed pre-operative imaging to under stage over 40% of patients^[17]. In the current study almost 30% were not compliant with Milan criteria on explant pathology. Interval tumour growth is a possible explanation for patients who meet criteria on imaging and then exceed them on pathology. However our cohort experienced a short waiting period of 3 mo and relatively small tumours (median 3 cm) which makes tumour doubling unlikely. Even with advances such as double-contrast MRI, extending the criteria based solely on size risks transplanting patients with tumours too large to benefit.

The limitation of established criteria is that they are based on tumour dimensions. While results from single

centre studies have justified its use for organ allocation, in a North American population study, a subgroup of patients with larger tumours within the Milan criteria had significantly poorer survival outcomes than those without HCC^[18]. It is imperative that any selection criteria be accurate in predicting prognosis to justify the large proportion of transplants undertaken for HCC in the setting of a shortage of organs. For example 25% of all United States OLT have been for HCC since the introduction of priority MELD scores for HCC in 2002^[18] and 11% of OLT in Ireland are for patients with HCC.

There is growing evidence that the biological behaviour of the tumour rather than size dictates recurrence. Patients with larger tumours beyond the Milan criteria but without micro-vascular invasion can have excellent survival, such as outlined in the "up-to-seven-criteria",

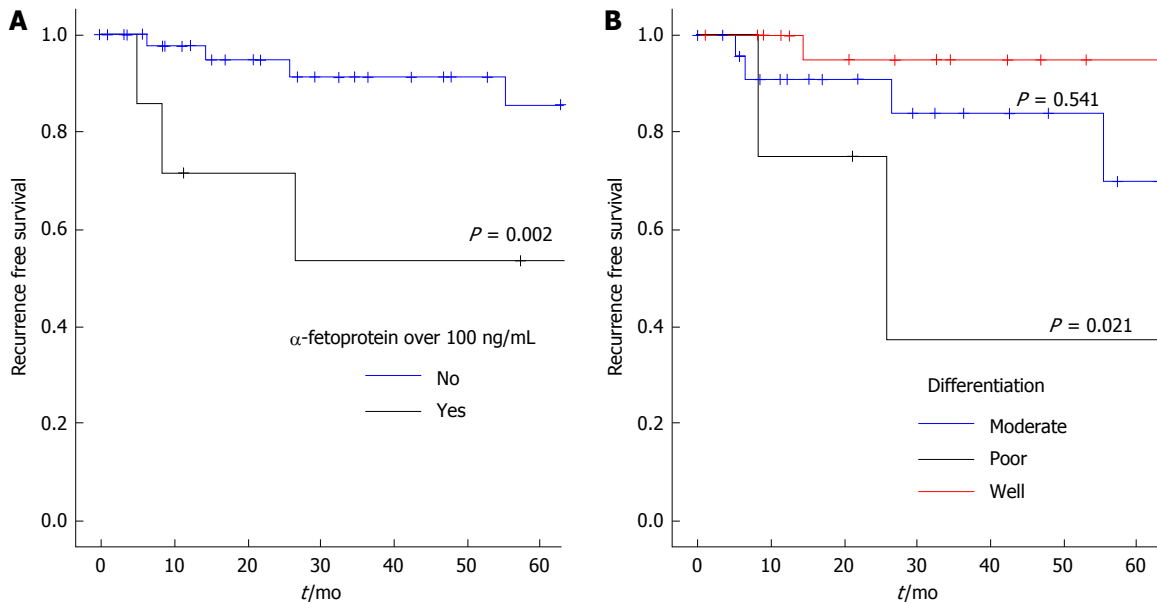


Figure 2 Kaplan-Meier estimates of recurrence free survival in relation to pre-operative α -fetoprotein (A) and tumour differentiation (B).

but this cannot be diagnosed pre-operatively^[19]. The impact of micro-vascular invasion was not found to be statistically significant in our cohort but in large studies it has been shown to double the risk of death^[7]. Pre-operative AFP may be the best available surrogate marker for micro-vascular invasion and the biological aggressiveness of the tumour. Several studies have identified a high pre-operative AFP as a risk factor for recurrence and reduced survival^[18,20-22]. We have shown AFP predicted reduced disease free survival, independent of both tumour size and micro-vascular invasion. Furthermore, patients exceeding Milan or even UCSF criteria experienced excellent overall and recurrence free survival with a pre-operative AFP < 100 ng/mL. This supports the finding of another group where an AFP level < 30 ng/mL predicted disease free survival in patients beyond Milan criteria^[23]. Both studies are limited by the small number of patients exceeding Milan criteria. Recent large studies have not examined the impact of AFP in the context of tumours beyond the Milan criteria. The largest study reporting survival in patients with tumours exceeding the Milan criteria (1112 patients) unfortunately did not examine the impact of AFP level^[7]. Analysis from the United Network for Organ Sharing on 2253 patients demonstrated a significant survival advantage in patients with low pre-transplant AFP (< 20 ng/mL) but this effect wasn't explored in patients with tumours outside Milan criteria^[24]. It would therefore be intriguing if AFP could be examined in a large population database or multicentre study to determine if patients with large tumours but low pre-operative AFP had higher survival rates. Only then can AFP be used to augment existing eligibility criteria to safely expand the pool of patients suitable for OLT.

In conclusion, in appropriately selected patients with HCC undergoing OLT, survival was comparable to non-HCC patients. A subgroup of patients with larger

tumours and low AFP may benefit from OLT but this association should be examined in larger, multicentre studies.

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COMMENTS

Background

Orthotopic liver transplantation (OLT) is the most effective treatment for hepatocellular carcinoma (HCC) in the setting of cirrhosis. Survival in well selected patients with a small burden of tumour is similar to patients undergoing OLT for non-cancer related indications.

Research frontiers

Existing selection criteria are based on the size and number of the tumour. Several datasets have demonstrated good survival outcomes in patients exceeding these criteria. The biological characteristics, for example micro-vascular invasion may just as important as the tumour dimensions. However these cannot be reliably detected pre-operatively.

Innovations and breakthroughs

This study also demonstrates that patients with larger tumours can still have good survival outcomes. Pre-operative alpha-fetoprotein (AFP) predicted tumour recurrence. AFP may be a useful surrogate marker for less favourable biological characteristics of the tumour.

Applications

The prognostic value of AFP could be evaluated in large, multi-centre datasets to determine its potential as an adjunct to existing selection criteria.

Terminology

OLT: Orthotopic liver transplant involves fully explanting the diseased liver immediately prior to the transplant; HCC: Hepatocellular carcinoma is the most common primary liver tumour. Because most cases occur in the setting of cirrhosis, it is often not amenable to resection; AFP: Alpha-fetoprotein has no known function in adults but it has clinical significance as a tumour marker in

the diagnosis of HCC.

Peer-review

This is an interesting attempt to evaluate the results of liver transplantation for HCC with regard to potential relation with pre-operative values of AFP. The paper is well written and results are clarified.

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Retrospective Study

Higher plasma bilirubin predicts veno-occlusive disease in early childhood undergoing hematopoietic stem cell transplantation with cyclosporine

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Informed consent statement: The authors petition BPG for waiver of informed consent because this study was not a clinical trial, but was retrospectively done using anonymized electronic medical records of the study subjects. In addition, authors declared that the study subjects were at no risk due to this study.

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Abstract

AIM: To analyze the association between plasma bilirubin levels and veno-occlusive disease (VOD) in non-adult patients undergoing hematopoietic stem cell transplantation (HSCT) during cyclosporine therapy.

METHODS: A total of 123 patients taking cyclosporine

were evaluated using an electronic medical system at the Seoul National University Children's Hospital from the years 2004 through 2011. Patients were grouped by age and analyzed for incidence and type of adverse drug reactions (ADRs) including VOD.

RESULTS: The HSCT patients were divided into three age groups: G#1 ≥ 18 ; 9 \leq G#2 ≤ 17 ; and G#3 ≤ 8 years of age). The majority of transplant donor types were cord blood transplantations. Most prevalent ADRs represented acute graft-vs-host disease (aGVHD) and VOD. Although the incidences of aGVHD did not vary among the groups, the higher frequency ratios of VOD in G#3 suggested that an age of 8 or younger is a risk factor for developing VOD in HSCT patients. After cyclosporine therapy, the trough plasma concentrations of cyclosporine were lower in G#3 than in G#1, indicative of its increased clearance. Moreover, in G#3 only, a maximal total bilirubin level (BILmax) of ≥ 1.4 mg/dL correlated with VOD incidence after cyclosporine therapy.

CONCLUSION: HSCT patients 8 years of age or younger are more at risk for developing VOD, diagnosed as hyperbilirubinemia, tender hepatomegaly, and ascites/weight gain after cyclosporine therapy, which may be represented by a criterion of plasma BILmax being ≥ 1.4 mg/dL, suggestive of more sensitive VOD indication in this age group.

Key words: Hematopoietic stem cell transplantation; Veno-occlusive disease; Cyclosporine; Adverse drug reaction; Total bilirubin

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Core tip: This study analyzed the association between plasma bilirubin and veno-occlusive disease (VOD) in childhood undergoing hematopoietic stem cell transplantation (HSCT) during cyclosporine therapy. Here, we report that age of 8 or under may be a risk factor for VOD in CsA-treated patients who underwent HSCT with differential clearance of CsA. Another finding is that a criterion of 1.4 mg/dL of plasma maximal total bilirubin level or higher content alone closely represents the incidence of VOD in early childhood patients with HSCT in CsA therapy. Information shown in this study would be of great help to understand VOD occurring during CsA medication and to find optimal pharmacotherapy in HSCT patients.

Kim KS, Moon A, Kang HJ, Shin HY, Choi YH, Kim HS, Kim SG. Higher plasma bilirubin predicts veno-occlusive disease in early childhood undergoing hematopoietic stem cell transplantation with cyclosporine. *World J Transplant* 2016; 6(2): 403-410 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/403.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.403>

INTRODUCTION

Cyclosporine is a major immunosuppressant for organ transplantation, and is widely used for the prophylactic treatment of acute graft-vs-host disease (aGVHD) after hematopoietic stem cell transplantation (HSCT)^[1]. However, the morbidity and mortality resulting from acute, or subsequently following chronic GVHD, and veno-occlusive disease (VOD), as indicated by hyperbilirubinemia, tender hepatomegaly, and ascites, are obstacles to the use of cyclosporine alone or in combination with other agents^[2]. Clinical studies on cyclosporine therapy demonstrated differences between neonate, child and adult populations in the incidence of adverse drug reactions (ADRs)^[3]. Since these events are closely linked to the metabolic burden and/or clearance of the drug, ADRs should be monitored and avoided depending on the types of transplantation, age groups and pharmacokinetic profiles. In particular, the dose regimens and therapeutic concentrations need to be appropriately adjusted for optimal efficacy and/or minimized ADRs.

Cyclosporine therapy should be carefully monitored as a therapeutic drug monitoring system^[4]. Monitoring of pharmacokinetic profiles, including oral bioavailability, has been claimed in the context of successful pharmacotherapy because intestinal absorption of cyclosporine varies depending on the type of transplantation, age, and other parameters of patients^[5-9]. In general, patients of a young age seem to be more at risk for ADRs to cyclosporine, and exhibit different ADR profiles^[10]. Therefore, the oral dose of cyclosporine required for the maintenance of therapeutic blood levels is significantly augmented in childhood patients^[5]. In addition to the narrow therapeutic range of cyclosporine, the types and incidences of cyclosporine-induced ADRs vary depending on the types and severities of diseases, as well as patient age^[11].

It has been recognized that wide variations exist in the plasma concentrations of cyclosporine among HSCT patients^[12]. A limited number of studies have been performed in cyclosporine-treated neonates and children who underwent HSCT in the context of ADR monitoring^[13]. In Seoul National University Hospital, the administered dose of cyclosporine was equally determined by the post-surgical day of HSCT, which frequently resulted in cyclosporine plasma concentrations being out of therapeutic range (150-250 ng/mL). Although the normalized doses of cyclosporine for transplant patients of childhood age were usually higher than those for adults, the plasma concentrations were significantly lower^[3]. This raised the contention that biotransformation and/or excretion of cyclosporine is accelerated in childhood patients, which may be linked to ADRs, such as GVHD, nephrotoxicity, and neurotoxicity^[13].

Age-different effects of cyclosporine therapy on the types and incidences of ADRs in HSCT patients are

Table 1 The characteristics of hematopoietic stem cell transplantation patients treated with cyclosporine (*n* = 123)

Characteristics	G#1 (<i>n</i> = 25)	G#2 (<i>n</i> = 70)	G#3 (<i>n</i> = 28)
Age (mean, SD)	20.3, 1.7	13.0, 2.5	5.8, 2.2
Initial body weight (mean, SD)	51.8, 11.1	37.8, 12.7	14.0, 4.0
Gender (M, %)	15 (60.0)	35 (50.0)	17 (60.7)
Liver function (mean, SD)			
ALT (mg/dL)	67.6, 95.8	67.1, 67.3	104, 133
AST (mg/dL)	70.2, 73.1	72.9, 61.5	161, 356
Donor types, <i>n</i> (%)			
Cord blood	15 (60.0)	41 (58.6)	21 (75.0)
Related donor	10 (40.0)	29 (41.4)	7 (25.0)
Types of disease, <i>n</i> (%)			
AA	5 (20.0)	6 (8.6)	1 (3.6)
ABL	4 (16.0)	5 (7.1)	2 (7.1)
ALL	10 (40.0)	28 (40.0)	7 (25.0)
AML	5 (20.0)	20 (28.6)	10 (35.7)
CML	0 (0.0)	2 (2.9)	0 (0.0)
JMML	0 (0.0)	0 (0.0)	4 (14.3)
MDS	1 (4.0)	2 (2.9)	1 (3.6)
Others	0 (0.0)	7 (10.0)	3 (10.7)
Observed events, <i>n</i> (%)			
aGVHD	13 (61.9)	26 (46.4)	13 (54.2)
cGVHD	1 (4.8)	4 (7.1)	0 (0.0)
VOD	2 (9.5)	13 (23.2)	7 (29.2)
DIC	4 (19.0)	4 (7.2)	2 (8.3)
Relapse	1 (4.8)	2 (3.6)	1 (4.2)
EF	0 (0.0)	7 (12.5)	1 (4.2)

Age groups: G#1, 18 years older; G#2, 9 to 17 years old; G#3, 8 years old or under. AA: Aplastic anemia; ABL: Acute biphenotypic leukemia; ALL: Acute lymphocytic leukemia; AML: Acute myelocytic leukemia; CML: Chronic myelocytic leukemia; JMML: Juvenile myelomonocytic leukemia; MDS: Myelo dysplastic syndromes; aGVHD: Acute graft versus host disease; cGVHD: Chronic graft versus host disease; VOD: Veno-occlusive disease; DIC: Disseminated intravascular coagulation; EF: Engraft failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

known^[14-16]. Nevertheless, more sensitive indicator(s) would be of great help to avoid or minimize serious ADRs and to accomplish successful pharmacotherapy, especially in patients of the childhood population who would be more prone to drug-induced harmful effects. This study analyzed the association between plasma bilirubin levels and VOD in non-adult patients undergoing HSCT during cyclosporine therapy. Here, we report that marginally high levels of total plasma bilirubin reliably indicate VOD during cyclosporine therapy in the HSCT patient of early childhood.

MATERIALS AND METHODS

Datasets

This study was approved by the Institutional Review Board of Seoul National University Hospital (SNUH; H-1112-087-390, 2012.3.17), a 1961-bed medical center, on March 17, 2012. The data collected had anonymous codes representing patient files comprising the following medical information: Age, gender, medical diagnosis codes, date of HSCT, absolute neutrophil count, post-transplantation day, donor types (cord blood and related donor), body weight, body surface area, body temperature, types of ADRs, peak

and trough concentrations of cyclosporine, serum hematocrit, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin levels, dates of labs drawn, medications (generic and brand name, prescription date), and duration of chemotherapeutic agent along with co-prescribed drugs.

Patients

The database contained records of 123 patients (ages ranging from 2 to 24 with 68 males and 55 females) who had been hospitalized in SNUH from September 15, 2004 to December 31, 2012 and had undergone measurements of plasma cyclosporine levels using a radioimmunoassay kit. Cyclosporine concentrations were monitored on day 1, 4, 11, 18, 24 and 28 after HSCT and at intervals of three or seven days after day 28 of HSCT in SNUH. Laboratory data were obtained from 123 patients, with three of them having HSCTs twice in this period. The total number of cyclosporine measurements was 2149, with an average of 17.5 measurements per patient.

HSCT patients who had been administered cyclosporine were divided into three groups based on age: G#1, 18 years of age or older; G#2, between 9 and 17 years of age; and G#3, 8 years of age or under. The median ages in G#1, G#2, and G#3 were 20, 13 and 6, respectively. Each group was additionally split into four subgroups by the levels of a maximal total bilirubin level (BILmax) [*i.e.*, BILmax (-), lower than 1.4 mg/dL of total plasma bilirubin; and BILmax (+), 1.4 mg/dL of total plasma bilirubin or higher] and VOD incidence [*i.e.*, VOD (-), no existing VOD; and VOD (+), existing VOD].

Statistical analysis

The Fisher exact test was chosen to determine differences in the frequency of BILmax \geq 1.4 mg/dL between VOD (-) and VOD (+) groups. Multivariate analysis was performed to find risk factors for drug therapy. Data represent the median (0.5-24.0 mg/dL). Results were considered statistically significant if the *P*-value was less than 0.05. Statistical analyses were conducted using the Duncan and Fisher's tests in SPSS Version 12.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

The characteristics of HSCT patients treated with cyclosporine (*n* = 123) are summarized in Table 1. Of the patients examined, cord blood transplantation constituted the majority of the transplant donor type (G#1, 60.0%; G#2, 58.6%; and G#3, 75.0%). The most prevalent ADR event observed was aGVHD (G#1, 61.9%; G#2, 46.4%; and G#3, 54.2%), whereas the second most frequent ADR event was VOD (9.5%-29.2%). Although the incidences of aGVHD, diagnosed as cytopenia and delayed immune reconstitution, did not vary much between the groups, the frequency ratios of VOD were significantly higher in G#3. Thus, being 8 years of age or under at the time of transplantation

Table 2 Median trough plasma concentrations and doses of cyclosporine in hematopoietic stem cell transplantation patients

Contents		G#1 (n = 25)	G#2 (n = 70)	G#3 (n = 28)
iv	Trough plasma concentration ^b (ng/mL)	535.6 (264.0-1214.0) ^a	448.9 (184.5-1070.0)	333.1 (152.5-819.0)
	Dose (mg/kg)	5.8 (3.9-7.7)	6.1 (3.5-14.2)	6.0 (3.8-9.3)
PO	Trough plasma concentration ^b (ng/mL)	345.9 (166.0-686.5)	247.7 (40.0-496.5)	204.4 (33.0-302.5)
	Dose (mg/kg) ^c	8.2 (3.4-11.4)	8.2 (1.6-17.5)	10.6 (6.0-24.6)

^aThe values in parenthesis represent the minimum and maximum trough plasma concentrations of cyclosporine; ^bG#1 was significantly different from G#2 or G#3 using Duncan test; ^cG#3 was significantly different from G#1 or G#2 using Duncan test. *iv*: Intravenous administration; *PO*: Per Os, which means oral administration.

Table 3 Two by two analyses between maximal plasma bilirubin contents and veno-occlusive disease in hematopoietic stem cell transplantation patients

	G#1 (n = 25)		G#2 (n = 70)		G#3 (n = 28)	
	BILmax (-)	BILmax (+)	BILmax (-)	BILmax (+)	BILmax (-)	BILmax (+)
VOD (+)	0	2	4	9	0	7
VOD (-)	5	18	28	29	17	4
P-value	1.00		0.356		0.0001	

BILmax (+): 1.4 mg/dL or higher; BILmax (-): Lower than 1.4 mg/dL. Data were analyzed using Fisher's test program. BILmax: A maximal total bilirubin level; VOD: Veno-occlusive disease.

would be a possible risk factor for VOD in patients who underwent HSCT from cord blood donors. In types of diseases, acute lymphoblastic leukemia and acute myeloid leukemia highly occurs in all three groups of patients, but there was no significant difference of the disease incidence rate depending on the age. Also the liver functions (*i.e.*, ALT and AST activities) were comparable in all groups of patients.

After intravenous administration, the trough plasma concentrations of cyclosporine were significantly lower (83.8% and 62.2% in G#2 and G#3, respectively, vs G#1) in G#2 or G#3 than in G#1, although the injected dose of cyclosporine was normalized to the patient body weight (Table 2). The trough plasma concentrations of cyclosporine were approximately 40% lower in G#3 than in G#1, indicative of its accelerated clearance in G#3. The trough plasma cyclosporine levels were similarly changed in the groups examined after oral administration; in this case, the oral dose was approximately 30% greater in G#3 than in G#1 (or G#2), suggesting the possibility that the bioavailability of cyclosporine was significantly lower in G#3 (Table 2). These results indicate that the clearance and/or turnover rate of cyclosporine in plasma might be augmented in G#3, whereas the oral bioavailability was lower in this group, implying the potential of increased detoxifying burden in the patients presumably due to accelerated biotransformation and excretion of cyclosporine.

Given the distinct difference in plasma cyclosporine concentrations and the potential of increased cyclosporine clearance in G#3, we next asked whether the incidences of VOD statistically correlated with total bilirubin levels in plasma among the patients examined. Setting the BILmax cutoff level at 2.0 mg/dL demonstrated an obvious increment in VOD incidences in

high BILmax groups when G#2, G#3 or the total population was analyzed, although it failed in demonstrating increased VOD incidences when G#1 was solely analyzed (data not shown). More importantly, setting the BILmax cutoff level at 1.4 mg/dL (a minimal significant value obtained empirically) revealed an augmented incidence of VOD in the high BILmax group in G#3 ($P < 0.0001$), but not in G#1 or G#2, as determined by two-by-two analyses (Table 3).

DISCUSSION

ADR-related admissions are a problem with a high prevalence^[17,18]. Pérez Menéndez-Conde *et al.*^[18] reported that 19.4% of admissions were direct consequences of ADRs, 65% of which were preventable^[19]. In particular, cyclosporine therapy causes various ADRs (*e.g.*, 20% of infectious complications during the therapy and 5% of severe GVHD)^[20,21], with approximately 6% of admissions eliciting permanent damage, including seizures or death^[22]. In general, the dose of cyclosporine is calculated for transplant patients primarily on the basis of body weight^[23]. However, this approach has limitations, such as the development of aGVHD, cGVHD, hepatotoxicity, gastrointestinal disorders, infections and hemorrhagic cystitis^[24]. Large variations in plasma cyclosporine concentrations exist in individuals (*i.e.*, 5-8 fold differences)^[25]. Since the biotransformation capacities of endogenous and exogenous substances vary depending on the stage of development and maturation, attention should be directed to cyclosporine clearance. The results of this study demonstrated the impact of age differences on the incidence and type of ADRs during cyclosporine therapy in HSCT patients of early childhood as compared to adolescent patients.

A major advantage of HSCT is the potential for therapeutic benefits from graft-vs-leukemia effects, which are mediated by donor T and natural killer cells^[26]. Unfortunately, graft-vs-leukemia effects are closely linked to aGVHD as the major limiting toxicity of allogeneic transplantation, which causes damage to the skin, gastrointestinal tract, and liver^[27]. Studies have shown that aGVHD frequently occurred when plasma concentrations of cyclosporine decreased to 125-200 ng/mL 12 h after treatment^[25,28]. Depletion of T cells from the graft effectively prevented aGVHD, but it also limited graft-vs-leukemia effects, possibly increasing the rate of graft failure^[29]. Therefore, plasma concentrations of immunosuppressant are currently one of the critical factors to maintain the proper balance between aGVHD and graft-vs-leukemia effects. The lowest plasma cyclosporine concentration (< 200 ng/mL) in the third week after transplantation showed a high risk factor related to aGVHD (grades II-IV) in HSCT patients^[30]. Thus, assessment of cyclosporine levels is a valuable diagnostic tool to predict aGVHD. In the present study, we observed that the incidences of aGVHD (*i.e.*, cytopenia and delayed immune reconstitution) were not much different among the groups examined, which supports the appropriateness of the pharmacotherapy.

Patients currently meet McDonald's VOD-Seattle Criteria by exhibiting two or more of the following criteria: Hyperbilirubinemia > 2 mg/dL, tender hepatomegaly, and either ascites or weight gain (> 2%). A key finding of this study is that VOD occurrences were significantly higher in G#3. Similarly, the incidences of VOD increased in childhood age^[31], whereas VOD was frequently observed day 18 (the median) after intravenous administration of cyclosporine^[32]. When we compared the plasma levels of cyclosporine and other pharmacokinetic parameters, the turnover rate of cyclosporine seemed to vary in different age groups. Our finding showing lower plasma cyclosporine level with higher occurrence of VOD in G#3 differs from the previous report that high plasma concentrations or high doses of drugs in pediatric HSCT patients related to the frequent and severe VOD in different therapy in HSCT patients^[33]. VOD occurrence seems to be associated with clearance of endogenous compounds as well as cyclosporine^[34]. It has also been suggested that the clearance rate of cyclosporine may affect VOD and total bilirubin levels in blood^[34]. This idea is consistent with the finding that the pharmacokinetic profile of cyclosporine was characterized by substantially faster elimination in children compared to adults, which necessitated more frequent dosing intervals and higher doses for younger children^[7,35]. So, low plasma cyclosporine levels in G#3 may reflect its high turnover rate. Overall, our results and others support the contention that the turnover rate of cyclosporine is increased, particularly in HSCT patients of early childhood.

Our finding that HSCT patients of 8 years of age or under were more at risk for the reactions of VOD, which was distinctively characterized by the plasma BILmax

level being ≥ 1.4 mg/dL, indicates that plasma BILmax alone may serve as a valuable marker of VOD in this particular patient population. Since a large fraction of cyclosporine binds with erythrocytes (41%-50%)^[36], cyclosporine-induced hyperbilirubinemia may result from destabilization and/or disruption of red blood cell membranes, with the consequent release of heme for biodegradation and excretion. It has also been shown that the clearance of red blood cells was slower, whereas the maturity and differentiation of red blood cells were lower in children compared to other groups^[37]. Disruption of canaliculi in children has also been shown to increase, even at lower cyclosporine concentrations^[38]. Therefore, the frequency of splenomegaly increases presumably due to the clearance of damaged red blood cells and debris, along with heme disposal, resulting in the subsequent production of bilirubin^[39]. Consistently, red blood cells may be impaired after cyclosporine therapy, especially during radiation therapy^[40].

Since cyclosporine is mainly oxidized *via* cytochrome P450s 3A4 (CYP3A4), followed by glucuronide conjugation *via* UDP-glucuronosyltransferase 1A1 (UGT1A1) and UGT2B7, total bilirubin levels in the blood would increase, enhancing the burden of detoxification^[41]. Cyclosporine is primarily metabolized by CYP3A4 in the liver, 95% of which is excreted *via* the biliary route. The main reason for the low bioavailability of cyclosporine may be due to its extensive intestinal metabolism by CYPs^[42]. The various rate and extent of cyclosporine metabolism, depending on age and drug interactions (60%-90%), may be related with polymorphisms of CYP3A4^[43]. The genetic associations between UGT variations and cyclosporine pharmacokinetics in patients would also affect its efficacy and ADRs (*e.g.*, GVHD, hepatic and/or gastrointestinal disorders) presumably due to unpredictable cyclosporine concentrations^[44]. Our results showed that plasma cyclosporine levels were significantly lower in G#3 despite the highest normalized dose. Clearance of endogenous bilirubin might also be reduced in the patients presumably due to relatively low rate of metabolism. Thus, cyclosporine biotransformation may change depending on the metabolic clearance of bilirubin, which would increase in early childhood compared to adolescents and/or adults^[45]. Alterations in red blood cell turnover and/or interference of biliary excretion of glucuronidated cyclosporine would also contribute to total plasma bilirubin levels^[46].

The value of pharmacist-provided drug-monitoring care to transplant recipients has been recognized as a beneficial service^[47]. Considering the complexity of pharmacotherapy, pharmacists need to implement clinically relevant interventions on the transplant unit^[48]. Although the dangers of ADRs are well recognized by clinicians and pharmacists, the efforts to elucidate the basis of ADRs still exist in clinical fields^[49]. This situation stimulated attempts to validate ways of ADR monitoring by developing new and critical indicators, algorithms and analytical tools^[50,51]. HSCT patients represent a population at high risk for drug-related problems^[52]. Our

results demonstrate that HSCT patients 8 years of age or under are at higher risk for developing the reactions of VOD after cyclosporine therapy, which may be indicated by plasma BILmax levels being ≥ 1.4 mg/dL, suggesting that this new criterion alone may be used as an indicator of VOD during cyclosporine therapy in HSCT patients of young childhood. A guideline for ADR-related problems and interventions may aid staffs working in the HSCT unit to optimize pharmaceutical care of patients, thereby reducing economic costs resulting from inappropriate drug utilization.

COMMENTS

Background

The incidence of veno-occlusive disease (VOD) differs from the ages of childhood, which is an obstacle of the use of cyclosporine, immunosuppressant for organ transplantation. Especially, the VOD incidence was higher in cyclosporine-treated neonates and children who underwent hematopoietic stem cell transplantation (HSCT). Therefore, the authors analyzed the association between plasma bilirubin levels and VOD in childhood patients undergoing HSCT during cyclosporine therapy.

Research frontiers

The sensitive indicator(s) would be of great help to avoid or minimize serious VOD and to accomplish successful cyclosporine therapy, especially in patients of the childhood population with higher VOD incidence. The results of this study contribute to clarifying the associations of bilirubin, VOD and cyclosporine concentrations.

Innovations and breakthroughs

Although age-different effects of cyclosporine therapy on various adverse drug reactions in HSCT patients are existing, the association between plasma bilirubin levels and VOD in non-adult patients undergoing HSCT during cyclosporine therapy was not reported yet. Thus, the authors report that marginally high levels of total plasma bilirubin reliably indicate VOD during cyclosporine therapy in the HSCT patient of early childhood.

Applications

A plasma BILmax levels being ≥ 1.4 mg/dL may be used as an indicator of VOD during cyclosporine therapy in HSCT patients of young childhood. A guideline for adverse drug reaction-related problems and interventions may aid staffs working in the HSCT unit to optimize pharmaceutical care of patients, thereby reducing economic costs resulting from inappropriate drug utilization.

Terminology

A maximal total bilirubin level (BILmax) (-): Lower than 1.4 mg/dL of total bilirubin during cyclosporine therapy; BILmax (+): 1.4 mg/dL of total plasma bilirubin or higher during cyclosporine therapy.

Peer-review

This review is well written, presenting a very significant issue of "an increased risk for developing VOD after cyclosporine treatments in younger (< 8 years old) generations". Authors also claimed that the plasma BILmax levels being ≥ 1.4 mg/dL would provide a useful indicator to recognize the development of VOD in those generations.

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Retrospective Study

Proposal of new expanded selection criteria using total tumor size and ¹⁸F-fluorodeoxyglucose - positron emission tomography/computed tomography for living donor liver transplantation in patients with hepatocellular carcinoma: The National Cancer Center Korea criteria

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Author contributions: Lee SD designed study and wrote the paper; Lee B and Joo J performed the statistical analysis; Kim SH designed the study and performed research; Kim SK, Kim YK and Park SJ performed the study.

Institutional review board statement: This study was reviewed and approved by the National Cancer Center Institutional Review Board.

Informed consent statement: This is the retrospective study and we analyzed data using only medical records. Therefore, waiver of informed consent for this study subjects might be justifiable. In our institute IRB, waiver of informed consent in this study was approved.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the first author at 1sd@ncc.re.kr. Participant's consent was not obtained but the presented data are anonymized and risk of identification is low.

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Abstract

AIM: To expand the living donor liver transplantation (LT) pool of eligible patients with hepatocellular carcinoma (HCC) using new morphological and biological criteria.

METHODS: Patients with HCC who underwent living donor LT (LDLT) from March 2005 to May 2013 at the National Cancer Center Korea (NCCCK) were enrolled. We performed the ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT)

before LDLT. Overall and disease-free survival analysis was done in patients to evaluate the usefulness of new NCCK criteria using PET/CT and total tumor size (10 cm).

RESULTS: We enrolled a total of 280 patients who pathologically confirmed to have HCC and performed the PET/CT before transplantation. Among them, 164 (58.6%) patients fulfilled the NCCK criteria and 132 patients (47.1%) met the Milan criteria. Five-year overall and disease-free survival rates for patients who fulfilled the NCCK criteria showed 85.2% and 84.0%, respectively, and were significantly higher than those beyond the NCCK criteria (60.2% and 44.4%, respectively; $P < 0.001$). The correlation analysis between preoperative imaging tests and pathologic reports using Cohen's Kappa demonstrated the better results in the NCCK criteria than those in the Milan criteria (0.850 *vs* 0.583). The comparison of disease-free analysis among the NCCK, Milan, and University of California, San Francisco (UCSF) criteria using the receiver operating characteristics curves revealed the similar area under the curve value criteria (NCCK *vs* Milan, $P = 0.484$; NCCK *vs* UCSF, $P = 0.189$ at 5-years).

CONCLUSION: The NCCK criteria using hybrid concept of both morphological and biological parameters showed an excellent agreement between preoperative imaging and pathological results, and favorable survival outcomes. These new criteria might select the optimal patients with HCC waiting LDLT and expand the selection pool.

Key words: Hepatocellular carcinoma; Living donor; Liver transplantation; Selection criteria

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Core tip: National Cancer Center Korea criteria using positron-emission tomography/computed tomography positivity and total tumor size (cutoff 10 cm) expanded the pool of living donor liver transplantation for patients with hepatocellular carcinoma. Patient identification on the bases of the criteria showed an excellent agreement between preoperative imaging and pathological results and favorable survival outcomes.

Lee SD, Lee B, Kim SH, Joo J, Kim SK, Kim YK, Park SJ. Proposal of new expanded selection criteria using total tumor size and ^{18}F -fluorodeoxyglucose - positron emission tomography/computed tomography for living donor liver transplantation in patients with hepatocellular carcinoma: The National Cancer Center Korea criteria. *World J Transplant* 2016; 6(2): 411-422 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/411.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.411>

INTRODUCTION

The application of selection criteria for liver transpl-

antation (LT) in patients with hepatocellular carcinoma (HCC) has changed the HCC treatment algorithm over the past 20 years. The Milan criteria proposed by Mazzaferro *et al*^[1] helped to increase the number of LTs in patients with HCC and demonstrated remarkably good survival outcomes for these patients. In particular, the Milan criteria, which use both tumor size and number are very useful and have been adopted as selection criteria. Based on these criteria, the patients for whom HCC was identified early had the best chance of being cured of cancer following LT. In Asian countries such as South Korea and Japan, the number of deceased donors is limited and living donor LT (LDLT) has become an important option for treatment in patients with HCC^[2,3]. As the amount of experience and evidence on LDLT for HCC has increased in recent years, the selection criteria for LT have gradually been expanded in large-volume centers. Various expanded criteria based on tumor number and size, such as the University of California, San Francisco (UCSF) criteria, have been proposed^[4-9]. Some Japanese centers have demonstrated that preoperative tumor markers such as the des-gamma-carboxy prothrombin (DCP) level and tumor size were associated with higher recurrence rates^[10,11]. These expanded criteria revealed that selected patients who did not fulfill the Milan criteria showed good overall survival (OS) and disease-free survival (DFS) rates compared with those who fulfilled the Milan criteria. Although the Milan criteria always guarantee the best survival rates in patients with HCC, they are too restrictive and use modalities.

In HCC patients, tumor characteristics, including differentiation grade and microvascular invasion, are well-known independent prognostic factors for OS and DFS following LT^[12]. However, these factors cannot be evaluated by preoperative imaging studies, which reveal the morphological characteristics such as number and size. Recently, several studies using ^{18}F -fluorodeoxyglucose positron emission tomography/CT (^{18}F -FDG PET/CT) demonstrated that ^{18}F -FDG PET/CT findings were a powerful prognostic marker in patients with HCC after LT and showed good correlation with pathological tumor characteristics, such as microvascular invasion and differentiation^[13-15].

In the present study, we performed a retrospective analysis to identify prognostic factors in patients with HCC who underwent ^{18}F -FDG PET/CT before LDLT. Based on this result, we developed new and simple expanded criteria [the National Cancer Center, Korea (NCCK) criteria], incorporating a hybrid concept of biological and morphological characteristics on PET/CT images, including total tumor size, and compared these criteria with the Milan criteria, which are based on only morphological evaluation.

MATERIALS AND METHODS

Patients

Patients who underwent LDLT due to HCC at NCCK

Table 1 Multivariable analysis of prognostic factors for overall and disease-free survival

Multivariable analysis		Overall survival			Disease-free survival		
		HR	95%CI	P	HR	95% CI	P
Variables							
AFP	> 400 ng/mL	1.145	0.543-2.418	0.722	1.003	0.556-1.811	0.991
PET/CT	Positive	2.652	1.384-5.085	0.003	2.517	1.481-4.279	0.001
Tumor number	> 3	0.647	0.294-1.425	0.280	0.814	0.425-1.557	0.534
Maximum tumor size	> 5 cm	0.696	0.307-1.580	0.386	1.551	0.836-2.877	0.164
Total tumor size	> 10 cm	2.909	1.230-6.880	0.015	3.003	1.536-5.870	0.001
Differentiation ¹	III-IV	1.206	0.616-2.358	0.585	1.010	0.594-1.717	0.972
Microvascular invasion	Present	1.269	0.522-3.084	0.599	2.148	1.064-4.336	0.033
Capsule formation	Present	0.439	0.166-1.162	0.097	0.737	0.353- 1.542	0.418
Major vessel invasion	Present	2.017	0.829-4.905	0.122	1.712	0.850-3.449	0.132
Ductal invasion	Present	0.907	0.265-3.100	0.876	1.409	0.534-3.720	0.489
Serosal invasion	Present	1.463	0.670-3.195	0.339	1.047	0.553-1.984	0.887
Intrahepatic metastasis	Present	1.471	0.595-3.640	0.404	1.519	0.752-3.070	0.244
Dysplastic nodule	Present	0.744	0.365-1.514	0.414	0.840	0.478-1.479	0.546

¹Edmondson-Steiner Grade. CT: Computed tomography; PET: Positronemission tomography; AFP: α -fetoprotein.

between March 2005 and May 2013 were collected using prospectively collected database. All patients were diagnosed as HCC by pathologic reports, and underwent ¹⁸F-FDG PET/CT to check biologic status of the primary tumor and the presence of metastasis within 1 mo before LDLT. Routine preoperative imaging tools for clinical staging in patients with HCC before LDLT were ultrasonography, multi-detector CT (MDCT), and/or dual contrast-enhanced magnetic resonance imaging (MRI) including PET/CT without protocol tumor biopsy. We reviewed the medical records for clinicopathological data, including age, sex, serum α -fetoprotein (AFP), viral markers, C-reactive protein, Model for End-Stage Liver Disease (MELD) score, PET/CT reports, tumor maximum standardized uptake value (SUVmax), pre-transplant therapies, and pathologic data such as Edmondson and Steiner grade; vessel, serosa, and duct invasion; capsule formation; cirrhosis; intrahepatic metastasis; and dysplastic nodules. Prognostic factors using clinicopathological data were analyzed for their effect on OS and DFS. This study was approved by the institutional review board of NCCK.

Our policy for selecting recipients with HCC for LDLT was basically based on the Milan criteria by preoperative imaging tools such as MDCT, MRI, or PET/CT. However, considering the specificity of living related donation, we performed LDLT on patients without major vascular invasion and extrahepatic metastasis on preoperative imaging tools even though they do not satisfy the Milan criteria. We do not recommend the downstaging or bridging therapy before LDLT even though the patient had advanced HCC. The operative techniques, immunosuppression, and management for hepatitis virus of donor and recipient have been described in detail in previous our reports^[16,17]. Patients were followed up periodically with interval 3 or 6 mo using imaging studies such as ultrasonography, abdomen, and chest MDCT with AFP and DCP level. As the tumor recurrence was suspected by imaging tools and serologic tests, additional PET/CT was performed to evaluate the

recurrent tumor and distant metastasis. For one or two nodules in the liver, lung, bone, or brain, we performed the resections. However, in case of multiple metastases, we treated tumors with a multimodality approach such as radiofrequency ablation, transarterial chemoembolization (TACE), radiation therapy, or chemotherapy.

¹⁸F-FDG PET/CT

Our protocol of ¹⁸F-FDG PET/CT was described in detail previously^[14]. In brief, ¹⁸F-FDG PET/CT was performed using a PET/CT scanner (Biograph LSO; Siemens Medical Systems and Discovery LS; GE Healthcare, New Jersey, United States). The mean period between PET/CT and LDLT was 14.8 d. All PET/CT images were analyzed by experienced nuclear medicine physicians. SUV was calculated as (decay-corrected activity kBq/mL of tissue volume)/(injected FDG activity kBq/body mass gram). SUVs of the lesions were checked by placing a region of interest (ROI) at the site of the maximum FDG uptake in the PET images. The ROI was drawn to encircle the highest activity of each tumor, by the results of the CT scans that were acquired from PET/CT or MRI scans. PET/CT positivity was defined by experienced nuclear medicine physicians by checking whether the SUVmax of the tumor by CT or MRI scans was higher than that in the surrounding noncancerous hepatic tissue. Mean SUVmax of tumors for PET/CT positivity and negativity in this study was 4.46 and 3.08, respectively ($P < 0.001$).

NCCK criteria

In a multivariable analysis of our data, we identified two significant prognostic factors by evaluating pathological examination results (Table 1). These were positive findings on PET/CT (HR = 2.652, 95%CI: 1.384-5.085, $P = 0.003$ for OS; HR = 2.517, 95%CI: 1.481-4.279, $P = 0.001$ for DFS) and total tumor size of > 10 cm (HR = 2.909, 95%CI: 1.230-6.880, $P = 0.015$ for OS; HR = 3.003, 95%CI: 1.536-5.870, $P = 0.001$ for DFS). Although microvascular invasion was a significant factor only for DFS (HR = 2.148, 95%CI: 1.064-4.336,

Table 2 Clinicopathologic characteristics of patients according to National Cancer Center Korea criteria

Variables		Within NCCK (n = 164)	Beyond NCCK (n = 116)	P value
Sex, n (%)	Male	138 (84.1)	97 (83.6)	1
	Female	26 (15.9)	19 (16.4)	
Age (yr), mean (SD)		54.2 (7)	54.7 (7.7)	0.561
MELD score, mean (SD)		14.4 (7.9)	12.5 (6.1)	0.029
C-reactive protein (mg/dL), mean (SD)		0.58 (1.11)	1.37 (2.67)	0.004
Tumor maximum SUV, mean (SD)		3.08 (0.64)	4.13 (1.79)	< 0.001
Tumor total size, n (%)	≤ 10 cm	164 (100)	56 (48.3)	< 0.001
	> 10 cm	0 (0)	60 (51.7)	
AFP, n (%)	≤ 400 ng/mL	151 (92.1)	88 (75.9)	< 0.001
	> 400 ng/mL	13 (7.9)	28 (24.1)	
PET/CT, n (%)	Negative	164 (100)	26 (22.4)	< 0.001
	Positive	0 (0)	90 (77.6)	
Pretransplant therapy, n (%)	No therapy	39 (23.8)	29 (25)	0.77
	Surgery only	8 (4.9)	4 (3.4)	
	TACE only	71 (43.3)	52 (44.8)	
	RFA only	7 (4.3)	2 (1.7)	
	Combination	39 (23.8)	29 (25)	
Viral hepatitis, n (%)	HBV	142 (86.6)	103 (88.8)	0.442
	HCV	9 (5.5)	8 (6.9)	
	NBNC	11 (6.7)	3 (2.6)	
	HBV + HCV	2 (1.2)	2 (1.7)	
Differentiation ¹ , n (%)	I - II	102 (62.2)	55 (47.4)	0.02
	III-IV	62 (37.8)	61 (52.6)	
Microvascular invasion, n (%)	Absent	127 (77.4)	47 (40.5)	< 0.001
	Present	37 (22.6)	69 (59.5)	
Capsule formation, n (%)	No complete	134 (81.7)	94 (81)	1
	Complete	30 (18.3)	22 (19)	
Ductal invasion, n (%)	Absent	161 (98.2)	109 (94)	0.123
	Present	3 (1.8)	7 (6)	
Serosal invasion, n (%)	Absent	146 (89)	72 (62.1)	< 0.001
	Present	18 (11)	44 (37.9)	
Intrahepatic metastasis, n (%)	Absent	129 (78.7)	55 (47.4)	< 0.001
	Present	35 (21.3)	61 (52.6)	
Cirrhosis, n (%)	Absent	10 (6.1)	11 (9.5)	0.407
	Present	154 (93.9)	105 (90.5)	
Dysplastic nodule, n (%)	Absent	120 (73.2)	81 (69.8)	0.633
	Present	44 (26.8)	35 (30.2)	

¹Edmondson-Steiner Grade. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: Non-hepatitis B and non-hepatitis C virus; B + C: Hepatitis B and C virus; NCCK: National Cancer Center Korea; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; PET/CT: Positron emission tomography/computed tomography; AFP: α -fetoprotein; MELD: Model for End-Stage Liver Disease; SUV: Standardized uptake value.

$P = 0.033$), it was not included because these data are typically not available before transplantation. We analyzed our data in comparison with the Milan and UCSF criteria using the NCCK criteria (negative findings on PET/CT and total tumor size < 10 cm vs others). The NCCK criteria were assessed both preoperatively and postoperatively.

Statistical analysis

Survival rates were estimated using Kaplan-Meier method, and survival curves were compared with log-rank test. Multivariable Cox proportional hazard regressions were fitted to identify factors that affected post-transplant survival. T -test and χ^2 test analyses were also used in comparing the differences between groups for continuous and categorical variables, respectively. Cohen's Kappa was used to assess classification consistency of each criteria. The prediction model of DFS using each criteria (the NCCK, Milan, and UCSF) adjusted for significant prognostic factors was developed using multivariable Cox proportional hazard regression. The

receiver operating characteristic (ROC) curves and the associated area under the curves (AUC) of these models predicting 1, 3 and 5 years DFS rates were evaluated to compare the discrimination ability of different criteria. Differences in AUCs were tested using Delong's method^[18]. All statistical analyses were performed using SAS software (9.2 version). P -value less than 0.05 was used to evaluate statistical significance.

RESULTS

Clinicopathological characteristics

During the study period, a total of 280 patients underwent LDLT for HCC. Among them, 116 (41.4%) patients did not fulfil the NCCK criteria. The comparisons of clinicopathological characteristics between patients who did and did not fulfill the NCCK criteria are presented in Table 2. C-reactive protein level, tumor SUVmax, total tumor size (> 10 cm), AFP (> 400 ng/mL), positive findings on PET/CT, differentiation (grade III-IV), microvascular invasion, intrahepatic metastasis, and serosal

Table 3 Comparison between preoperative imaging and explant pathology by the Milan and National Cancer Center Korea criteria

Milan criteria		NCCK criteria		Preoperative imaging	
				Within	Beyond
Explant	Within			120 (42.86)	12 (4.29)
Pathology	Beyond			47 (16.79)	101 (36.07)
		Explant	Within	161 (57.50)	3 (1.07)
		Pathology	Beyond	17 (6.07)	99 (35.36)

Cohen's Kappa = 0.850. NCCK: National Cancer Center Korea.

invasion were significantly greater in patients who did not fulfill the NCCK criteria compared with those who did. The mean C-reactive protein levels in two groups were 0.58 mg/dL and 1.37 mg/dL, and tumor SUVmax were 3.08 and 4.13, in patients who did and did not fulfill the NCCK criteria, respectively. On the other hand, patients who did not fulfill the NCCK criteria had significantly lower MELD scores compared to those within the NCCK criteria (12.5 vs 14.4, respectively, $P = 0.029$). Pre-transplant therapy type, viral hepatitis type, ductal invasion, capsule formation, dysplastic nodules, and cirrhosis were not significantly different between the two groups.

NCCK criteria: Survival rates and comparison between preoperative imaging and explant pathological reports

OS and DFS according to the NCCK criteria are presented in Figure 1. Patients fulfilling the NCCK criteria according to preoperative imaging findings revealed significantly higher OS and DFS than those who did not fulfill the NCCK criteria (five-year OS: 83.6% vs 59.8%, $P < 0.001$; five-year DFS: 80.7% vs 45.1%, $P < 0.001$). In patients who fulfilled the NCCK criteria according to explant pathological reports, five-year OS and DFS were 85.2% and 84.0%, respectively; these values were significantly higher than those among patients who did not fulfill the NCCK criteria (60.2% and 44.7%, respectively, $P < 0.001$).

The number of patients who fulfilled the NCCK criteria according to preoperative imaging and explant pathology reports were 178 (63.6%) and 164 (58.6%). According to the Milan criteria, these were 167 (59.6%) and 132 (47.1%) patients (Table 3). The NCCK criteria exhibited 95.0% accuracy of preoperative imaging and explant pathological reports; in contrast, the Milan criteria demonstrated only 78.9% accuracy. Compared with the Milan criteria, the NCCK criteria exhibited almost perfect agreement between preoperative imaging and explant pathological reports (Cohen's Kappa 0.850 vs 0.583).

Comparative survival analysis among the NCCK, Milan, and UCSF criteria

In a survival analysis including all patients, five-year OS and DFS were 75.2% and 67.7% (Figure 1). The patients who fulfilled the Milan criteria according to

Table 4 Area under the curves and 95%CI for the Milan, University of California, San Francisco, and National Cancer Center Korea criteria for the prediction of 1, 3, and 5 years disease-free survival

Diagnostic approach	Criteria	AUC (95%CI)		
		1 yr	3 yr	5 yr
Preoperative imaging	Milan ¹	0.814 (0.754, 0.873)	0.804 (0.750, 0.858)	0.799 (0.747, 0.851)
	UCSF ²	0.812 (0.754, 0.871)	0.800 (0.747, 0.853)	0.793 (0.741, 0.844)
	NCCK ³	0.810 (0.753, 0.867)	0.806 (0.755, 0.857)	0.802 (0.753, 0.852)
	Milan ⁴	0.824 (0.767, 0.880)	0.815 (0.764, 0.866)	0.807 (0.757, 0.856)
	UCSF ⁵	0.819 (0.761, 0.877)	0.811 (0.759, 0.863)	0.803 (0.752, 0.853)
	NCCK ⁶	0.823 (0.769, 0.878)	0.817 (0.767, 0.866)	0.810 (0.762, 0.857)
Explant pathology	Milan ⁴	0.824 (0.767, 0.880)	0.815 (0.764, 0.866)	0.807 (0.757, 0.856)
	UCSF ⁵	0.819 (0.761, 0.877)	0.811 (0.759, 0.863)	0.803 (0.752, 0.853)
	NCCK ⁶	0.823 (0.769, 0.878)	0.817 (0.767, 0.866)	0.810 (0.762, 0.857)

¹Adjusted by PET, X, Y and Z; ²By PET, X and Y; ³By maximum tumor size, X, Y, and Z; ⁴By PET, total tumor size, X and Y; ⁵By PET, X, Y, and Z; ⁶By total tumor size, X, Y, and Z. X: Microvascular invasion; Y: Major vessel invasion; Z: Intrahepatic metastasis; AUC: Area under the curves; UCSF: University of California, San Francisco; PET: Positron emission tomography; NCCK: National Cancer Center Korea; 95%CI and P value were calculated by Cox PH regression analyses adjusted by the following covariates for each criteria.

preoperative imaging and explant pathological reports showed good five-year OS and DFS (83.4% and 82.0% according to preoperative imaging; 85.5% and 84.4% by explant pathological reports, Figure 2). These survival results are very similar to those of patients fulfilling the NCCK criteria, particularly with regard to explant pathological reports. There were 34 (12.14%) patients who did not fulfill the NCCK criteria but fulfilled the Milan criteria according to preoperative imaging findings, and 22 (7.9%) according to explant pathological reports. This group showed a trend toward low five-year OS and DFS according to both preoperative imaging and explant pathological reports, compared with those who fulfilled the NCCK criteria; however, the differences between the two groups were not statistically significant ($P = 0.148$ in OS and $P = 0.212$ in DFS according to preoperative imaging findings; $P = 0.658$ in OS and $P = 0.376$ in DFS according to explant pathological reports, Figure 3).

ROC curve and AUC of the Milan, UCSF and NCCK criteria for the prediction of one, three, and five years DFS are presented in Figure 4 and Table 4. The value of AUC by three criteria was similar in both preoperative imaging and explant pathological reports, and there were no significant differences in the area under the ROC curve at one, three, and five years by three groups (five-year DFS, Delong's $P = 0.267$ for Milan vs NCCK, $P = 0.213$ for UCSF vs NCCK in preoperative imaging; $P = 0.484$ for Milan vs NCCK, $P = 0.189$ for UCSF vs NCCK in explant pathological reports).

DISCUSSION

In the present study, the NCCK criteria were associated

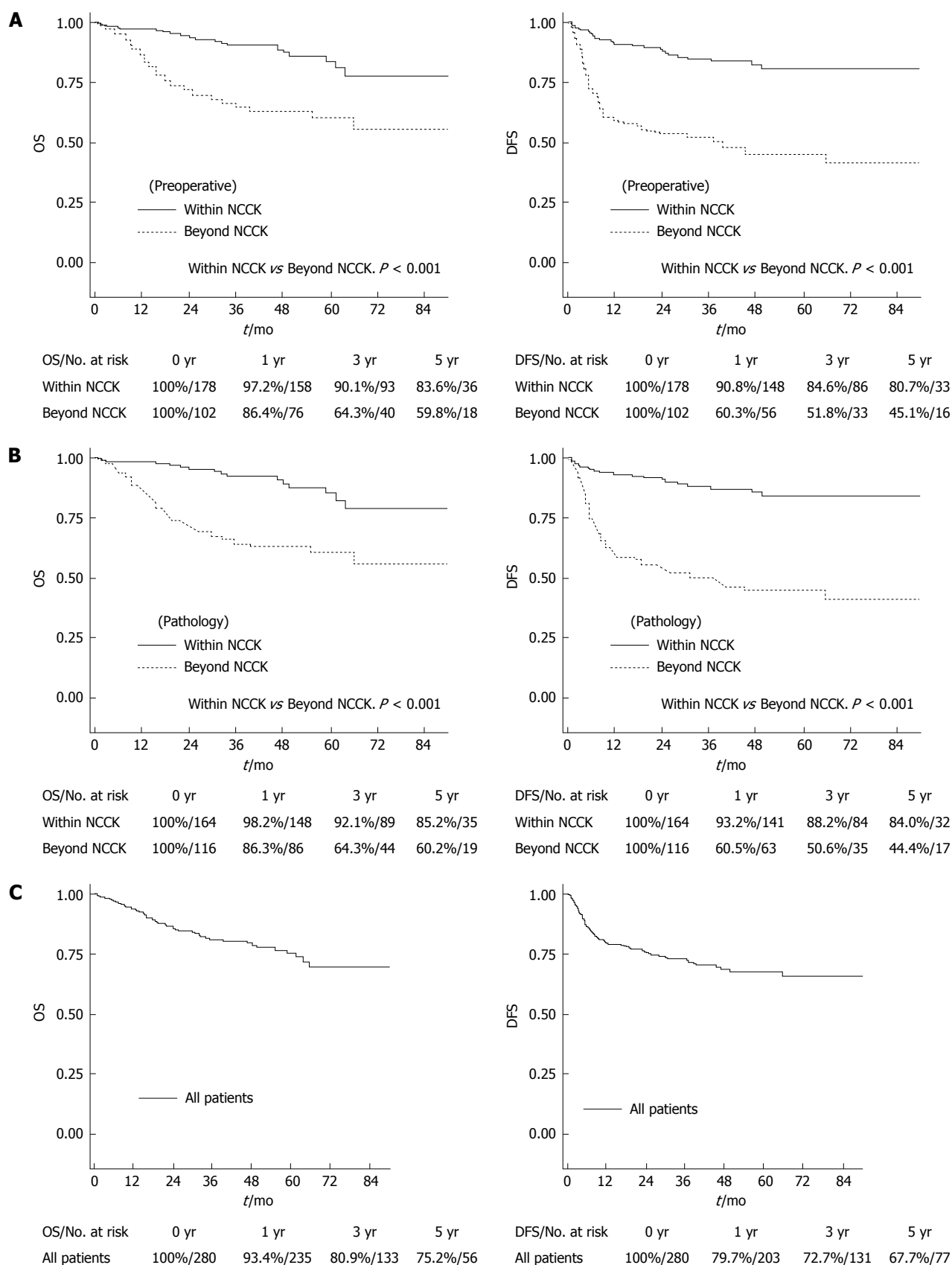


Figure 1 Overall and disease-free survival rates according to the National Cancer Center Korea criteria. A: By preoperative imaging; B: By explant pathology; C: OS and DFS rates for all patients. OS: Overall survival; DFS: Disease-free survival; NCCK: National Cancer Center Korea.

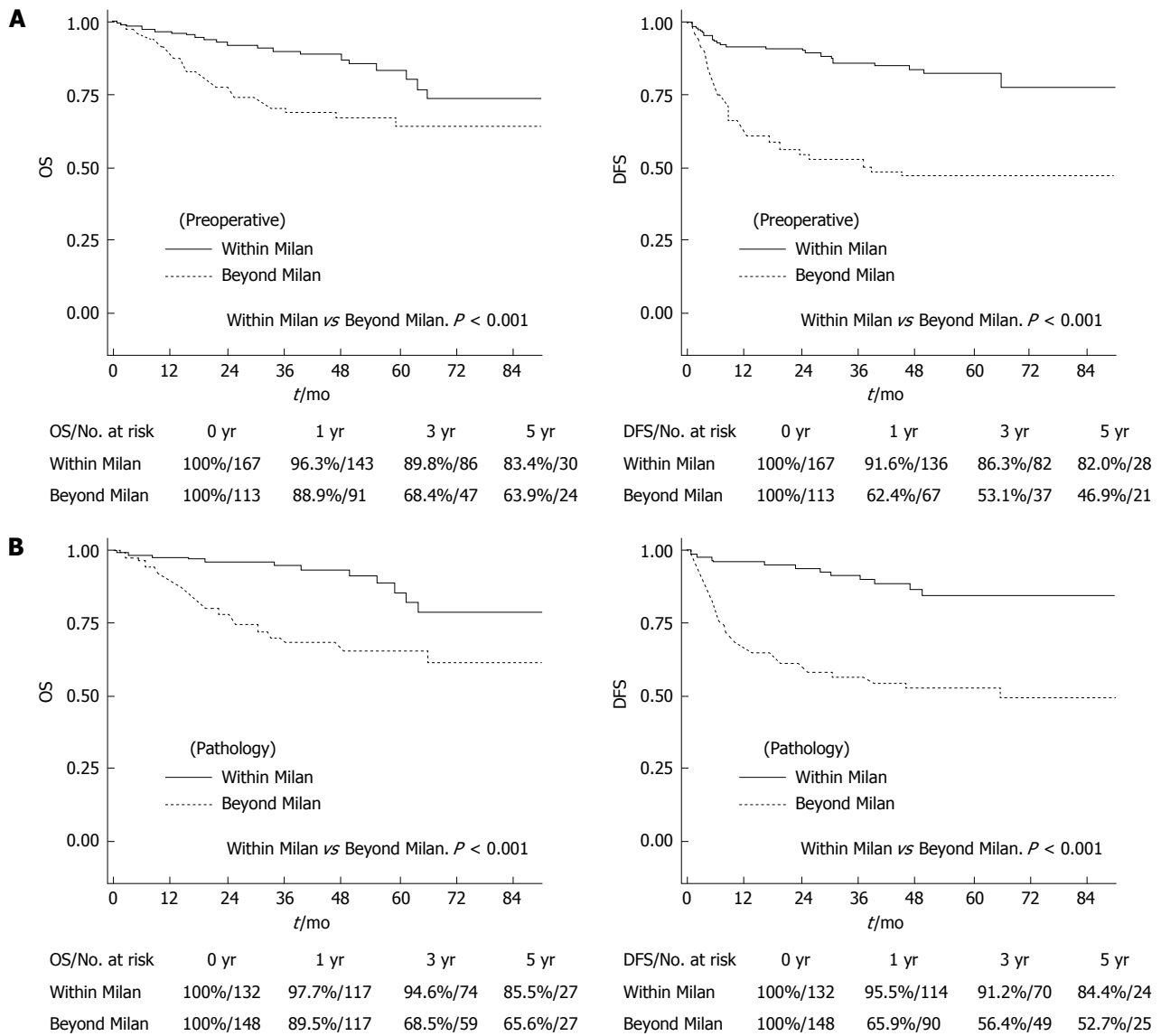


Figure 2 Overall and disease-free survival rates according to the Milan criteria. A: By preoperative imaging; B: By explant pathology. OS: Overall survival; DFS: Disease-free survival.

with favorable survival outcomes and expanded the selection pool for LDLT among patients with HCC. Over the past 10 years, the Milan criteria have been regarded as a well-established tool for assessing the prognosis of HCC for LT. However, limited selection and inaccurate assessment using preoperative imaging modalities, such as CT, have been constantly recognized as a limitation of the criteria. Tumor biological characteristics, such as microvascular invasion and differentiation, are strong predictive factors for HCC recurrence. ^{18}F -FDG PET/CT findings are a useful marker to predict these factors before LT, as well as to detect extrahepatic metastases. Furthermore, total tumor size itself can be simple and relatively accurate measure rather than using both tumor number and size which are used in the Milan and UCSF criteria. The proposed NCCK criteria, therefore, presented with better correlation with preoperative imaging and explant pathological reports than the Milan criteria.

There were several expanded criteria for patients with HCC beyond the Milan criteria. The main factors that were present in these criteria were tumor size and number. The UCSF, Tokyo, and "up-to-seven" criteria are based on tumor morphological characteristics using preoperative imaging or explant pathological reports^[4,8,19]. However, recent studies reported the expanded criteria using markers of tumor aggressiveness as well as tumor morphological characteristics. These included responses to TACE, the degree of differentiation, the gene-expression profile, the presence of microvascular invasion, and the levels of tumor markers, including AFP or DCP^[11,20-24]. In particular, it is well known that microvascular invasion and the degree of differentiation are associated with decreased survival and an increased risk of recurrence following LT. However, these pathological examination results are not routinely available before LT because fine-needle biopsy before surgery has not shown significant correlations with explant

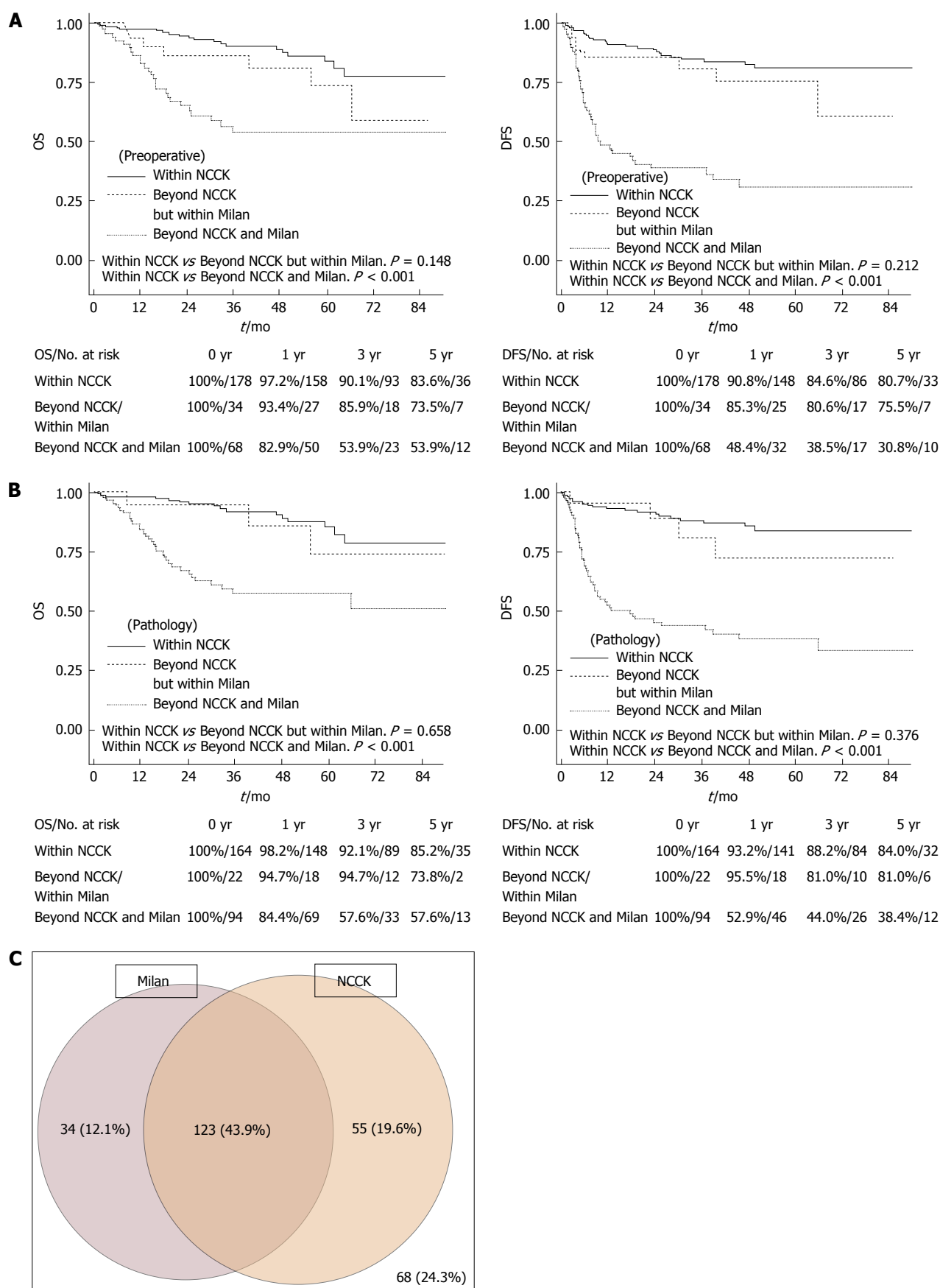


Figure 3 Overall and disease-free survival rates according to three groups (within the National Cancer Center Korea criteria, Beyond the National Cancer Center Korea but within the Milan criteria, Beyond both the National Cancer Center Korea and Milan criteria). A: By preoperative imaging; B: By explant pathology; C: The diagram of the portion of patients in Milan and NCCK criteria by preoperative imaging. OS: Overall survival; DFS: Disease-free survival; NCCK: National Cancer Center Korea.

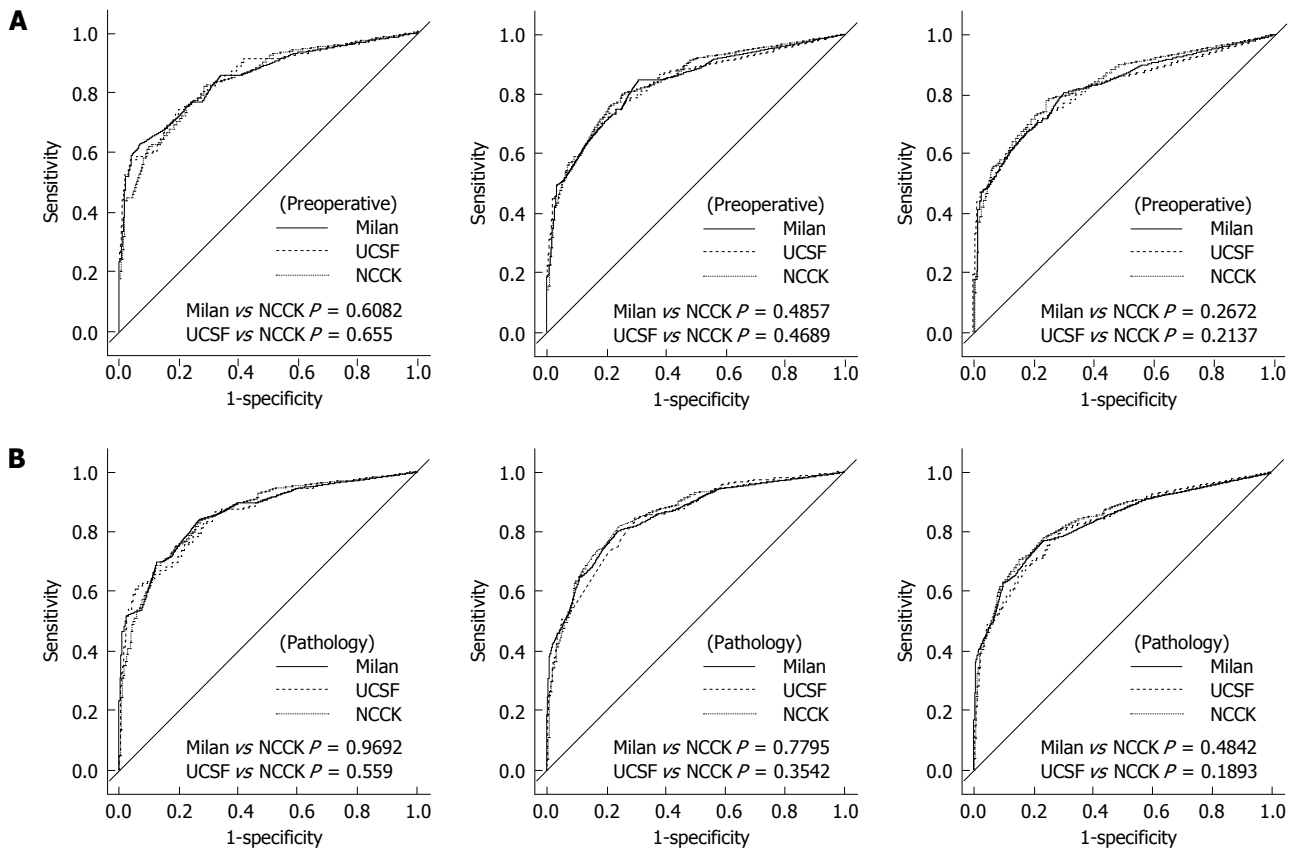


Figure 4 Receiver operating characteristic curves of three criteria (the National Cancer Center Korea, Milan and University of California, San Francisco) at 1, 3, and 5 years. A: By preoperative imaging; B: By explant pathology. UCSF: University of California, San Francisco; NCCK: National Cancer Center Korea.

pathological reports^[25]. Some promising attempts to identify microvascular invasion before LT through ¹⁸F-FDG PET or PET/CT have been reported^[13,14,26]. Moreover, positive findings on PET/CT in patients with HCC predicted the prognosis and tumor recurrence after LT^[13-15]. In the present study, the patients beyond the NCCK criteria, including positive findings on PET/CT, showed more microvascular invasion (59.5% vs 22.6%, $P < 0.001$) and poor differentiation (52.6% vs 37.8%, $P = 0.02$). One concern regarding the use of PET/CT in patients with HCC is that the sensitivity is low for the primary detection of HCC compared with many other cancers, because glucose metabolism is high in liver tissue^[27,28]. On the other hand, PET/CT has been shown to differentiate between well-differentiated and poorly-differentiated HCC, and is useful in the detection of extrahepatic metastases and recurrence of HCC after transplantation^[29].

The concept of the NCCK criteria began from the observation that good survival rates without recurrence could occur in patients who did not fulfill the Milan criteria. In our data, patients beyond the Milan criteria who also had negative findings on PET/CT showed significantly better survival rates than those who had positive findings on PET/CT (five-year OS, 74.6% vs 51.4%, $P < 0.001$; five-year DFS, 73.3% vs 37.5%, $P < 0.001$). When another significant factor for survival in multivariable analysis (total tumor size < 10 cm) was

considered, patients who did not fulfill the Milan criteria with negative findings on PET/CT and total tumor size < 10 cm showed similar OS and DFS compared with those who met the Milan criteria (OS: mean 90.7 mo vs 83.8 mo, $P = 0.235$; DFS: mean 94.4 mo vs 84.4 mo, $P = 0.076$). Furthermore, positive findings on PET/CT and total tumor size were significant prognostic factors of OS and DFS for all patients (Table 1). Therefore, we applied the NCCK criteria to all patients and analyzed their usefulness and associated survival rates as new expanded criteria that could be used instead of the traditional Milan criteria.

Numerous expanded criteria based on tumor number and size have been reported, but are not used widely due to limited clinical usefulness. The major reason for this is that the risk of underestimating tumor status is considerable regardless the recent developments of new technologies in radiological assessment of liver tumors^[30]. Freeman *et al.*^[31] studied the results from the United Network for Organ Sharing database on 789 LT recipients to analyze the accuracy of imaging findings compared with the explant pathological reports. In that report, radiological imaging underestimated tumor staging in 26.6% of cases, and the risk of overestimation was almost 30%. The overall preoperative accuracy was approximately 50%, regardless of the radiological technique used. In our data, among 167 patients who fulfilled the Milan criteria according to preoperative

imaging modalities, 47 patients (28.1%) were found as not fulfilling the Milan criteria in explant pathological reports. Therefore, some authors proposed that total tumor volume or size was more likely to result in accurate staging before LT^[32-34]. We also used the total tumor size (cutoff 10 cm), which was a significant prognostic factor in multivariable analysis for the NCCK criteria. In our study, among a total of 243 patients with preoperative total tumor size < 10 cm measured with imaging modalities, only 27 patients (11.1%) were confirmed to have a total tumor size of > 10 cm according to pathological reports. Compared with the Milan criteria, the percentage of underestimation in the NCCK criteria using total tumor size (cutoff 10 cm) was lower (9.6%), and Cohen's Kappa was high (0.850), explaining the near-perfect agreement between preoperative imaging and explant pathological reports (Table 3).

In particular, the survival rates of patients who fulfilled the NCCK criteria were quite good and showed similar outcomes compared with the Milan and UCSF criteria (five-year DFS; 80.7% according to preoperative imaging findings, 84.0% in explant pathological reports, Figure 2). Furthermore, the number of patients who fulfilled the NCCK criteria was higher than the Milan criteria [preoperative imaging findings, 178 (63.6%) vs 164 (58.6%) patients; explant pathological reports, 167 (59.6%) vs 132 (47.1%) patients]. The patients who did not fulfill the NCCK, but fulfilled the Milan criteria did not show statistically significant differences compared with those who fulfilled the NCCK criteria; however, a trend toward low five-year OS and DFS according to both preoperative imaging and explant pathological reports was observed (Figure 3). This result was likely because of the fact that the Milan criteria are too restrictive and limited. There was no significant difference observed when the values of AUC and ROC curves for predicting DFS at one, three, and five years were compared among the three criteria (NCCK, Milan, and UCSF) (Figure 4 and Table 4).

There are some limitations to the present study. First, we analyzed LDLT patients without including deceased donor LT patients; therefore, comparison with other studies that included deceased donor LT patients was not possible. However, we included a considerable proportion of patients who were beyond the Milan criteria; thus, the dilution effect on the analysis was less than that in other studies. Second, the present study was retrospective in nature, and selection bias could have influenced the survival analysis. However, we enrolled all consecutive cases and performed routine PET/CT before LDLT in patients with HCC. Therefore, exclusions during the study period were rare.

In conclusion, our data show that the NCCK criteria, utilizing total tumor size and PET/CT findings, successfully expanded the recipient pool and demonstrated better ability of tumor assessment before LT and similar survival rates compared with the well-known criteria, such as the Milan and UCSF. These criteria represent

a new approach to selection for LT that incorporates both tumor biological and morphological characteristics. Therefore, the NCCK criteria are simple and useful expanded criteria for LDLT in HCC, showing excellent agreement between preoperative imaging and explant pathological reports and favorable survival outcomes.

COMMENTS

Background

Several expanded criteria based on morphological features have been proposed to identify appropriate candidates for liver transplantation (LT). However, the definitions are still complex, and the benefit of expanding the pool remains controversial. In this study, the authors evaluated the new criteria using positron-emission tomography/computed tomography (PET/CT) and total tumor size, called as National Cancer Center Korea criteria.

Research frontiers

The expanding criteria for living donor liver transplantation (LDLT) for hepatocellular carcinoma (HCC) is issued recently. The results of this study contribute to clarifying exact criteria using PET/CT and tumor morphologic characteristics.

Innovations and breakthroughs

In this study, they used the PET/CT for all patients underwent LDLT in their institute before transplantation. These results are so unique and included relatively large number of patients. PET/CT is very useful tool for selecting recipients with HCC in LDLT.

Applications

This study suggested that PET/CT is useful for selecting recipient and total tumor size is simple for marker in preoperative imaging tests. If a patient is diagnosed with HCC and waiting the LDLT, PET/CT can be chosen for diagnostic metastasis and prediction of prognosis.

Peer-review

The author this paper evaluated the usefulness of PET/CT and total tumor size for predicting the prognosis after LDLT for HCC, and showed the expanded criteria using these tools. Further trials using these criteria in large population of LDLT will be valuable.

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Observational Study

Deceased donor organ procurement injuries in the United States

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, who will provide a permanent, citable and open-access home for the dataset. In addition, a copy of the signed statement should be provided to the BPG in PDF format.

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Abstract

AIM: To determine the incidence of surgical injury during deceased donor organ procurements.

METHODS: Organ damage was classified into three tiers, from 1-3, with the latter rendering the organ non-transplantable. For 12 consecutive months starting in January of 2014, 36 of 58 organ procurement organization's (OPO)'s prospectively submitted quality data regarding organ damage (as reported by the transplanting surgeon and confirmed by the OPO medical director) seen on the procured organ.

RESULTS: These 36 OPOs recovered 5401 of the nation's 8504 deceased donors for calendar year 2014.

A total of 19043 organs procured were prospectively analyzed. Of this total, 59 organs sustained damage making them non-transplantable (0 intestines; 4 pancreata; 5 lungs; 6 livers; 43 kidneys). The class 3 damage was spread over 22 (of 36) reporting OPO's.

CONCLUSION: While damage to the procured organ is rare with organ loss being approximately 0.3% of procured organs, loss of potential transplantable organs does occur during procurement.

Key words: Organ procurement; Deceased donations; Organ procurement organization; Organ injury; Organ transplantation

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Core tip: This study represents a unique report looking into the incidence of surgical injuries during deceased donor organ procurement. There is no other large scale study reporting this. This represents a multi-organizational study, collecting data prospectively over a period of a year. This study will hopefully help define the problem and contribute to the development of basic standards that organ procurement organizations can follow across the country.

Taber TE, Neidlinger NA, Mujtaba MA, Eidbo EE, Cauwels RL, Hannan EM, Miller JR, Paramesh AS. Deceased donor organ procurement injuries in the United States. *World J Transplant* 2016; 6(2): 423-428 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/423.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.423>

INTRODUCTION

Organ transplantation remains one of the enduring miracles of modern medicine. The ability to replace a dysfunctional organ with a functional allograft that returns the recipient to health is truly an impressive feat. Human organ transplantation essentially started in 1954 with the first successful kidney transplant^[1]. Early transplant successes were limited by the lack of availability of adequate immunosuppression. With the advent of cyclosporine in 1983, the modern era of transplantation began^[2]. Organ transplantation, since that time, has been limited less by the ability to maintain viability of allografts post-transplant than by the supply of transplantable organs^[3]. As most organ transplants are deceased organs, the willingness of potential donor families to agree to organ donation has become paramount. Despite the altruism of these families, over time, there has developed a mismatch of supply and demand with the current waitlist (April 2016) of patients for a solid organ transplant exceeding 121000. Hence, there has been an imperative to ensure that any organ procured should be uninjured during the procedure in

order to maximize utilization. Little data exists in the literature regarding procurement injury. The aim of this study was to determine the incidence of procurement injury in the United States. Organ procurement organization (OPO) system and to further stratify the impact of these injuries by developing a graded scoring system directly linked to the extent of loss.

MATERIALS AND METHODS

There are currently 58 OPOs in this country performing organ procurement. The Association of OPOs (AOPO) serves to unify these individual OPO's and to assist in the sharing of knowledge of best practices in the many tasks performed by the OPOs. Within each OPO, organ procurement is overseen by a medical director to whom each is extended an offer for membership within the AOPO medical council. It is within this medical council that, in 2013, a discussion culminated in the desire to ensure that the "gift of life" of an organ donation should be protected. The medical directors agreed upon a national standard of measurement of organ damage. These levels of damage were agreed upon and range from a level of "0" (no damage); level "1" (minimal damage sustained upon procurement requiring no intervention); level "2" (damage sustained upon procurement requiring some surgical repair but not rendering the allograft non-transplantable); and finally level "3" (damage sustained upon procurement rendering the allograft non-transplantable). These levels of damage would be reported by the transplanting surgeon and reviewed and agreed upon by the medical director of the procuring OPO in consultation with the medical advisory board within that OPO (as deemed necessary by the individual medical director).

After the aforementioned preliminary agreement was reached, this study commenced and included all deceased donors from whom solid organs were procured for transplantation from January 1, 2014 through December 31, 2014. All 58 OPOs were encouraged to prospectively collect data during this period. Data was sent to the AOPO national office where it was transferred to a database and separated by month and OPO. Data was collected for transplantable solid organs: Heart, lung, liver, kidney, pancreas and intestine. Data was subsequently analyzed in an organ-specific fashion. Only data collected for the entire 12 mo of the study was included for evaluation. As noted above, levels of damage were defined as class 1, class 2 or class 3. For each level 3 injury, a written description of the injury was provided to AOPO.

All data for this analysis were collected prospectively in our OPO database. Continuous variables are presented as mean/median. Number and type of organ procured at each OPO and class of injury were reviewed. Class of injury was expressed as 1, 2 or 3 and reported as a frequency at each OPO. Chi square test was used for categorical variables. A two-tailed *P* value of < 0.05 was considered to be significant. The program - graph pad

Table 1 Participating organ procurement organizations

Arkansas Regional Organ Recovery Agency
Donor Network West
Life Sharing - A Donate Life Organization
Donor Alliance Inc.
Life Choice Donor Services
Washington Regional Transplant Community
Life Alliance Organ Recovery Agency
Life Quest Organ Recovery Services
LifeLink of Florida
Legacy of Life Hawaii
Indiana Donor Network
Louisiana Organ Procurement Agency
New England Organ Bank
The Living Legacy Foundation of Maryland
Gift of Life Michigan
Life Source
Mid-America Transplant Services
Mississippi Organ Recovery Agency
Midwest Transplant Network
Carolina Donor Services
Nebraska Organ Recovery System
New Jersey Organ and Tissue Sharing Network
Live-On-NY
Lifebanc
Life Connection of Ohio
Lifeline of Ohio
Life Center Organ Donor Network
Life Share Transplant Donor Services of Oklahoma
Pacific Northwest Transplant Bank
Center for Organ Recovery and Education
Tennessee Donor Services
Life Gift Organ Donation Center
Southwest Transplant Alliance
Life Center Northwest
Wisconsin Donor Network
UW Organ and Tissue Donation

prism - was used to perform statistical evaluation.

The need for consent in the United States is regulated by local Institutional Review Boards. The consent for brain dead (BD) donors for research is not legally required when no additional tissue, *etc.*, is taken from the donor^[4]. For that reason this study was Institutional Review Board (IRB) exempt and IRB consent was not requested.

RESULTS

A total of 36 OPOs (out of a potential 58) participated in the prospective collection of data (Table 1). An additional 3 OPOs submitted data but were not included in the analysis as this data was not a complete years' collection. By excluding partial year's data, we aimed to minimize selection bias. OPO size (donors/year) varied from 43 to 305 donors/year (mean 147.5; median 141). These 36 OPOs recovered a total of 5401 of the nation's 8594 deceased donors in 2014. From these donors, 19043 procured organs' data was analyzed. Of the donors, 4347 were BD donors and 870 were donation after cardiac death (DCD) donors. Data was reported in terms of both recovered and transplanted organs. The

Table 2 Recovery data with Injuries

Recovered intestine	128
Transplanted intestine	77
Type 1	2
Type 2	0
Type 3	0
Recovered pancreas	855
Transplanted pancreas	648
Type 1	7
Type 2	3
Type 3	4
Recovered heart	1726
Transplanted heart	1617
Type 1	6
Type 2	2
Type 3	1
Recovered lung	2437
Transplanted lung	2004
Type 1	16
Type 2	1
Type 3	5
Recovered liver	4396
Transplanted liver	3928
Type 1	58
Type 2	16
Type 3	6
Recovered kidney	9501
Transplanted kidney	7889
Type 1	156
Type 2	86
Type 3	43

Table 3 Number of type 3 injuries (one year) by organ procurement organization

OPOs with 1 injury	7
OPOs with 2 injuries	5
OPOs with 3 injuries	6
OPOs with 4 injuries	2
OPOs with 6 injuries	1
OPOs with 10 injuries	1

OPO: Organ procurement organization.

most frequent type of injury was class 1 (Table 2). Class 2 injuries were usually but not always intermediate in number between class 1 and class 3 injuries. In order of increasing incidence of injury, type 3 injuries were compared to recovered organs and occurred in the following frequencies: Intestine: 0/128 (0%); heart 1/1726 (0.05%); liver: 6/4396 (0.14%); lung: 5/2437 (0.21%); kidney: 43/9501 (0.42%); pancreas: 4/855 (0.47%). A total incidence then of class 3 injury in the 19043 organs procured was 0.3%. Among individual OPOs, there were a total of 22 OPOs that reported at least one type 3 injury (Table 3). The median number of class 3 injuries per OPO (in OPOs that had at least 1 injury) was 2.0 with a mean of 2.7 and a mode of 1. One OPO reported 10 class 3 injuries during the year of data collection, one OPO reported 6 and 2 OPOs reported 4 class 3 injuries. The remaining OPOs reporting class 3 injuries fell in the range of 1-3 injuries for the year (#18).

Table 4 Causes of class 3 injury

Organ	# injuries	Cause
Intestine	0	N/A
Pancreas	4	Vascular injury (2) Traction injury to organ (2)
Heart	1	Vascular injury (1)
Lung	5	Vascular injury (2) Inadequate trachea for anastomosis (1) Not specified (1)
Liver	6	Vascular injury (3) Capsular tear (2) Not specified (1)
Kidney	43	Vascular injury (27) Capsular tear (7) Ureteral transection (5) Not specified (3) Failure to flush artery adequately (1)

In looking at OPO size as being predictive of the number of class 3 injuries, 3 of 4 of the OPOs having at least 4 class 3 injuries were larger than the median OPO size in the total cohort (147.5 donors/OPO) but this did not reflect their frequency. The incidence of class 3 injury within this subset of OPO's having at least 4 injuries ranged from 1.3% (of procured organs) to 4.4% with the highest incidence occurring in the OPO with 10 class 3 injuries. Further evaluation of this subgroup of 22 OPOs with class 3 injuries, 7 had only 1 and 5 only had 2. In the subgroup of OPOs with at least 3 class 3 injuries (#10), only 6 of the OPOs had an incidence of over 2.1%. In looking at the highest incidence of injury, 4 OPOs had an incidence of at least 3.9% (range 3.9%-4.7%). In contrast to that noted above in regards to total injuries and OPO size, 3 of these 4 OPOs were smaller OPOs as defined by annual donor numbers (< 147.5 donors/year). Finally, arbitrarily using a 2% injury rate irrespective of number of injuries, there were 7 OPOs that fell within this parameter. Of those OPOs, 5 were in the smaller OPO group (again - as defined as < 147.5 donors/year) and 2 were in the larger group. From a statistical analysis standpoint, using chi square testing, a higher incidence of class 3 injury was observed in the smaller OPOs (grouped together: < 147.5 donors/year) vs larger OPOs (> 147.5 donors/year) with a *P* value of 0.044.

As class 3 injuries rendered the allograft unable to be transplanted, a summary was received for each lost organ (Table 4). In all allografts (with the exception of pancreas that sustained a "traction" injury) vascular damage was the most common injury rendering the organ non-transplantable. The total BD vs DCD donors were noted but the only data regarding donor type supplied on failed organs was in the narrative. Of note, however, 2 of 6 livers felt to be non-transplantable were noted to be DCDs and 4 of the 43 kidneys. Unfortunately, this data was gleaned from the narrative and not specifically collected so a comparison of DCD vs BD donors reflecting the likelihood of class 3 injuries cannot be made.

DISCUSSION

There have been retrospective reviews regarding surgical damage during procurement but to our knowledge, this is the first prospective look at the surgical outcome of organs procured from deceased donors gathered at the United States OPO level^[5,6]. For that reason, an acceptable degree of surgical damage seen during procurement could not be known. The technique required in procuring organs for donation requires the skills of a vascular surgeon and the insights of a transplant surgeon. Surgical damage may be related to the procurement procedure itself or may be related to the cause of death of the donor (trauma). Damage rendering the organ non-transplantable may be related to parenchymal damage, injury to the vasculature or other parts of the organ (ureter, etc.). The surgeon is required to procure the organ without injury to any of these structures^[7]. In addition, they must obtain enough of the vasculature to allow for anastomosis into the recipient. This desire for adequate vessel length, though, must be balanced with the needs of the other procuring surgeons. Frequently vessel lengths are shared between donor surgeons and a degree of communication and cooperation is required and almost always achieved. Anomalous anatomy also may play a part in organ injury^[8]. This is especially true in the procurement of small organs (pancreas)^[9]. Finally, the insight of the transplanting surgeon should not be overlooked in the determination of transplantability of the organ. If a marginal organ is procured and found to have a significant injury that could potentially impact its function, the transplanting surgeon might be more disinclined to transplant this organ. This could especially be the case in the procurement of a marginal or DCD organ as has been seen previously in DCD kidneys^[10]. Unfortunately, this study was not designed to compare damage seen in BD vs DCD donors. In some cases this information was contained in the narrative describing the injury but as this was not consistent, that information is not reported here.

Despite all the enumerated pitfalls involved in organ procurement, the frequency of organ injury during procurement is rare. The motivation and the skill of the procuring and transplanting surgeon combine to make this outcome predictable. In looking for trends within class 3 injuries, the very scarcity of these injuries made such efforts difficult. What was seen, however, in the OPOs with the highest levels of class 3 injuries was that the injuries tended to cluster within months and then disappear in the months following. In reading the narrative associated with injuries, it was evident that procurement injuries resulted in feedback to the procuring surgeons. It was likely then that such feedback either improved the future focus of the procuring surgeon or resulted in a change or a call for mentorship (in at least one case) in the procuring team. This study would then support the importance of a collegial

discussion with the procurement team in the instance of organ injury.

In looking at class 3 injuries, as noted previously, 14 of the 36 participating OPOs had no such injuries while 3 of 4 of the highest raw number of injuries occurred in larger OPOs (> 147.5 donors/year). However in looking at the frequency of injury of > 2%, smaller OPOs made up the majority of this subset (see above). It does appear then that smaller OPOs by size tend to have a statistically significantly higher likelihood of having a greater frequency of class 3 injury - again arbitrarily defined as a frequency of > 2%. At least one of the reasons for this can be the smaller margin for error when fewer donors are procured. Other potential causes for this would be speculative without further data collection.

Certainly the vast majority of this discussion has been focused on class 3 injuries. The numbers of class 1 and 2 injuries certainly exceed class 3 but, as these do not result in a lost allograft, there is a lessened imperative to examine these events. However, it is likely that these events may be harbingers of class 3 injuries. As no narrative was provided for class 1 and class 2 injuries, it is unknown as to whether OPOs have these discussions after these events. By providing feedback to individual procuring surgeons not just in class 3 but also in the event of a class 1 or 2 injury, there would seem to be potential for improving an individual's procurement surgeon's skills and so avoid future type 3 injuries. These events therefore should continue to be reviewed on an individual OPO level.

Finally, the collection of this data provides OPOs a perspective on their effectiveness in organ procurement. Individual OPOs can, by continuing to follow their surgical injury rate, have an idea as to where their injury rate falls within the national benchmarks. While the goal for surgical damage continues to be the lack of damage, careful review of the frequency of different damage levels will give individual OPOs continuous feedback on at least one aspect of their quality.

The strengths of this study include the prospective data collection, the inclusion of 36 of 58 OPOs as well as the use of the entire 12 mo of data during the collection period. The inclusion and review of the narrative also gave insight into the individual OPOs efforts in enhancing quality. The weaknesses of this study include the lack of participating would have shown a higher level of surgical injury but that outcome again would not be a fait accompli. Additionally, expanding data collection to include determination of BD vs DCD donors, names of procurement teams and levels of experience of these teams would have been helpful in interpreting the data. Finally, as the degree of damage was first quantitated by the transplanting surgeon, there is a potential for under-reporting type 1 and 2 injuries if the procuring team were from the transplanting center. This degree of underreporting should not be seen, however with type 3 injuries as the loss of an organ would be evident to the on-site OPO coordinators. Taking all of these concerns

into account, the goal of this study was to establish a standard in the description of procurement surgical damage and a baseline of injury rate. Examined in this light, this study achieved its goals.

A 12 mo collection of surgical damage data from 36 of 58 OPOs in the United States was reviewed. In the entire group, surgical damage was a rare event with the loss of allograft seen in less than 0.5% of procured organs. The majority of the surgical damage seen was related to vascular injuries. Incidence of class 3 injury appears to be higher in OPOs with smaller donor volumes.

COMMENTS

Background

There is a paucity of information about organ injuries during deceased donor procurements. This has significant importance into the numbers of organs that are transplanted every year. This study set out to document and report, for the first time, the incidence and grades of surgical injury to organs during their procurement across the United States. This has the potential to set national standards for quality and offer future ideas for research.

Research frontiers

As the waiting list for organ transplants gets bigger, there has been recent impetus for research to look at more ways to obtain such organs. One such important way would be to identify the numbers of organs that are lost to injury during procurement.

Innovations and breakthroughs

There have been no previous large scale reports of this kind, hence the novelty of this study.

Applications

This study offers for the first time, a national perspective on procurement organ injuries. It helps define a national problem, which the authors know little about. This offers the potential to establish national standards for organ procurement organizations, training of transplant surgeons, and even insurance and regulatory purposes.

Terminology

OPO: Organ procurement organizations. These are independent organizations, contracted with the United Network of Organ Sharing in the United States. Their purpose is the responsibility of procurement and distribution of organs from deceased donors in their assigned geographic area; AOPO: Association of OPOs. A national organization representing all of the 58 OPOs across the United States.

Peer-review

This study is a large scale multi-organizational report looking into the incidence of surgical injuries during deceased donor organ procurement, collecting data prospectively over a period of a year. Organ damage was classified into three tiers, with the latter rendering the organ non-transplantable.

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Randomized Controlled Trial

Exercise manual for liver disease patients

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Abstract

AIM: To increase inspiratory muscle strength and improve the quality of life of candidates for liver transplantation.

METHODS: Twenty-three candidates for liver transplantation participated in the control group and 14 made up the intervention group. The control group consisted of 18 men and 5 women, body mass index (BMI) 27.3 ± 4.5 kg/m² and Model for End-Stage Liver Disease (MELD) 18.2 ± 6.1 . The intervention group consisted of 11 men and 3 women, BMI 28.6 ± 5.4 kg/m² and MELD 18 ± 4.5 . The presence or absence of ascites was identified in the first patient evaluation and after three months. We evaluated maximal inspiratory pressure (MIP) and maximal expiratory pressure, spirometry, root mean square (RMS) of diaphragm and rectus abdominis, and the quality of life. The exercises were performed daily by patients at home for three months and were supervised at distance monthly. The manual consisted of diaphragmatic breathing exercises, diaphragmatic isometric exercise, Threshold IMT®, lifting upper limbs with a bat and strengthening the abdomen.

RESULTS: There was significant difference ($P = 0.01$) between the first (initial) and the third month (final) MIP in the control group and in the intervention group, but there was no difference ($P = 0.45$) between the groups.

The RMS of the diaphragm was lower ($P = 0.001$) and the functional capacity was higher ($P = 0.006$) in the intervention group compared to the control. The general health and mental health domains received higher scores after three months in the control group ($P = 0.01$) and the intervention group ($P = 0.004$), but there was no significant difference between them. The comparison between the presence of initial ascites with the presence of ascites was performed after three months in the control group ($P = 0.083$) and intervention group ($P = 0.31$). There was no significant difference, in relation to the presence of ascites after three months between groups ($P = 0.21$). In the intervention group, patients with ascites at the end of the time period had decreased scores on the social aspects SF-36 domain ($P = 0.023$) compared to those who had no ascites.

CONCLUSION: The proposed exercises provide an increase in the inspiratory muscle strength and improve functional capacity, consequently bettering the quality of life of liver disease patients.

Key words: Respiratory muscles; Pre-operative period; Electromyography; Muscle strength; Breathing exercises

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Core tip: Studies on the effects of exercises, mainly those on breathing for liver transplant patients on the waiting list, are rare in the literature. This study proposes a manual of exercises for this group in order to increase muscle strength and improve their quality of life, as sarcopenia found in these patients contributes to a worsening of quality of life and is associated with mortality. The results are encouraging and may represent the beginning of further studies in the area and the establishment of exercise protocols for liver diseases.

Limongi V, Dos Santos DC, Oliveira da Silva AM, Boin IFSF, Stucchi RSB. Exercise manual for liver disease patients. *World J Transplant* 2016; 6(2): 429-436 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/429.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.429>

INTRODUCTION

Liver transplantation means a chance of survival for individuals with advanced chronic liver diseases or acute liver failure when there are no clinical treatments available^[1-3]. However, there is a disproportion between the supply of organs^[4] and those in need of transplantation, increasing the time on the waiting list and the chance of complications^[5] such as fatigue, decreased aerobic capacity, malnutrition, sarcopenia^[6] and impaired ventilator mechanics due to ascites^[7].

Sarcopenia may be associated with mortality in cirrhotic patients^[8,9] and contributes to an impaired of

quality of life in these patients^[10-13].

Probably also due to loss of muscle mass, according to the authors Oliveira da Silva *et al.*^[7], and da Silva *et al.*^[14], the liver disease patients showed on average higher RMS of the diaphragm when compared to healthy subjects. This means that the respiratory muscles of patients with liver disease should try harder to gain the best resistance in the basal ventilation profile, in order for the electrical activity of the diaphragm to be higher.

Studies of Dharancy *et al.*^[15], Pieber *et al.*^[16] and Wiesinger *et al.*^[17], suggest that a change from the predominance of aerobic metabolism to anaerobic metabolism occurs early during exercise in individuals with cirrhosis compared to healthy subjects^[8].

The findings in cardiopulmonary exercise testing, early termination of exercise with low peak VO_2 (oxygen consumption), hyperventilation precocious and reduced or unattainable ventilatory threshold^[15,18] may correspond to a fatigue at the beginning of exercise or indicate deconditioning thus hampering the exercise. This reflects the difficulty that cirrhotic patients have to performing everyday activities, as well as feeling fatigue^[17] even when they are hospitalized.

All these complications in the preoperative period, which also influence the recovery after transplantation, can be mitigated with well-defined and specific intervention programs for this group.

Therefore, the aim of the study was to increase inspiratory muscle strength and improve quality of life for liver disease patients with the proposed manual of breathing exercises.

MATERIALS AND METHODS

In this prospective, randomized and controlled trial, data collection was performed at the Unit of Liver Transplantation, Hospital de Clinicas, State University Campinas (Unicamp). The study protocol followed the Ethics Committee of the Medical Sciences Faculty, Unicamp, CEP: 922/2009. Each study participant signs the Informed consent statement.

Liver disease patients were included, men and women, aged over 18 years, with or without a diagnosis of cardiorespiratory disease and those with any Model for End-Stage Liver Disease (MELD) score obtained. All patients filled out a form for identification, age, gender and diagnosis of liver disease. The MELD and body mass index (BMI) were calculated. The presence or absence of ascites was identified in the first patient evaluation and after three months.

Exclusion criteria were: The inability to understand verbal commands, patients with poor general condition (for example, bed reset condition), the failure to perform the evaluations and acute liver failure diagnosis.

The study population was selected from the liver transplant waiting list from August 2012 to February 2014. From the 49 patients evaluated, 27 individuals were chosen through a random draw for participation in the control group. However, four patients were

Table 1 Demographic and baseline characteristics of the patients

Features	Control (<i>n</i> = 23)	Intervention (<i>n</i> = 14)	<i>P</i>
Male/female	18 (78.3%)/5 (21.7%)	11 (78.6%)/3 (21.4%)	1.00
Age (yr)	55.4 ± 9.9	55.8 ± 5.4	0.97
BMI (kg/m ²)	27.3 ± 4.5	28.6 ± 5.4	0.58
Diagnosis			
HCV	5 (21.7%)	4 (28.6%)	
HCC + HCV	4 (17.4%)		
Alcohol	3 (13%)	3 (21.4%)	
HCC	3 (13%)		
Alcohol + HCV	3 (13%)	2 (14.2%)	
Alcohol + HCC	1 (4.3%)		
Alcohol + HCV + HCC	1 (4.3%)	2 (14.2%)	
Autoimmune hepatitis	1 (4.3%)		
Polycystic liver disease	1 (4.3%)		
Cryptogenic cirrhosis		1 (7.1%)	
Sclerosing cholangitis		1 (7.1%)	
HBV + HCV		1 (7.1%)	

BMI: Body mass index; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

excluded; three died and one was submitted to a liver transplantation. Thus, 23 patients made up the control group. Twenty-two patients were randomly picked to take part in the intervention group, through a random draw. However, eight patients were excluded as three died, two had liver transplant operations and three individuals declined to perform the exercises. Thus, 14 patients constituted the intervention group. Software for randomization and allocation was not used; the names of the patients were placed in identical envelopes and drawn by the researcher, one by one, to make up the control group and intervention.

The control group was composed of 18 men (78.3%) and five women (21.7%) and the intervention group consisted of 11 men (78.6%) and three women (21.4%). Table 1 shows the demographic and baseline characteristics of the patients.

The respiratory pressures, maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured using an analog manometer Gerarmed® (SP, Brazil), with unit scale in cmH₂O, coupled to a mouth-piece and nose clip, always with the patient seated. The data were always collected by the same researcher.

To measure the MIP was requested a maximum exhalation until residual volume and after, a maximal inspiratory effort. To measure the MEP a maximal inspiratory effort was asked for in order to achieve the level of total lung capacity, and then a maximum expiratory effort. The maneuvers were repeated three to five times at intervals of 30 s and it was considered the highest value obtained^[19].

The surface electromyography EMG System of Brazil Ltda®, Series 00405, Model 210C (SP, Brazil) was used to obtain the electrical activity of the diaphragm and rectus abdominis, represented by the root mean square (RMS). Electrodes 3M Brazil® (Sumare, SP, Brazil) were used for the study of electrical activity in these muscles.

The electronic circuit acquisition captures and processes the signals, making them available to the EMG

System of Brasil® software, it was installed on a computer Intelbras I21® (SP, Brazil).

The participants were positioned at 45° in order to study the electrical activity of the diaphragm. A passive electrode was adapted in the paraxiphoid position about 5 cm from the xiphoid process and another 16 cm from the right costal margin. To measure the rectus abdominis an electrode was adapted in the rectus abdominis muscle 5 cm away from the umbilicus and another about 15 cm along the involved muscle^[7]. On the left hand side was positioned a ground electrode. Participants breathe normally while the electrical activity was recorded for ten seconds. A heavy breathing was requested every three seconds. For rectus abdominis was used 500 Hz of frequency and 500 μV of the sensitivity of signal amplitude^[7]. For the diaphragm was used 300 Hz frequency and 300 μV of the sensitivity of signal amplitude^[7].

Through EasyOne Diagnostic Spirometer World® (Zurich, Switzerland), it was possible to perform spirometry, following the Guidelines for Pulmonary Function Tests^[20]. Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁) and forced expiratory flow rate at 25%-75% of FVC curve (FEF_{25%-75%}) were measured.

The "short form 36" (SF-36)^[21] was used to evaluate the quality of life of the participants. The questionnaire consisted of 36 items related to eight domains covering different concepts of health, functional capacity, physical role, pain, general health, vitality, social aspects, emotional role and mental health.

Participants in the intervention group received a manual with illustrations and explanations to be held at home for three months and they received orientation from the therapist at the time of the delivery of the material. The first evaluation was made at this time; the second was made after three months. Figure 1 shows the prepared manual.

The therapist remained available for any questions

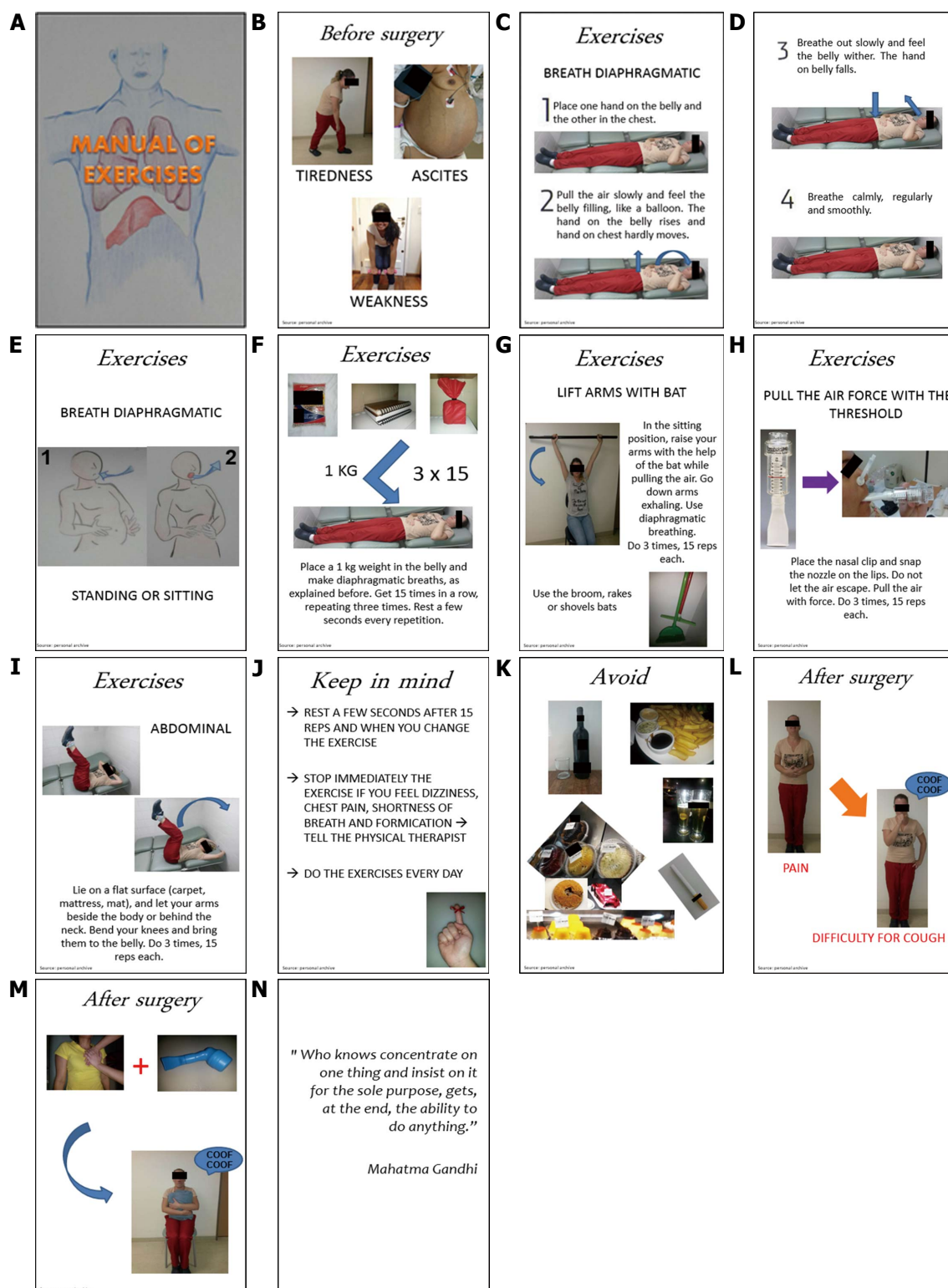


Figure 1 Manual of exercises for liver disease patients. A: Manual of exercises; B: Complications before the liver transplantation; C: How to do breath diaphragmatic; D: How to do breath diaphragmatic; E: Breath diaphragmatic standing or sitting; F: Breath diaphragmatic with weight on the belly; G: Lift arms with bat; H: Training with Threshold IMT®; I: How to do abdominal exercises; J: Instructions for patients; K: To avoid alcoholic beverages, tobacco, frying and pastries; L: Complications after liver transplantation; M: Physical therapy after surgery; N: Incentive phrase for patients.

and followed up these patients monthly by phone.

Patients were aware regarding diaphragmatic breathing and instructed to perform this breathing in all the exercises.

In addition to the diaphragmatic breathing, the

exercises described in the manual were: Diaphragmatic isometric exercise with the patient in the supine position and 1 kg of weight placed on the diaphragm muscle, exercise with Threshold inspiratory muscular training (IMT)® (Philips Respironics®), elevation of upper limbs

Table 2 Comparison between the control and intervention groups

	Control (<i>n</i> = 23)		Intervention (<i>n</i> = 14)		<i>P</i>
	Initial	Final	Initial	Final	
MIP (cmH ₂ O)	88.5 (44.1)	98.3 (39.2)	101.1 (34.4)	117.9 (43)	0.45
MEP (cmH ₂ O)	108.3 (46.3)	116.5 (51.8)	113.6 (31)	128.2 (35)	0.61
EMG rectus (μV)	52.9 (51.1)	46.1 (29.7)	32.5 (12.4)	28.8 (7.9)	0.65
EMG diaphragm (μV)	43.8 (14.9)	53.8 (22.4)	55.7 (34.7)	35.6 (15.8)	0.001 ¹
FVC (%)	84.6 (13.9)	85.5 (16.1)	88.3 (14.5)	92.6 (14.2)	0.42
FEV ₁ (%)	84.6 (15.1)	85.4 (14.5)	88.6 (20)	90 (14.1)	0.5
FEF _{25%-75%} (%)	92.4 (31.2)	94.7 (24.6)	100.7 (47.1)	102.9 (44.2)	0.72
Functional capacity	68.5 (24.5)	71.7 (21.7)	69.3 (21.4)	84.6 (14.5)	0.006 ²
Physical role	52.2 (39.1)	45.7 (38.9)	60.7 (38.9)	55.4 (38.2)	0.92
Pain	61 (32)	61.3 (21.1)	62 (27.9)	56.7 (30.1)	0.78
General health	52.8 (26.2)	58.4 (26.3)	59.3 (20.1)	68.4 (19.3)	0.4
Vitality	61.7 (23.9)	59.8 (23.2)	58.9 (15.2)	65 (25.2)	0.33
Social aspects	58.2 (34.9)	67.9 (29.9)	68.8 (37.3)	75.9 (30)	0.93
Emotional role	60.8 (39.8)	56.5 (44.3)	45.1 (44.5)	61.9 (36.6)	0.16
Mental health	59.1 (26.4)	64.5 (24)	64.9 (20.7)	78.3 (22)	0.14

¹Difference between groups; ²Difference in the intervention group after three months. MIP (cmH₂O): Maximal inspiratory pressure; MEP (cmH₂O): Maximal expiratory pressure; EMG: Electromyography; FVC: Forced vital capacity; FEV₁: Forced expiratory volume in one second; FEF_{25%-75%}: Forced expiratory flow rate at 25%-75%.

with the help of a bat and strengthening the abdominal muscles.

The manual contained information of the possible complications during the postoperative period. It was highlighted the importance of bronchial hygiene and the proper way to cough in the postoperative period. Patients were instructed to perform the exercises in three sets of fifteen repetitions.

The control group did not perform the exercises; the second evaluation was done three months after the first.

The patients' tolerance was a parameter for the choice of load for training with the Threshold IMT[®], since it is already established in the literature that liver disease patients have fatigue^[17,22,23] and interrupt the exercise early with low peak VO₂^[15,18].

The manual also contained orientation for patients regarding the avoidance of alcohol, cigarettes, sweets and fried foods and had information about the importance of exercises during postoperative recovery from liver transplantation, especially concerning bronchial hygiene and effective cough.

Statistical analysis

The Statistical Analysis System (SAS) System for Windows (SAS Institute Inc, Cary, NC, United States), version 9.2 was used for statistical analysis.

Position and dispersion measures were used for numerical variables and frequency tables for categorical variables, for descriptive analysis.

For comparison of proportions, the χ^2 test or Fisher's exact test were used when necessary. For comparison of numerical measurements between two groups the exact Mann-Whitney test was used. For comparison of measurements between groups and times, ANOVA for repeated measurements was employed or post hoc transformation. To compare changes in proportions, the

McNemar test was used. The significance level used was $P < 0.05$.

RESULTS

There was significant difference ($P = 0.01$) between the first (initial) and the third month (final) MIP in the control group and in the intervention group, but there was no difference ($P = 0.45$) between the groups.

After three months, the electromyography of the diaphragm represented by RMS decreased in the intervention group ($P = 0.001$) compared to that of the control group.

The score of the domain functional capacity (SF-36) was not statistically different between the groups; however, in the intervention group there was a significant increase in the score ($P = 0.006$) after three months.

The general health and mental health domains received higher scores after three months in the control group ($P = 0.01$) and the intervention group ($P = 0.004$), but there was no significant difference between them.

The descriptive analysis and comparison between groups are detailed in Table 2.

In the first evaluation, 10 patients had ascites in the control group and 3 had ascites in the intervention group. After three months, 13 patients had ascites in the control group and 5 patients had ascites in the intervention group.

The comparison between the presence of initial ascites with the presence of ascites was performed after three months in the control group ($P = 0.083$) and intervention group ($P = 0.31$). There was no significant difference in relation to the presence of ascites after three months between groups ($P = 0.21$).

The presence or absence of ascites three months after the first assessment was compared with age, BMI, MIP, MEP, RMS of the diaphragm and rectus abdominis,

FVC, FEV₁, FEF_{25%-75%} and the SF-36 domains.

There was no significant difference between the variables in the control group. In the intervention group, patients with ascites at the end of the time period had decrease of scores on the social aspects domain ($P = 0.023$) compared to those who had no ascites.

DISCUSSION

Patients on the waiting list for liver transplantation waiting a long time for the new organ and consequently, there may be complications in this period, such as sarcopenia. Therefore, rehabilitation becomes an important alternative in order to reduce inactivity, increase muscle performance, as well as exercise tolerance, and to avoid complications in the post-operative period^[24].

The results of this study showed that most patients were men, aged above 50 years and BMI revealing overweight. These findings are consistent with other studies^[9,25].

Several authors^[26,27] have recommended IMT in order to minimize respiratory muscle dysfunction in the postoperative period of cardiac, thoracic and abdominal surgery. Despite the literature employing a 40%^[28] initial MIP load for IMT and increasing it over time training, patients' tolerance has been responsible for the choice of load for training with the Threshold IMT®, since it is already established in the literature that liver disease patients have fatigue^[20-23].

In the current study, there was a significant increase of MIP in the intervention group after the final evaluation.

In the study of Gosselink *et al.*^[29], a meta-analysis was performed on the effects of IMT in patients with chronic obstructive pulmonary disease. The study revealed better results in the inspiratory muscle strength, functional capacity and dyspnea after strength training.

In the study by Serón *et al.*^[28], the Threshold IMT® was effective for strengthening inspiratory muscles.

One possible explanation for the non-significant increase in MEP in the present study is that the main focus of the prepared manual was to strengthen the inspiratory muscles. Unlike what was expected, the control group also showed a significant increase in MIP after three months. One possible explanation is that the patients were not discouraged from performing physical activities or were advised to stop exercising because they were participating in the research.

The intervention proved to be effective in this study; after three months there was a reduction of the RMS of the diaphragm in the intervention group, and due to the increase of the inspiratory muscle strength, the diaphragm needed to perform less force in order to overcome the same resistance. In other words, the action's potential decreased since only a small amount of fibers were needed to be recruited during normal breathing. No articles on the effects of inspiratory muscle training on electromyography of the diaphragm have been found; therefore, further studies are required for a

broader discussion on the issue.

The exercise program also provided relevant improvement in functional capacity domain. This means that the difficulty in performing daily life activities decreased, and individuals became more active and willing. Regarding liver transplant, two authors^[30,31] proved that the quality of life can be improved with physical exercises.

The general and mental health areas received higher scores after three months in the intervention and control groups, demonstrating that the patients' perception of their health improved. The control group may have presented positive changes in the mentioned aspects for the same reasons already explained above.

Also, one must consider, on average, an increase of some values of variables (FVC, FEV₁, FEF_{25%-75%}, vitality, social aspects and emotional role) at the end time, in the intervention group, showing the positive effects of the intervention performed.

The two groups were not ideally matched, because the incidence of ascites was lower in the intervention group in the first evaluation and after three months. However, the presence of ascites did not affect the respiratory variables evaluated. In the intervention group, patients with ascites had worse scores on the social aspects domain. In the final stage of cirrhosis, ascites causes the appearance of symptoms that can impair the performance of activities of daily living^[32]. All these factors contribute to social isolation being away from work and low self-esteem. According to Saab *et al.*^[33], the ascites, associated or not with encephalopathy, was associated with poorer quality of life.

Certainly, new studies on the benefits of breathing exercises will be necessary after liver transplantation. However, the results of the present study are satisfactory regarding the improvement of quality of life as well as the electrical diaphragm activity result using the exercises learnt in the preoperative manual. This study is the beginning of exercise protocols developed specifically for this group, and it may prompt new research with a larger population sample.

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COMMENTS

Background

Some changes usually affect the quality of life of patients with chronic liver disease, such as fatigue, malnutrition and predominance of anaerobic metabolism. In order to improve the functionality, muscle strength and physical conditioning of the liver disease patients and physically prepare them for transplantation, minimizing possible postoperative complications, specific preoperative rehabilitation programs for this population become necessary.

Research frontiers

The liver transplant waiting list patients belong to the Unit of Liver Trans-

plantation at the Hospital de Clinicas/Unicamp, and are from several cities in the state of Sao Paulo, and other regions of Brazil. Therefore, it was difficult to weekly or even monthly require patients to participate in evaluations or in the respiratory intervention group at the Unit of Liver Transplantation. In addition, these patients are constantly doing exams, have difficult schedules, and often need help for locomotion. As a result, these patients were followed up by phone each month. Despite these hardships, in the current study, only three participants were excluded from the trial, since they declined to perform the exercises. The other patients satisfactorily agreed to the exercises. Due to lack of financial resources, the authors used the analog manometer and some Thresholds inspiratory muscular training (IMT)[®] were donated by Philips Respironics[®]. Each participant remained with the Threshold IMT[®] for three months. This contributed to the reduced sample in the intervention group, in addition to other factors, such as death, abandonment or the transplant itself.

Innovations and breakthroughs

An illustrative and explanatory manual was prepared with breathing exercises to be performed by patients at home, for a period of three months. Monthly, they were accompanied by the same researcher by telephone, and doubts were resolved.

Applications

The results found in the group that performed the exercises were encouraging; there was a decrease in the electrical activity of the diaphragm and increase some scores of the short form 36 domains. These results represent a start for new rehabilitation programs which are developed preoperatively. Still, the proposed manual in this article may be used in other studies, with extended samples, and further positive results may be found.

Terminology

The manual of the exercises was prepared by the researchers and consisted of breathing exercises, including the Threshold IMT[®]. The Threshold is a device designed for respiratory muscle training in which the load is independent of the air flow. It consists of a chamber where at the distal end there is a valve which is held closed by the positive pressure (graduated in cmH₂O) of a spring. If a negative pressure with an absolute value greater than the spring pressure is generated, the valve will open and allow the air passage.

Peer-review

Studies on the effects of exercises, mainly those on breathing, in liver disease awaiting transplantation are rare in the literature. This issue can spark interest in other researchers who want to study the manual exercises in an enlarged sample of liver disease patients and who also want to follow up these patients postoperatively, evaluating the effects of exercises in this period. Another possibility is to use this study as a basis for development of new specific exercise programs before surgery for this population.

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Islet autotransplantation in a patient with hypercoagulable disorder

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Abstract

Total pancreatectomy and islet auto transplantation is a good option for chronic pancreatitis patients who suffer from significant pain, poor quality of life, and the potential of type 3C diabetes and pancreatic cancer. Portal vein thrombosis is the most feared complication of the surgery and chances are increased if the patient has a hypercoagulable disorder. We present a challenging case of islet auto transplantation from our institution. A 29-year-old woman with plasminogen activator inhibitor-4G/4G variant and a clinical history of venous thrombosis was successfully managed with a precise peri- and post-operative anticoagulation protocol. In this paper we discuss the anti-coagulation protocol for safely and successfully caring out islet transplantation and associated risks and benefits.

Key words: Islet transplantation; Autoislet transplant; Pancreatectomy; Chronic pancreatitis; Hypercoagulable disorder; Heparin

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Core tip: Total pancreatectomy and islet auto-transplantation is an option for select patients with chronic pancreatitis. Portal vein thrombosis is the most feared surgical complication and chances are increased if the patient has a hypercoagulable disorder. The paper describes important topics like the management of the anticoagulation in the peri-operative period.

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INTRODUCTION

Patients with chronic pancreatitis suffer from significant pain and associated decrease in the quality of life and also a potential of forming type 3C diabetes and the pancreatic cancers^[1-4]. It is an inflammatory disease, which is characterized by irreversible, morphological changes that cause permanent loss of function, and fibrosis and development of severe pain and complications. Over time, fibrosis in the pancreas, results in destruction of the islet cells, and patients are at risk of diabetes^[1,3,4]. The risk of pancreatic cancer is 10 to 15 fold higher in chronic pancreatitis patients and if it is associated with hereditary pancreatitis with genetic mutations, then the lifetime risk is 75%^[2,5]. Many surgical, medical, endoscopic and intervention radiological treatments are applied to these patients, despite which many still suffer from continuous dependence on narcotics and bad quality of life.

Removal of the pancreas followed by autologous islet cell transplantation is a great option for selected patients with chronic pancreatitis^[6-14]. Islet auto transplantation helps to take care of 3 Ps that are necessary for this disorder: (1) Pain relief; (2) Prevention of the brittle diabetes mellitus; and (3) Prevention of pancreatic cancer^[15]. At times, the results of the autologous islet cell transplantation are criticized because the variable insulin independence rate reported^[16,17]. We have previously argued that the insulin independence is not the only marker of the success, the wide marker of the success would be euglycemia, preventing cancer and having better quality of life^[15].

Good outcomes of islet auto transplantation are based on various factors from selection of the case to performing safe surgery, good isolation and safe injection of the cells followed by good engraftment of the islet cells. Once the islets are isolated and brought back to the patient, a small angiocatheter is introduced in one of the vessels either the splenic vein stump or any vessels draining into the superior mesenteric vein to infuse these cells into the portal vein so that they can flow to the liver. Safety is important in terms of decreasing the risk of thrombogenesis in these vessels by paying attention to the details of the procedure, the physiology of the patient, and the liver pathology^[18]. Surgical complications are most dreaded compared to the long-term outcome and insulin dependency because they can add to significant morbidity and therefore poor quality of life to the patient. Porto-venous thrombosis would arguably be the most important complication. It can vary in magnitude from a segmental vein to thrombosis of the main portal vein and potentially complete thrombosis of the superior mesenteric access requiring a bowel resection and consequent problems^[19,20]. The risk of portal vein thrombosis will be increased if the patient has a hypercoagulable disorder.

We report a case from our new program with physiological challenge in the context of issues described. These include a case of islet autotransplantation per-

formed in a patient with a hypercoagulable disorder. To our knowledge, it is the first such case in the literature.

CASE REPORT

The patient was a 29-year-old lady (body weight 83 kg, body mass index 29.3 kg/m²) with a history of chronic abdominal pain related to chronic pancreatitis. At the time of her initial visit she was in the emergency room or hospitalized on a weekly basis. Her history dated back 13 years and she had been on narcotics for 6 years. She had undergone 7 endoscopic retrograde cholangiopancreatographys over the years and magnetic resonance imaging (MRI) had shown pancreas divisum. Our own MRI scoring system^[21] indicated minimal pancreatic damage (atrophy, 1/6). The pre-operative C-peptide was 1.75 ng/mL and hemoglobin A1c was 5.5%. We also considered gall stone disease, alcohol and completed a genetic analysis for common hereditary gene mutations that are causally associated with chronic pancreatitis. She had also reported having developed thrombosis related to PICC line placement on multiple occasions at an outside institution. During her evaluation we obtained hypercoagulability studies, which included factor V Leiden mutation, prothrombin gene mutation, plasminogen activator inhibitor-1 (*PAI-1*) gene mutation and level, clotting factor VII, protein C, protein S levels, methylenetetrahydrofolate reductase (*MTHFR*) gene mutations and an autoimmune thrombophilia screen. She was found to be homozygous for the 4G variant of the *PAI-1* gene and heterozygote for the *MTHFR* A1298C.

Her surgery was performed using the technique described earlier^[22] and islet infusion was also done through splenic vein stump. Islet preparation was performed at the current good manufacturing practice facility in the Islet Cell Laboratory at the Georgetown University Hospital.

The pancreas was explanted post 1 and half min of warm ischemia time and placed immediately into an ice-cold Viaspan solution in a sterile container and delivered to the lab on ice. On arrival of the lab, the pancreatic duct was cannulated after trimming. The pancreas was then divided into two portions at the neck. On the cut surface both openings of the pancreatic duct were cannulated with a 14-gauge cannula. An enzyme solution containing collagenase HA and Thermolysin (Vitacyte, Indiana, United States) was infused into the pancreas through the cannula and connected with a 60 cc syringe through an extension tube. In addition, the parenchyma was then repeatedly injected with the enzyme solution using a 60 cc syringe. The thoroughly distended pancreas was then digested using the semi-automated method of Ricordi^[23]. The pancreas weighed 65.9 g. The total cold ischemia time from removal of the pancreas to completion of trimming was 51 min. The digestion rate was 92.2% post 18 min of digestion. After purification using a modified continuous density gradient method with cell processor COBE2991^[24], the

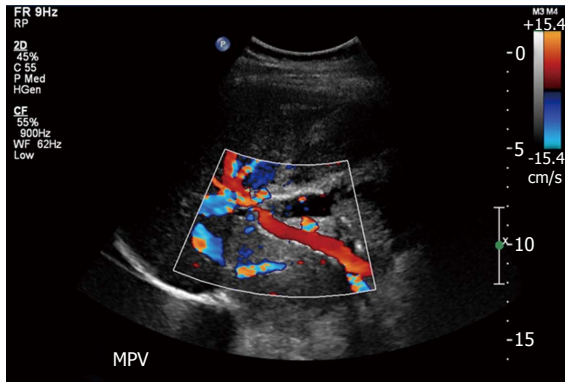


Figure 1 Post-operative Doppler ultrasound of the liver demonstrating widely patent portal vein with normal direction of blood flow. MPV: Main portal vein.

final pellet was reduced from 36 to 12 mL^[25]. The total islet yield was 459164 islet equivalents (IEQ) which was quantified as IEQ by normalizing the islet mass to an islet size of 150 μ m diameter. The islet recovery was 7552 IEQ/g of pancreas tissue. The final pellet was suspended in the transplantation media (5% human serum albumin) containing 35 units of Heparin per kilogram of patient body weight. In total, 5532 IEQ per kilogram recipient body weight (IEQ/kg) of islets were available.

The islet infusion in to the liver involved a venous catheter placed in a splenic vein stump and advanced intravenously towards the portal vein. In order to reduce complication rates of acute portal hypertension and thrombosis in this case at the most, low-volume (12 mL pellet) prepared through purification procedure, was infused. We gave the patient 35 U/kg intravenously in addition to the 35 U/kg of Heparin along with islet infusion; the patient therefore received a total dose of 70 U/kg of heparin. Portal pressures were closely monitored during infusion, because of an established tenfold (1.52%-15.2%) increase in the risk of thrombosis with portal pressure changes above 25 cm H₂O^[25]. The pre infusion portal pressure was 4.5 cm/saline and the post infusion pressure was 15 cm/saline.

Heparin was started intra-operatively. Fifty IU/kg of body weight bolus before the infusion of islet cells followed by 25000 IU mixed with 500 mL of D5 1/2 normal saline at the rate of 10 IU/kg per hour. Postoperatively, the patient was continued on a heparin drip according to our protocol and activated thromboplastin time was maintained in the range of 50 to 60 s. At the end of three days when she started on clear liquid diet, we continued the patient on low molecular weight heparin and monitored with anti-Xa activity factors maintained between 0.6 to 1 international units/mL. Postoperative Doppler ultrasound of the liver was performed on day 1, 2 and 5 and once weekly for one month and biweekly for another two months. Specifically, the doppler studies during the first week demonstrated patency and normal flow in the portal veins, hepatic arteries and veins; the main portal vein peak velocities

ranged between 25-38 cm/s, left and right portal vein velocities ranged from 11-27 cm/s (Figure 1). The patient was discharge home after 14 d. At three months the patient was off insulin with a C-peptide of 1.95 ng/mL. At the end of three months, the dose of low molecular weight heparin was reduced to maintain anti-Xa level between 0.3 to 0.6 international units/mL. Six months after the surgery, the low molecular weight heparin was discontinued after consultation with hematology. The patient did not develop venous thrombosis of any form during follow-up and was able to resume a normal life.

DISCUSSION

Total pancreatectomy and islet auto transplantation has been described by some as a radical option though it has a clear role for patients with chronic pancreatitis. Patients undergo multiple endoscopic procedures and fail to get a satisfactory outcome and all the time their narcotic requirement keeps escalating. This definitive procedure is feared because of surgical complications like portal vein thrombosis and also the failure of the islets to prevent diabetes.

Hypercoagulability is a significant risk factor for portal vein thrombosis. In one study 28% of patients with portal vein thrombosis had an inherited thrombophilic disorder^[26]. Of this factor V Leiden mutation was the most common (11%) followed by anti-thrombin III deficiency (11%) and protein c deficiency (8%). Pro-thrombin gene mutations are also commonly implicated in venous thrombosis^[27]. The PAI 4G variant and MTHFR mutations are considered less severe though do have an increased risk for venous thrombosis after major surgery including transplantation. Such situations are challenging because of the post-operative risk of thrombosis leading to graft failure or bleeding from anti-coagulation. However, many such transplants are carried out in a safe manner. Our patient had a *PAI-1* gene mutation, which was only diagnosed after diligent history taking helped us to obtain the risk in this case. The authors have previously worked at different auto islet cell transplantation centers and as with other surgeries it was not routine to do a hypercoagulable workup since obtaining this panel in every patient is very expensive and may not be cost effective^[15,18,22].

Portal vein thrombosis after islet auto-transplant though uncommon, can be risky and life threatening. There are few previous individual reports of portal vein thrombosis after islet auto-transplantation^[20] and one series that indicated a prevalence of 3.7% after clinical islet transplantation^[28]. There is however no systematic study of the cause of thrombosis in such cases. In a previous publication we have noted that there may be unrecognized mild fibrosis and or steatosis^[18]. We were however unable to show that any specific histologic pattern was more susceptible to venous thrombus formation. To prevent portal venous thrombosis in patients such as ours above with pre-existing risk factors it is imperative to identify at risk patients and manage these

patients with therapeutic anticoagulation with heparin. Heparin also has advantage in the islet engraftment process and hence it has dual advantage, but has a significant risk of post-operative bleeding and hence it is very important that the surgery is performed with good hemostasis. Heparin is given by almost all the centers performing auto-islet cell transplant to their patients. However, there are no consensus guidelines on the amount and duration it needs to given. We adapted an approach in which we start a heparin drip in operating room at the time of starting islet infusion after giving bolus. It is continued for the next three days maintaining the activated thromboplastin time in the range of 50 to 60 s. At the end of three days when the patient starts taking clears, we continue with low molecular weight heparin two times a day dose based on patient's weight with anti-Xa activity factors maintained between 0.6 to 1 international units/mL. Patient's postoperative Doppler ultrasound on the liver is done on postoperative day 1, 2 and 5 and subsequently was done once weekly for one month and then twice weekly for another two months if they are at high risk. High risk is defined by three main factor: (1) hypercoagulable disorder; (2) previous history of deep venous thrombosis other than segmental splenic vein thrombosis related to chronic pancreatitis (even if the hypercoagulable panel is normal); and (3) high portal pressure after infusion (more than 25 cm of saline). If the patient is high risk then at the end of three months, low molecular weight heparin dose is reduced to maintain anti-Xa level to be between 0.3 to 0.6 international units/mL. Six months after the surgery, the low molecular weight heparin is discontinued after consultation with hematology. If the patient is not at high risk then after two weeks dose is reduced and then stopped after another two weeks.

In summary, islet auto transplantation in itself is a challenging procedure and even more challenges can arise medically if there are physiological challenges like a hypercoagulable disorder. Despite all these challenges with careful teamwork and experience, these patients can be safely managed.

Islet auto transplantation is a challenging procedure and even more challenges can arise medically; if there are physiological challenges like a hypercoagulable disorder. Despite all these challenges with careful teamwork and experience, these patients can be safely managed.

COMMENTS

Case characteristics

Total pancreatectomy and islet autotransplantation complicated by primary hypercoagulability that presented as repeated thrombosis of indwelling venous lines.

Clinical diagnosis

The presentation was characterized by symptoms of chronic pancreatitis and a history of deep venous thrombosis.

Differential diagnosis

An alternative explanation to a primary hypercoagulability to account for thrombosis if intravenous lines would be that the presence of intravenous lines themselves was the cause of catheter thrombosis.

Laboratory findings

Screening for hypercoagulability included plasma proteins, genetic defects and autoimmunity as potential causes of thrombosis with the patient having a plasminogen activator inhibitor-1 variant.

Imaging diagnosis

Serial ultrasounds were used to monitor for portal vein thrombosis after islet infusion in to the portal vein after total pancreatectomy.

Pathological diagnosis

Confirmation of chronic pancreatitis as the cause for abdominal pain.

Treatment

Heparin infusion followed by low molecular weight heparin and aspirin as prophylaxis for a prothrombotic state.

Related reports

There are previous cases of a hypercoagulability giving rise to deep venous thrombosis, most notably with factor V Leiden mutation.

Term explanation

Hypercoagulability refers to a pathological increase in the tendency to form intravascular clots. Patients undergoing major intraabdominal operations should be screened for a hypercoagulable state if there is any history of abnormal venous clot formation.

Peer-review

This a successful case of islet autotransplantation performed in a chronic pancreatitis patient suffered from significant pain with a hypercoagulable disorder. It is imperative to identify at risk patients and manage these patients with therapeutic anticoagulation with heparin to prevent portal venous thrombosis in patients with pre-existing risk factors. The author's careful teamwork and experience is helpful for safely managing these patients.

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Acute bacterial sternoclavicular osteomyelitis in a long-term renal transplant recipient

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Abstract

Kidney transplantation is the treatment of choice for a significant number of patients with end-stage renal disease. Although immunosuppression therapy improves graft and patient's survival, it is a major risk factor for infection following kidney transplantation altering clinical manifestations of the infectious diseases and complicating both the diagnosis and management of renal transplant recipients (RTRs). Existing literature is very limited regarding osteomyelitis in RTRs. Sternoclavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria. We present an interesting case of acute, bacterial sternoclavicular osteomyelitis in a long-term RTR. Blood cultures were positive for *Streptococcus mitis*, while the portal entry site was not identified. Magnetic resonance imaging of the sternoclavicular region and a three-phase bone scan were positive for sternoclavicular osteomyelitis. Eventually, the patient was successfully treated with Daptomycin as monotherapy. In the presence of immunosuppression, the transplant physician should always remain alert for opportunistic pathogens or unusual location of osteomyelitis.

Key words: Bacterial infections; Immunosuppression; Renal transplantation; Osteomyelitis

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Core tip: Although immunosuppression therapy improves kidney allograft and patient's survival, it is a major risk factor for infection following kidney transplantation, altering the clinical manifestations of the infectious diseases and complicating both the diagnosis and management of renal transplant recipients (RTRs). Existing literature regarding osteomyelitis in RTRs is very limited while sternoclavicular osteomyelitis is a rare entity presenting with its own unique set of risk factors and complications. Infections caused by unconventional

pathogens with unconventional infection sites are being increasingly diagnosed in RTRs and the physician should always remain alert when dealing with these patients.

Dounousi E, Duni A, Xiromeriti S, Pappas C, Siamopoulos KC. Acute bacterial sternoclavicular osteomyelitis in a long-term renal transplant recipient. *World J Transplant* 2016; 6(2): 442-446 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i2/442.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i2.442>

INTRODUCTION

Kidney transplantation is the treatment of choice for a significant number of patients with end-stage renal disease. Renal transplant recipients (RTRs) benefit from a longer life expectancy and a better quality of life. Despite, recent accomplishments in the field of kidney transplantation, both short- and long-term medical complications still exist. Infectious diseases constitute one of the most common complications after kidney transplantation and the second most common cause of death among RTRs with a functioning graft^[1]. Even though immunosuppressive therapy improves graft and patient survival, it has been reported that the increasing load of maintenance immunosuppression predisposes RTRs to clinically important infectious sequelae. The plethora, diversity and consequences of infectious complications in kidney transplantation have led to the accumulation of a growing amount of evidence describing the problem and trying at the same time to establish guidance for optimal management and support of these patients^[1].

Existing literature comprises of a very small number of cases reporting osteomyelitis in RTRs. Traditional risk factors for osteomyelitis include trauma to the bone and trauma near a site of infection, the presence of sickle-cell disease, rheumatoid arthritis, diabetes mellitus, dialysis and related procedures, as well as immunosuppression. Most cases of osteomyelitis in adults are of hematogenous origin and primarily affect the spine^[2]. The sternoclavicular joint is less commonly associated with osteomyelitis but presents its own unique set of risk factors and complications. We present a rare case of an adult long-term RTR who was diagnosed with acute, hematogenous sternoclavicular osteomyelitis due to streptococcus bacteremia whereas remarkably a portal entry site was not identified.

CASE REPORT

A 50-year-old male RTR, presented at the emergency department of our Tertiary University Hospital complaining about fever, chills and pain over the left sternoclavicular area, radiating to the shoulder and neck for the last two days. He denied any recent history of trauma, intravenous drug administration or dental procedure. Physical examination revealed pyrexia and

marked tenderness over the left sternoclavicular area which appeared warm, red and swollen. Laboratory exams showed an elevated white blood cell count and C-reactive protein (Table 1), while the cervical spine and chest X-rays were unremarkable. The patient was directly admitted to the Renal Unit Ward and serial blood cultures were taken.

The patient was a long-term RTR regularly followed up at the renal transplant outpatient clinic (OC) of our Hospital during the last year. On his last visit a month ago, he was asymptomatic with unremarkable clinical findings and stable renal function, with an estimated glomerular filtration rate (eGFR) of 41 mL/min per 1.73 m² (Modification of Diet in Renal Disease equation). Maintenance immunosuppression therapy included cyclosporine (75 mg bid, C2 levels of 436 ng/mL), mycophenolate mofetil (1 g bid) as well as prednisolone (5 mg qd). The patient was diagnosed with chronic kidney disease of unknown etiology more than twenty years ago, was treated with hemodialysis for approximately 7 years and subsequently received a renal allograft from a cadaveric donor 14 years ago. Three months after the transplantation, the patient had suffered an acute rejection episode, which was successfully treated with intravenous pulses of steroids. The rest current medical history included well controlled arterial hypertension (antihypertensive treatment: Amlodipine 10 mg qd) and hip osteopenia diagnosed by a Dual-energy X-ray absorptiometry scan (DEXA) (Alfacalcidol 0.25 µg qd).

Immediately after admission, imaging of the sternoclavicular area excluded the presence of a fluid collection that could be aspirated. Considering the patient's clinical findings and his long-term immunocompromised status, empirical treatment for septic arthritis with Vancomycin (dose adjusted to eGFR) and Ciprofloxacin was commenced. Further diagnostic workup included a dental examination which did not reveal a possible portal entry site for the bacteria. Abdominal ultrasound findings were unremarkable while ultrasound of the renal allograft was within normal. Urine cultures were negative. Transthoracic echocardiography revealed mild mitral regurgitation and calcifications of the aortic cusps and mitral annulus. A transesophageal ultrasound was subsequently performed, ruling out concomitant endocarditis.

All blood cultures became positive within 48 h for *Streptococcus mitis* (Viridans group streptococcus) with a good sensitivity profile, including Glycopeptides (Vancomycin minimum inhibitory concentration < 1 mg/L) and Daptomycin. Vancomycin treatment, targeting trough blood levels of 15-20 mg/L, was continued whereas Ciprofloxacin was stopped. In order to further evaluate the sternoclavicular joint and differentiate between septic arthritis and osteomyelitis, magnetic resonance imaging (MRI) (no gadolinium administration) of the region was performed. The MRI showed bone edema of the left intraarticular surface of the sternum and the clavicle together with soft tissue

Table 1 Patient's laboratory findings at admission and on discharge

	At admission	On discharge
Hemoglobin (g/dL)	13.3	11.3
WBC (/μL)	11760	11900
Neutro-Lympho-Mono (%)	83-6-10	83-7-7
PLT (/μL)	163000	290000
ESR (mm/h)	41	15
CRP (mg/L)	130	17
eGFR (mL/min per 1.73 m ²)	36	40
Urea (mg/dL)	86	76
Sodium (mEq/L)	136	139
Potassium (mEq/L)	4.2	4.3
PTH (pg/mL)	75	80
Phosphate (mg/dL)	3.9	2.7
Albumin (g/L)	3.4	3.4

WBC: White blood count; PLT: Platelets; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; eGFR: Estimate glomerular filtration rate (calculated by CKD-EPI formula); PTH: Parathyroid hormone.

edema, findings suggestive of acute sternoclavicular osteomyelitis (Figure 1). No intraarticular fluid collection was observed. A three-phase whole body bone scan (technetium-99m methylene diphosphonate) was subsequently performed, which showed focally intense, increased activity over the left sternoclavicular area, a finding positive for osteomyelitis (Figure 2).

A week from admission the patient continued to have low grade fever and was dependent on analgesics for pain control, despite achieving adequate Vancomycin trough levels. Considering the diagnosis of acute bacterial osteomyelitis with an unconventional location, the patient's clinical course, the need of long-term intravenous antibiotic treatment, the difficulties of Vancomycin treatment (monitoring levels and possible related nephrotoxicity) and practical issues (patient's residence was far from the hospital), the decision of switching antimicrobial treatment to Daptomycin as monotherapy (dose 4 mg/kg per 24 h) was taken. The patient became afebrile within a few days, inflammatory markers gradually declined and his physical status progressively improved (Table 1). No surgical debridement was performed as there was no evidence of a soft tissue abscess or subperiosteal collection, and no concomitant joint infection was diagnosed. The patient was discharged from the hospital a fortnight after admission, with recommendations for continuation of antimicrobial treatment for a total period of 6 wk and close medical follow-up at the renal transplant OC.

The patient remains asymptomatic and with preserved renal function six months after the completion of the antimicrobial treatment.

DISCUSSION

In the modern era of renal transplantation infectious diseases remain a major cause of morbidity and mortality in RTRs^[1]. The introduction of new immunosuppressant agents in renal transplantation along with the increasing

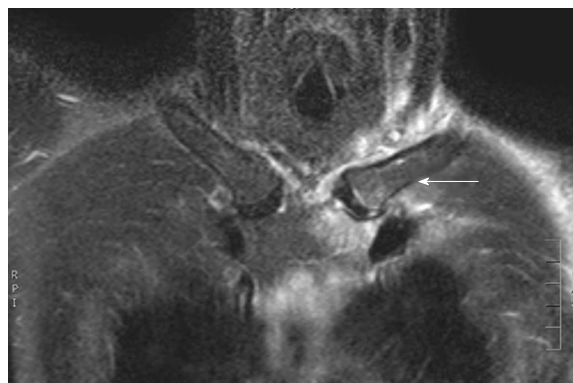


Figure 1 Magnetic resonance imaging of the sternoclavicular area showing edema on the left intraarticular surface of the sternum and the clavicle together with edema of the surroundings soft tissues (arrow).

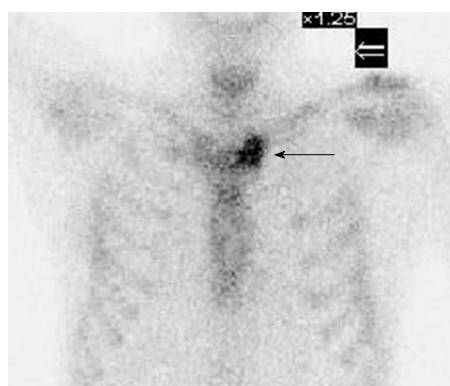


Figure 2 Three-phase bone scan (technetium-99m methylene diphosphonate) showing intense increased focal activity uptake over the left sternoclavicular area (arrow).

resistance of pathogens to antimicrobial agents worldwide are partially responsible for the emergence of rare infectious clinical cases which constitute a major challenge for the transplant clinicians. Here, we report an interesting, noteworthy case of acute bacterial sternoclavicular osteomyelitis in a long-term adult RTR, with no portal entry site for the bacteria which was successfully treated with Daptomycin as monotherapy.

In general, traditional risk factors for osteomyelitis include trauma to the bone and trauma near a site of infection, the presence of sickle-cell disease, rheumatoid arthritis, diabetes mellitus, dialysis and related procedures, as well as immunosuppression. Most cases of osteomyelitis in adults are of hematogenous origin and primarily affect the spine^[2]. The clavicle contains scanty red marrow and sparse vascular supply. It is an exceedingly rare site for osteomyelitis, especially of hematogenous origin^[3]. Clavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria^[4]. Thus, there are reports in the literature of sternoclavicular osteomyelitis following central line placement^[5], major head and neck surgery and radiation therapy to head and neck tumors^[6]. Intravenous drug abusers are an especially high risk group for clavicular

osteomyelitis and septic arthritis^[7].

With regard to the responsible pathogens, *S. aureus* is the most commonly isolated organism in most types of osteomyelitis, affecting 50%-70% of cases, while other gram positive cocci and gram negative bacilli are identified less often, accounting for approximately 20%-25% of acute osteomyelitis cases respectively^[8,9]. Treatment of osteomyelitis requires prolonged antimicrobial therapy and frequently adjunctive surgical therapy for the debridement of necrotic material in order to eradicate the infection. Antibiotic therapy should be adjusted to culture and susceptibility results. If culture results are not obtainable, broad spectrum empiric therapy, including Vancomycin together with an agent with activity against gram negative organisms, should be administered^[10-12].

Regarding selection of antimicrobial treatment in our patient, Daptomycin was finally chosen as it has exhibited activity in the treatment of gram positive bone and joint infections^[13]. It is rapidly bactericidal and appears effective against multidrug-resistant gram positive pathogens, commonly found in osteomyelitis and joint infections, even when other first-line antibacterial treatments have failed^[14-16]. Daptomycin is well tolerated; it has a relatively safe side effect profile, no interactions with calcineurin inhibitors, and a low risk of spontaneous resistance. The mode of action, rapid *in vitro* bactericidal activity against growing and stationary-phase bacteria, a once-daily dosing regimen, and no requirement for drug monitoring contribute to its potential therapeutic utility^[17].

Existing literature comprises of a very small number of cases reporting osteomyelitis in RTRs, which involve locations such as the ankle, the symphysis pubis or the vertebral column^[13,18-21], whereas there are no reports in the literature regarding sternoclavicular osteomyelitis in RTRs. The additive effect of long-term immunosuppression treatment and possibly osteopenia (although previous routine DEXA scans revealed only hip localized osteopenia) rendered our patient among patients' subgroups with increased risk for osteomyelitis. Remarkably, a portal entry site for the bacteremia was not identified. Finally, the sternoclavicular bone was the solitary site of infection as demonstrated from the imaging studies.

Considering the immune suppressed status as a predisposing factor for infections as well as the growing number of RTRs, we might come across more cases of unconventional pathogens and sites of infection in the future^[22]. Prevention, vigilance and deep knowledge of the diagnostic and therapeutic management of infections could potentially mitigate the consequences for RTRs.

COMMENTS

Case characteristics

A 50-year-old male renal transplant recipient complained about fever, chills and pain over the left sternoclavicular area, radiating to the shoulder and neck for

the last two days.

Clinical diagnosis

Physical examination revealed pyrexia and marked tenderness over the left sternoclavicular area which appeared warm, red and swollen.

Differential diagnosis

Differential diagnosis was between septic arthritis and osteomyelitis.

Laboratory diagnosis

Laboratory exams showed an elevated white blood cell count and C-reactive protein and all blood cultures became positive within 48 h for *Streptococcus mitis*.

Imaging diagnosis

Magnetic resonance imaging (no gadolinium administration) of the region showed bone edema of the left intraarticular surface of the sternum and the clavicle together with soft tissue edema, without intraarticular fluid collection and a three-phase whole body bone scan [technetium-99m methylene diphosphonate (⁹⁹Tc-MDP)] showed focally intense, increased activity over the left sternoclavicular area, findings positive for osteomyelitis.

Treatment

Empirical treatment for septic arthritis with Vancomycin and Ciprofloxacin was commenced and subsequently switched to treatment with Daptomycin as monotherapy.

Related reports

Clavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria as occurs following central line placement, major head and neck surgery and radiation therapy to head and neck tumors.

Term explanation

A three-phase whole body bone scan is a ⁹⁹Tc-MDP based diagnostic test is used in nuclear medicine in order to detect different types of pathology in the bones. The three phases are the flow phase, the blood pool image and the delayed phase. Differential diagnosis is based on differential image processing from the three phases; Calcineurin inhibitors: Cyclosporine and Tacrolimus - are a class of immunosuppressive drugs which are used as first line agents for maintenance therapy after kidney transplantation.

Experiences and lessons

Considering the immune suppressed status as a predisposing factor for infections as well as the growing number of renal transplant recipients, the authors might come across more cases of unconventional pathogens and sites of infection in the future.

Peer-review

The authors describe a very unusual complication occurring late after renal transplantation. The case report is well written and useful for the reader just because of the unusual complication.

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Cavitary lung lesion 6 years after renal transplantation

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Abstract

The differential diagnoses of a cavitary lung lesion in renal transplant recipients would include infection, malignancy and less commonly inflammatory diseases. Bacterial infection, Tuberculosis, Nocardiosis, fungal infections like Aspergillosis and Cryptococcosis need to be considered in these patients. Pulmonary cryptococcosis usually presents 16-21 mo after transplantation, more frequently in patients who have a high level of cumulative immunosuppression. Here we discuss an interesting patient who never received any induction/anti-rejection therapy but developed both BK virus nephropathy as well as severe pulmonary Cryptococcal infection after remaining stable for 6 years after transplantation. This case highlights the risk of serious opportunistic infections even in apparently low immunologic risk transplant recipients many years after transplantation.

Key words: Lung cavity; Immunosuppression; Renal transplantation

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Core tip: Here we discuss an interesting patient who never received any induction/anti-rejection therapy but developed both BK virus nephropathy as well as severe pulmonary Cryptococcal infection after remaining stable for 6 years after transplantation. This case highlights the risk of serious opportunistic infections even in apparently low immunologic risk transplant recipients many years after transplantation.

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INTRODUCTION

Fungal infections causing cavitory lung lesions usually manifest in transplant recipients who have received a high level of cumulative immunosuppression. We describe an unusual case, where a low risk transplant recipient who had been stable for 6 years developed severe pulmonary Cryptococcal disease and BK virus nephropathy.

CASE REPORT

A 40-year-old Indian man was admitted with low grade fever and dry cough for one month. He had end stage renal disease due to unclassified primary disease and had a live related renal transplantation with his sister as the donor in 2009. He was detected hepatitis B surface antigen (HBsAg) positive before transplantation and has been on Tenofovir since then. He received no induction and was initially maintained on Tacrolimus, Mycophenolate Mofetil (MMF) and Steroids. After a year, MMF was changed to Azathioprine due to financial constraints. He received Trimethoprim-Sulfamethoxazole for 6 mo after transplantation but no primary prophylaxis for Cytomegalovirus (CMV), Tuberculosis (TB) or fungal infection. His postoperative course was uneventful and he maintained serum creatinine of 1.1-1.2 mg/dL. He is a non smoker.

Clinically, the patient was febrile, hemodynamically stable and hypoxemic (SPO₂ 92% on room air) requiring oxygen by mask. Investigations revealed pancytopenia (Hb 7.4 g/dL, total leucocyte count -3400/cu mm, platelet count -87000/cu mm) and high serum creatinine (2.5 mg%). Azathioprine was stopped. Tacrolimus trough level was 3.7 ng/mL. Urinalysis was unremarkable. Graft biopsy showed BK virus (BKV) nephropathy and serum BKV plasma load was more than 10⁴ copies/mL.

He was started empirically on broad spectrum antibiotics. Blood and urine cultures and quantitative CMV PCR assay were non-contributory. A non-contrast CT thorax showed bilateral, multiple, diffuse centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with a larger cavity in apico posterior segment of upper lobe of left lung (Figure 1). Bronchoscopy with bronchoalveolar lavage (BAL) fluid cultures was unrevealing. Serum Cryptococcal antigen was negative. Serum and BAL fluid galactomannan were negative.

Since patient continued to be febrile, computed

tomography guided biopsy of the cavitory lesion in the left lung was done and the histopathology (Figure 2) showed Cryptococcal infection. He was treated with liposomal Amphotericin for 6 wk and given Fluconazole prophylaxis. Flucytosine was not available at that time. Patient showed clinical as well as radiologic improvement and was discharged on oral fluconazole. His pulmonary infection has subsequently recurred and now he is being treated with a combination of Amphotericin and Flucytosine.

DISCUSSION

A renal transplant recipient may present with a cavitory lung lesion due to infection, malignancy (post-transplant lymphoproliferative disorder) or inflammatory disease, though infections are the predominant causative factor^[1-3]. TB is the commonest cause of cavitory lung lesions in endemic areas like India and patients may receive empiric anti-TB therapy if the index of suspicion for rarer infections is not high and investigations are non-contributory. Aspergillosis (either angioinvasive or chronic necrotizing form) is the most common fungal infection associated with cavitation. Other causes are Nocardiosis, Cryptococcosis, Actinomycosis and rarely Legionella pneumophila. In a sick patient, the possibility of septic emboli has to be kept in mind^[1,2].

Cryptococcosis is the third most common fungal infection seen in transplant recipients^[4,5]. It typically occurs late with median time to onset being 16 to 21 mo after renal transplantation. However our patient presented very late - 6 years after transplantation. So besides TB and fungal infection, post-transplant lymphoproliferative disease was an important differential diagnosis considered. All factors which increased the cumulative immunosuppression in patients increase the risk of disseminated Cryptococcal disease. Presence of chronic liver disease and use of steroids, T cell depleting antibodies and Alemtuzumab are specifically associated with increased risk of Cryptococcosis. Calcineurin inhibitor based regimens are believed to be protective, being associated more commonly with Cryptococcosis limited to lungs with less likelihood of dissemination^[5,6].

Our patient is HBsAg positive. But he had not received induction, had no history of rejection requiring pulse steroid therapy and has not been on MMF for 5 years. Though the apparent dose of immunosuppressive drugs given seems to be low, his cumulative immunosuppression level is definitely high as is suggested by the onset of late BKV associated nephropathy.

Cryptococcal infection commonly presents with neurologic disease (meningitis) or pneumonia. But it may also involve the skin and soft tissue, bones, joints and other organs like the liver and the kidney. Isolated pulmonary disease is uncommon seen in only 33% of the patients. Serum Cryptococcal antigen has 90% sensitivity in disseminated disease but may be

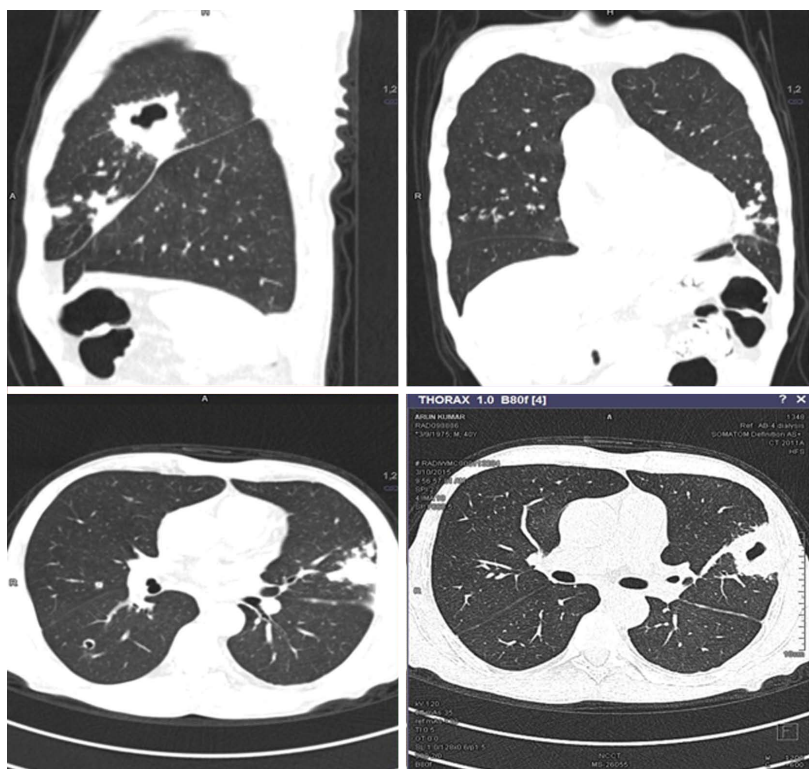


Figure 1 Multiple diffuse bilateral centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with larger cavity in apico posterior segment of upper lobe of left lung.

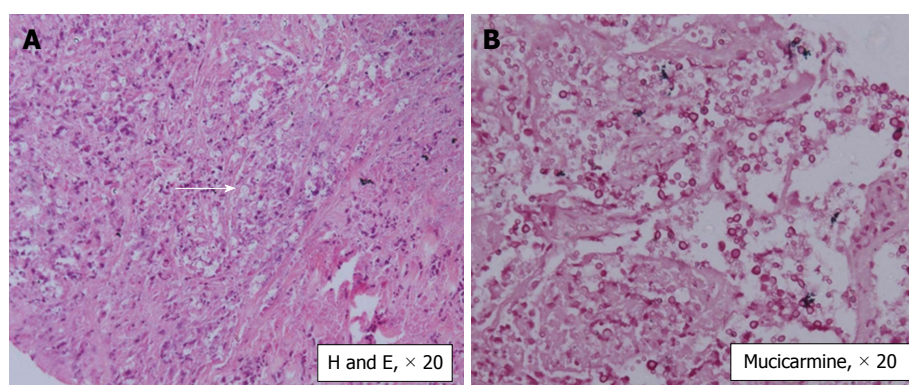


Figure 2 The histopathology showed cryptococcal infection. Histopathology of the lung lesion shows: A: Large area of necrosis with numerous capsulated yeast forms of fungi (arrow) morphologically resembling *Cryptococcus*; B: Special histochemical stain (Mucicarmine) highlights its polysaccharide capsule.

negative in immunosuppressed patients especially with isolated pulmonary disease^[5] as seen in our patient. The final diagnosis is by tissue biopsy and/or culture. The organism can be recognized by its oval shape, and narrow-based budding on histopathology. With the use of mucicarmine staining, the *Cryptococcal* capsule will stain rose to burgundy in color and help differentiate *Cryptococcus neoformans* from other yeasts, especially *Blastomyces dermatitidis* and *Histoplasma capsulatum*^[5].

Choice of antifungal therapy depends on the severity and extent of the disease. In patients with severe pulmonary infection, neurological involvement and disseminated disease, combination of liposomal Amphotericin with Flucytosine for 2 wk followed by Fluconazole for

12 mo is recommended. If Flucytosine is not available, which was the case initially in our patient, Amphotericin should be given for a minimum of 4-6 wk^[5].

Cryptococcal infection has an overall mortality of 14% in solid organ transplant recipients^[6]. Early diagnosis and initiation of treatment is the key to survival. A high index of suspicion and step-wise approach to diagnosis including a lung biopsy is required as the duration of therapy differs significantly from other fungal infections.

COMMENTS

Case characteristics

A 40-year-old male renal transplant recipient presented with low grade fever

and dry cough for one month.

Clinical diagnosis

A febrile patient with respiratory symptoms.

Differential diagnosis

Chest infection-bacterial/Tuberculosis/fungal.

Laboratory diagnosis

Pancytopenia with high serum creatinine.

Imaging diagnosis

Non contrast computed tomography scan of chest showed bilateral, multiple, diffuse centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with a larger cavity in apico posterior segment of upper lobe of left lung.

Pathological diagnosis

Biopsy from the lung lesion showed Cryptococcal infection and graft kidney biopsy showed BK virus associated nephropathy.

Treatment

He was treated with liposomal Amphotericin and Flucytosine.

Related reports

A renal transplant recipient may present with a cavitory lung lesion due to infection, malignancy (post-transplant lymphoproliferative disorder) or inflammatory disease, though infections are the predominant causative factor.

Term explanation

Cryptococcal infection is the third most common fungal infection seen in transplant recipients. It commonly presents with neurologic disease (meningitis) or pneumonia, but may also involve the skin and soft tissue, bones, joints and

other organs like the liver and the kidney.

Experiences and lessons

Tissue biopsy or culture is required to diagnose isolated pulmonary cryptococcosis. Early diagnosis and initiation of treatment is essential for survival.

Peer-review

The case discusses an important issue in patients with kidney transplantation.

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Deceased organ donation for transplantation: Challenges and opportunities

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Abstract

Organ transplantation saves thousands of lives every

year but the shortage of donors is a major limiting factor to increase transplantation rates. To allow more patients to be transplanted before they die on the wait-list an increase in the number of donors is necessary. Patients with devastating irreversible brain injury, if medically suitable, are potential deceased donors and strategies are needed to successfully convert them into actual donors. Multiple steps in the process of deceased organ donation can be targeted to increase the number of organs suitable for transplant. In this review, after describing this process, we discuss current challenges and potential strategies to expand the pool of deceased donors.

Key words: Consent; Eligible death; Imminent brain death; Organ procurement; Potential organ donor

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Core tip: An increase in the number of donors is necessary to allow more patients to be transplanted before they die on the wait-list. Multiple steps in the process of deceased organ donation can be targeted to increase the number of organs suitable for transplant.

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INTRODUCTION

Several obstacles have been overcome over the last few decades to make organ transplantation an effective life-saving treatment for many patients. Among them, the refinement of surgical techniques and the availability of effective immunosuppressive regimens against rejection

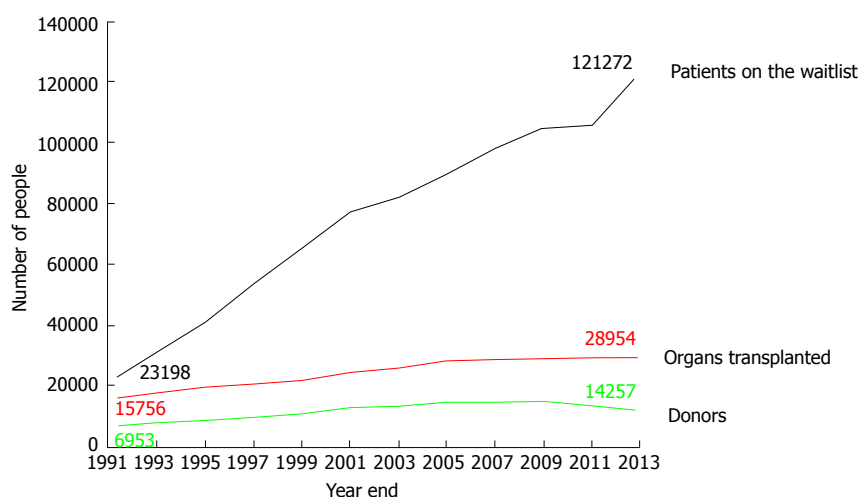


Figure 1 The gap between organs needed and organs available continues to grow. Available from: URL: <http://www.organdonor.gov/about/data.html>.

have played a major role. However, only the availability of donated organs from deceased persons (DD) has made it possible for organ transplantation to become an established, worldwide treatment for patients with organ failure. Without the “gift of life” from deceased donors, it is difficult to imagine how so many lives could have been saved. Currently, the shortage of organs is a major obstacle to making organ transplantation more accessible to a larger number of candidates. Only 30973 transplants from 15064 donors have been performed in the United States in the year 2015, while more than 121000 candidates were waiting for a transplant^[1]. Furthermore, the gap between the number of patients on the wait list and the limited number of available organs continues to widen. As a consequence, more than 6000 patients die every year while waiting for a transplant. In the ideal situation of an unlimited organ supply, virtually no patient would die on the wait list. Instead, due to the persistent scarcity of organs, a candidate for transplant has a 10%-30% chance of dying, depending on the organ, while on the wait list to receive an organ.

The common parameter adopted in different countries to measure the activity of organ donation has been traditionally the number of donors/million population. Although this metric is prone to the flaws of regional variations in health status, it is still used worldwide^[2]. In this review, because our observations are limited to the United States, we will refer instead to the total number of donors/year.

The shortage of organs has been recognized worldwide as a major limiting factor to organ transplantation. The World Health Organization and several international agencies have addressed organ shortage at different levels^[3-7]. Over the past decade, several initiatives have been put into place in the United States to address the shortage of organs. Among them, The Organ Donation Breakthrough Collaborative, funded by the Division of Transplantation in the Health Resources and Services Administration of the Department of Health and Human Services, was launched in September 2003 with the

intent of increasing the number of organs available for transplant. The goal of this initiative was to achieve a donor conversion rate (*i.e.*, from eligible to actual donor, see below) of 75% or higher across the country. Since its inception, more than 180 hospitals have met or exceeded this goal. Another goal proposed in this initiative was to increase the number of organs transplanted per donor. Subsequently, the Institute of Medicine (IoM) published the document “Organ Donation: Opportunities for Action”^[8]. This report emphasized that the current system of organ donation could be greatly improved and offered a number of specific recommendations to help increase the supply of transplantable organs. Given the wide variation in consent rate, ranging between 30% and 70%, across Organ Procurement Organizations (OPO), the IoM recommended the identification of best practices and their dissemination among institutions in the organ-procurement and transplantation system. In addition, the IoM report suggested to devote research efforts to identify new ways to improve the system and increase donation rates. Importantly, among them, it was recommended to integrate organ donation in the process of end-of-life care, recognizing that patients and their families should be offered the opportunity to donate as part of the standard care at the end of life. Still, after those and other efforts, over the last decade the donation rate from deceased donors has remained stagnant in the United States (Figure 1).

Brain dead donors

The vast majority (80%-90%) of organs from DD are procured after declaration of death by neurologic criteria (or “brain death”, BD). Brain death is determined after irreversible cessation of brain stem activity documented by bedside neurologic tests (reflexes, Table 1).

The oxygenation of a comatose person who suffered a devastating irreversible brain injury fulfilling the criteria for brain death is maintained by mechanical ventilation, while cardio-circulatory activity and organ perfusion is supported, if needed, by inotropic medications.

Table 1 Brain stem reflexes

Corneal reflex
Cough reflex
Facial motor response to painful stimuli
Gag reflex
Oculocephalic reflex ("Doll's eyes")
Oculovestibular reflex (caloric response)
Pupillary response to light

Donation after cardiac death

Unlike BD donors, a proportion of DD, currently 16% of the organs procured nationally, are recovered after declaration of death by circulatory criteria [donation after cardiac death (DCD)]^[9]. In this scenario, patients who have suffered severe brain injury but do not fulfill the criteria for brain death, may still be organ donors if the patient, by advance directive, or the patient's family decides to withdraw life support. In these circumstances, after consent for organ donation has been obtained, the patient is brought to the operating room where ventilation is disconnected and life-sustaining medications are withdrawn. After the cessation of cardio-circulatory activity for 2-5 min, depending on the local protocol, the patient is pronounced dead by a member of the primary team. After declaration of death the organ procurement team arrives to the operating room and begins organ recovery. The different dynamics involved in BD and DCD pathways and their implications on organ allocation and function are beyond the scope of this review. For historical purposes, it is interesting to note that at the beginning of organ transplantation in the 1960s all organs were procured from DCD donors, since the concept and legislation of brain death had not been developed. Only in 1968, an *ad hoc* committee at Harvard Medical School defined brain death as the state of irreversible coma with unresponsiveness and lack of reactivity, absence of movement and breathing and absence of brain-stem reflexes^[10]. Since then, the vast majority of DD have been BD. Only over the past decade there has been an increase in the proportion of DCD from 7% in 2005 to the current 16% of all deceased donors, with wide regional variation ranging between 7%-30%. The recent increase in the proportion of DCD donors has paired with only a small increase in the total number of DD. This has raised the legitimate concern whether the BD pool is curtailed as a result of more DCD donors being pursued. Specifically, the question is raised whether some of the DCD donors could/would have progressed to BD had life support been continued for a sufficient time to allow BD to occur. In a multicenter report from 27 European countries participating in a survey on organ donation, including 10 countries with established DCD programs, the number of both DBD and DCD overall increased during the interval 2000-2009. However, DBD decreased of about 20% in three countries with a predominant DCD activity, implying that DCD might have negatively impacted on DBD activity^[11]. Ideally, in order to increase the overall

donation rate, the expansion of the DCD pathway should have an additive rather than detrimental effect on DBD, so that, in aggregate, more potential donors become actual donors compared to the DBD pathway alone. Indeed, a recent study from the New England Organ Bank, one of the top ranking OPOs in the United States by percentage of DCD (> 30%), reports a 5-year experience with 331 DCD donors without a concomitant reduction of DBD, suggesting that a DCD program may actually expand the donor pool rather than curtailing it. The results of this study also show that overall more potential donors had been identified that would have not been realized without the DCD program^[12]. Regardless, DCD alone and/or in combination with current DBD practices are unlikely to bridge the gap between current organ availability and need. In addition to DCD, other strategies to optimize the current limited organ pool are needed, including the use of less-than-ideal organs ("marginal organs") and split techniques (in case of the liver). While these strategies partially mitigate the donor shortage, still do not resolve the problem of organ shortage and call for additional initiatives. Among them, a considerable attention has been given lately in several countries to the pool of potential donors.

"Potential" deceased donors

Multiple recent studies from different countries, including the United States, have documented the potential for increasing the number of deceased donors. The Iberoamerican Network/Council on Donation and Transplantation has reported a 52% increase in deceased donation in less than 10 years in Central and South America^[3], indirectly demonstrating that the pool of potential donors was previously incompletely exploited. According to a report from Spain, 2.3% of hospital deaths and 12.4% of deaths in the intensive care unit could yield potential donors, making the number of actual donors up to 21% higher if all potentials were to be identified and followed^[13]. The Spanish donation system, among the top performing worldwide, has been widely recognized as a valid model in both BD and DCD pathways and includes an internal hospital chart review of patients who died in ICU performed by transplant coordinators followed by an external periodic audit. Although the plain application of the Spanish model to other national donation systems would not necessarily lead to increased donation rates due to several socio-economic and cultural differences between countries, nonetheless the Spanish experience in recent decades and published studies from other countries indicate that the donor potential is probably not fully exploited. A few definitions currently used in the organ donation literature and protocols are reported in Table 2.

Although with different definitions, the number of potential donors has been estimated in previous studies. According to the IoM report, the number of donor-eligible deaths has been estimated in the range between 10500 and 16800 per year, significantly higher than the actual 8500-9000 deceased donors/year over

Table 2 Definitions^[14]

Donor	A person from whom at least one organ was procured for the purpose of transplant, regardless of whether the organ was transplanted
Eligible death	Death of a person aged 70 yr or younger, legally declared brain dead according to hospital policy and without exclusions listed in OPTN policy
Imminent neurological death	70 yr or younger, ventilated, with severe brain injury and without exclusion criteria, lacking 3 brain stem reflexes but not fulfilling BD criteria
Potential donor	Patient with devastating irreversible brain injury apparently medically suitable for organ donation and suspected to fulfill BD criteria

BD: Brain death; OPTN: Organ Procurement Transplantation Network.

the last two years^[8,15]. In other reports, the potential for brain dead donors has been estimated between 10000 and 26000 per year, depending on the study modality based on either mortality records or hospital chart review^[16-20]. In 2010 the Health Resources and Service Administration of the Department of Health and Human Services commissioned UNOS to conduct the Deceased Donor Potential Study to estimate the number of potential donors in the United States. According to the results of this study, the pool of potential donors is larger than previously estimated with as many as 35000 to 40000 potential donors each year meeting basic criteria for donation^[21]. Although the true potential could have been over-estimated due to the lack of more detailed medical information, nevertheless this study confirms that there is an untapped pool of potential donors. Another interesting finding in this study was that, among people who met basic medical criteria for deceased donation, the actual donation rate was considerably lower (10%) in the age group 50 to 75 years compared to those age 18 to 34 (50%), implying that more donors could be potentially obtained in the age group 50-75 years.

The potential for donation varies across geographic areas of the United States with a four-fold difference in eligible death/million population reported to OPTN by OPOs (national mean 31 eligible death/million population, ranging from 15 to 61) based on the existing geographical variability in mortality (91-229 deaths/million population from cerebro-vascular accident and trauma)^[2]. Importantly, this study highlighted that the number of eligible deaths is correlated to the number of deaths from cerebro-vascular accidents and trauma in that specific area (r square = 0.79).

Outside the United States, studies from Europe, Canada and other countries have documented similar findings regarding potential donors. In Belgium, Roels *et al*^[22] found that 57% of deceased potential donors were missed along the process due to non-identification or missed referral or lack of consent. Likewise, a study from Canada based on discharge data submitted to the Hospital Morbidity Database reported that only 1 in 6 potential donors (17%) became actual donor^[23]. Even assuming that the study methodology overestimated the number of potential donors due to the limitations of analyzing abstract data rather than actual patient chart review, nevertheless this study confirms that the potential to increase the number of deceased donors exists. Regardless of the definition of potential donor,

it is evident from several studies that the number of actual donors represents only a small proportion of the pool of potential donors (Figure 2).

Therefore, a major challenge to increase donation rates would consist of expanding the pool of actual donors to include potential donors. The process of organ donation and potential strategies to expand the pool of actual donors will be discussed below.

THE PROCESS OF ORGAN DONATION (DECEASED DONORS)

Currently, organs for transplant are recovered after determination of the donor's death. This standard practice, commonly known as the "dead donor rule", requires that the intended donor be declared dead before the removal of any life-sustaining organs^[24]. This rule was introduced to protect the person's life before death and to prevent that lives were ended for the purpose of procuring organs. This rule is important to maintain the public trust in organ donation and transplantation and to avoid the misconception that care is withdrawn from potential donors in order to expedite death for the purpose of organ recovery. Recently, however, the dead donor rule has been reconsidered^[25]. In the opinion of some ethicists, while the "dead donor rule" assures patients, families and health professionals that a patient is dead before removing organs, therefore making organ transplantation legally and ethically acceptable, on the other hand it may jeopardize donation in selected cases. As an example, it is quoted the case of a DCD potential donor with prolonged agonal phase (the interval between withdrawal of support and cardiac arrest) that prevented organ recovery and transplantation due to prolonged ischemia. It is argued by some that, after the decision of withdrawing support has been reached, organs be procured without waiting for the declaration of death by circulatory criteria (*i.e.*, cardiac arrest). The advantage of this pathway would be to give patients the opportunity to donate even before death is declared, when death is imminent ("near death") and donation is desirable, in order not to jeopardize the viability of donated organs for transplant. It is argued that, when death is very near, some patients may want to die in the process of helping others to live, even if that means altering the timing or manner of their death. Regardless of this debate about the dead donor rule, it is important that ICU physicians, transplant professionals and organ

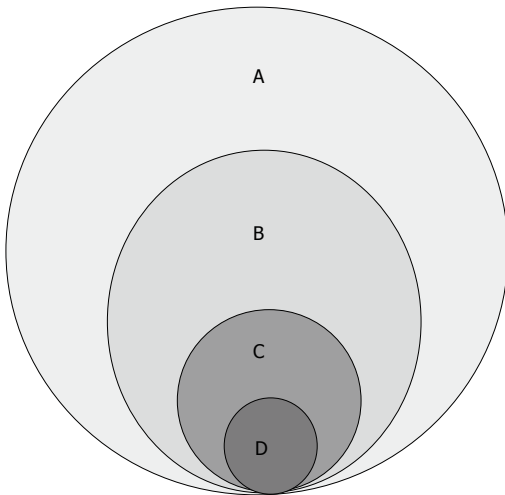


Figure 2 The number of actual organ donors is only a small proportion of the pool of deaths. A: Total deaths; B: Imminent deaths; C: Eligible deaths; D: Actual donors.

procurement organizations make every effort towards maintaining public trust. Mistrust from the general public regarding the procurement of organs will likely result in reduced consent rates for donation based on the perceived fear by the donor's family that treatment is withdrawn from their loved one in order to obtain organs. In other words, fearful people will assume that physicians care more about obtaining organs than saving the patient's life. In addition, this debate on the dead donor rule emphasizes the importance of a previous recommendation by the IoM about the integration of organ donation with end-of-life care. By this integration, the donation process starts before the occurrence of the donor's death, at the time when the potential donor with irreversible devastating brain injury is referred but is not yet declared dead. Since every actual donor has been a potential donor sometime before in the process, it is likely that the coordination of end-of-life care and organ donation would allow to identify and manage potential donors early in the process, increasing the chances of donation. The process leading from donation to transplantation can be described in the following 6 steps: Brain injury, referral, brain death, consent, organ recovery and organ transplantation (Figure 3).

The process of organ donation for transplantation has been described before^[11]. In this review we will limit our considerations to deceased organ donation in the United States.

Brain injury

Organ donors are patients with extensive brain injury resulting, most commonly, from cerebro-vascular accident or trauma or anoxia. Only a small proportion of those patients who suffered extensive and irreversible brain injury become actual organ donors because of the variable impact, in terms of intensity and timing, of brain injury on neurological functions and on brain stem activity. As a result, the occurrence of brain death

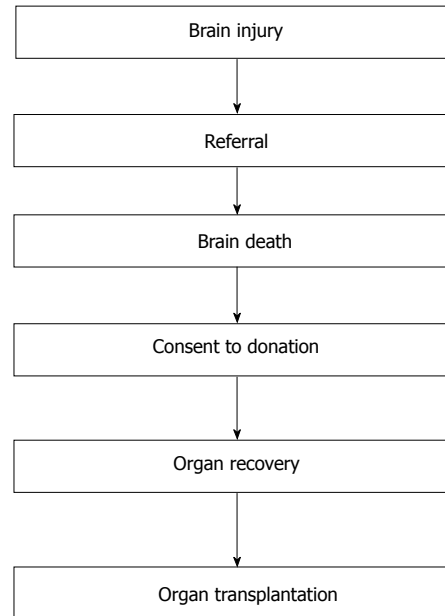


Figure 3 The process of deceased organ donation.

is more or less likely and more or less rapid in different patients. As an example, a patient with large intracerebral hemorrhage or a bilateral pontine hemorrhage is more likely to progress to brain death within a relatively short timeframe than a patient with diffuse anoxic injury without intracranial hypertension^[26]. Consequently, the time interval between brain injury and brain death varies, impacting on the management of the potential donor and costs. In addition, during the time interval between brain injury and brain death the patient is exposed to the systemic adverse effects of brain injury, including hemodynamic instability, diabetes insipidus, and others. In this context, the management of the potential donor while in ICU is paramount and has been described elsewhere^[27].

Referral

Among all patients with brain injury as described above, the medical suitability for organ donation is determined according to established criteria and represents the second step of the process leading to the referral of the potential donor. Federal rules require hospitals to notify the OPO of an individual whose death is imminent or who has died in the hospital^[28]. A network of 58 OPOs constitutes the liaison system designated by the United States federal government to coordinate the organ donation process. The criteria (or triggers) for referral from the hospital to the local OPO are reported in Table 3.

The referral of a potential donor to the OPO can occur as early as on patient presentation to the Emergency department^[29]. After referral, the OPO is involved with the management of the potential donor by coordinating the logistic, medical and regulatory aspects of donation. Importantly, an OPO representative approaches the family of the donor providing support from the time of referral through donation and after

Table 3 Criteria for referral of a potential donor

Every ventilated patient with
Glasgow coma scale of 5 or less without sedation
Brain death testing being considered/pursued
Do-not-resuscitate or comfort care being considered
Withdrawal of support being considered
Family initiates conversation about donation
Every cardiac death within 1 h

donation. The potential donor is considered medically suitable for donation based on established criteria of transplantability of the organs except in cases with potentially transmittable diseases, such as infections or cancer, as indicated in the UNOS policy^[30].

Brain death

Once exclusion criteria have been ruled out, the potential donor becomes eligible for donation after declaration of brain death, which is the third step of the process. Established neurologic tests allow the determination of death by neurologic criteria (brain death tests) and therefore determine eligibility for donation. According to UNOS definition (see above), an eligible death for organ donation is defined as the death of a patient 70 years old or younger, without any exclusion criteria for donation, legally declared brain dead according to hospital policy independent of family decision regarding donation or availability of next-of-kin, independent of medical examiner or coroner involvement in the case, and independent of local acceptance criteria or transplant hospital practice.

The concept of brain death has been introduced in 1968 following the proposal by an *Ad Hoc* Committee that a person could be declared dead after irreversible cessation of the function of the entire brain^[10]. Before the introduction of this concept, the death of a person was declared after irreversible cessation of circulatory and respiratory function. After the introduction of brain death, it became accepted that a person requiring mechanical ventilation can be declared dead even while maintaining heart beating. This is an important aspect to discuss with the donor's family given that the concept of death in the public opinion is mainly associated with arrest of cardio-circulatory activity.

Consent to donation

After brain death, in observance of the principles of autonomy and non-maleficence, the consent to donation is sought from the patient, the family or the next of kin before proceeding with organ recovery. This represents the fourth step in the process and an important focus for future strategies to increase donation (see below). Several aspects of the step of obtaining consent to donation are crucial, including the timing, the method and the approach. Usually, the donor's family is approached after declaration of brain death. However, in selected cases it may be indicated to approach the family before brain death, as in the case of an unstable

donor where rapid deterioration of organ function may occur. This critical step of communicating with the family highlights the importance of effective coordination of end of life care between ICU providers and OPO personnel. In some countries outside the United States, regulations allow the procurement of organs based on the presumed consent of the donor in absence of documented objection to donation. In the United States system, which is based on explicit rather than presumed consent, it is important that the approach to the family and the process of obtaining consent for donation is conducted in a culturally-sensitive way. It is becoming increasingly clear that a better understanding of the donor's family language, culture, faith, and values is critically important to increase consent rates^[31]. The current consent rate is on average 76% ranging between 62% and 93% across OPOs^[32]. Little is known about the factors associated with such variability across regions. In addition, the reasons for denied consent to donation by the donor's family are still poorly understood and represent an opportunity for action in order to increase deceased donation (see below).

Organ recovery

After consent is obtained, the OPO, in collaboration with the donor hospital, allocates suitable organs and arranges for the operation of organ recovery, which represents the fifth step of the process. Typically, multiple organs are procured in different combinations including heart, lungs, liver, kidneys, pancreas and intestine from the same deceased donor during a multi-team operation lasting several hours. Each team carries the burden of recovering the respective organ in the best possible condition for their intended recipient. Therefore excellent communication and coordination between teams is essential during procurement. Typically the teams recovering the thoracic organs and the abdominal organs proceed simultaneously. The intra-operative management of the donor during organ recovery has been reviewed elsewhere^[33]. It is critical to assess and correct, when necessary, the hemodynamic, metabolic, hormonal and pro-inflammatory alterations occurring in the setting of brain death. Studies have documented that the quality of donor management impacts on the quality of the procured grafts and on graft function^[34]. The different techniques of multi-organ procurement have been described extensively and vary among countries.

Organ transplantation

The allocation and transplantation of the procured organs represents the final step of the process. In the United States organ allocation is regulated by organ-specific policies following the criteria of urgency as indicated by the degree of disease severity of transplant candidates. Although the vast majority of recovered organs are subsequently transplanted, not all recovered organs are always transplanted. The reasons for failure to transplant procured organs are multiple and include

damage to the organ during procurement, organ unsuitability discovered during or after procurement, sudden unsuitability of the intended recipient to receive the allocated organ and others. Regardless, to maximize the use of this scarce resource it is important to prevent organ "discard" after recovery. The conversion rate, which reflects the proportion of eligible donors that becomes actual donors and is one of the parameters monitored by the OPO, is an indirect way to assess discard rate of procured organs. Accordingly, actual donors are considered those in which at least one organ has been successfully transplanted. Multiple factors impact on conversion rates and are beyond the scope of this review. Each step of the process of organ donation from deceased donors as outlined above can potentially be the target of strategies to increase donation rates, as discussed below.

CHALLENGES AND OPPORTUNITIES TO INCREASE DECEASED ORGAN DONATION

The "imminent" death

The number of deceased organ donors per year has remained relatively stable over the last decade with only a small annual increase over the years from 8016 deceased organ donors in the year 2006 to 8143 in 2012 and 8596 in 2014^[35]. At the same time, the number of patients added to the wait list has increased at a faster pace every year, making the gap between need and supply of organs wider every year (Figure 1). One of the strategies to narrow this gap is to increase the number of donors for transplant, especially deceased donors. Being the pool of potential donors larger than the number of actual donors, as outlined above, and considering that all donors were "potential" at some point during the process, it is reasonable to focus efforts on identifying and managing potential donors in order to increase donation rates. This would require a novel and broader approach to deceased donation to include not only those fulfilling brain death criteria (eligible deaths) but also those closed to it ("near brain death" or "imminent death"). According to OPTN, imminent donor is a potential donor who is imminent to fulfill the criteria for the determination of death by neurologic criteria (BD). Currently, imminent deaths are being monitored by OPOs, although their definition varies among regions and hospitals. It would be important to have a uniform characterization of imminent deaths and, more importantly, to have a better understanding of their evolution in terms of progression to BD.

Several challenges have been identified at each step of the process of deceased organ donation that could potentially be the target of action to improve donation rates. These include: Missed clinical triggers for referral, premature withdrawal of support before BD testing, cardiac death during evaluation, lack of consent, donor instability and death during organ recovery, organ

damage at procurement or organ unsuitability discovered after recovery and others. At the very beginning of the process of organ donation from deceased donors it is crucial that the potential donor is recognized early after presentation to hospital and referred promptly to the local OPO. The determination of the suitability for donation based on initial demographic (age) or clinical parameters and co-morbidities of patients with devastating brain injury should be deferred to the OPO representative rather than to the primary ICU team. An early referral allows the OPO sufficient time to evaluate the potential donor for medical suitability and to approach the family^[36].

The donor's family

The donor's family plays a key role in the donation process. Within the OPO, a dedicated team of trained personnel approaches the family in a sensitive way. Even in case of registered donors, the family is always consulted before organ procurement. Although legally the donor's consent is sufficient to allow organ recovery, nevertheless the wishes of the family are always taken in consideration and usually organ recovery is not pursued in case of opposition from the donor's family. Respect for the donor's family is important to maintain the public trust: It would be deleterious to pursue organ donation against the family wishes, even in presence of donor's consent. In addition, it is important to understand the motivations behind the declined consent by the donor's family. Factors associated with declined consent include donor age (older), ethnic minority, time interval between certification of brain death and approach to the family and the amount of time spent by the coordinator with the family^[37,38]. The education of families from ethnical minorities using a culturally-sensitive approach seems particularly important, since minority groups are disproportionally represented on the transplant waiting list and unfortunately also suffer from disparities in deceased and living donation. Barriers to donation in minority groups include decreased awareness of transplantation, religious or cultural distrust of the medical community, fear of medical abandonment and fear of racism^[39]. Culturally sensitive communication and interventions are needed to overcome these barriers^[40].

"CPR" for organs

After referral, the ideal management of the potential donor involves both ICU team and OPO personnel. This combined approach provides the best chances to effectively integrate organ donation as part of end of life care, as recommended by the Institute of Medicine. Although prognostic factors have been studied and identified^[41], still the likelihood and timing of progression to BD in patient with brain injury remains incompletely understood. Further studies are needed to better identify early predictors of brain death.

BD is associated with a plethora of systemic manifestations including hemodynamic, metabolic and endo-

crine disturbances. Guidelines have been developed to assist the donor management before organ recovery. Occasionally, eligible donors are lost due to intercurrent hemodynamic instability and cardiac arrest. As part of the integration of end-of-life care with organ donation, it would be important to identify risk factors for cardiac arrest, treat disimbalances and discuss with the donor's family the code status of the donor, including the possibility of hemodynamic support and, if necessary, cardio-pulmonary resuscitation in order to maintain organ perfusion until organ recovery occurs.

CONCLUSION

An increase in deceased organ donation is necessary to make organ transplantation accessible to more candidates. Among others, new strategies to manage the pool of potential donors are needed in order to increase donation rates.

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***Cryptosporidium* infection in solid organ transplantation**

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Abstract

Diarrhea is a common complication in solid organ transplant (SOT) recipients and may be attributed to immunosuppressive drugs or infectious organisms such as bacteria, viruses or parasites. *Cryptosporidium* usually causes self-limited diarrhea in immunocompetent hosts. Although it is estimated that cryptosporidium is involved in about 12% of cases of infectious diarrhea in developing countries and causes approximately 748000 cases each year in the United States, it is still an under recognized and important cause of infectious diarrhea in SOT recipients. It may run a protracted course with severe diarrhea, fluid and electrolyte depletion and potential for organ failure. Although diagnostic methodologies have improved significantly, allowing for fast and accurate identification of the parasite, treatment of the disease is difficult because antiparasitic drugs have modest activity at best. Current management includes fluid and electrolyte replacement, reduction of immunosuppression and single therapy with Nitazoxanide or combination therapy with Nitazoxanide and other drugs. Future drug and vaccine development may add to the currently poor armamentarium to manage the disease. The current review highlights key epidemiological, diagnostic and management issues in the SOT population.

Key words: *Cryptosporidium*; Solid organ transplantation; Diarrhea; Nitazoxanide; Antiparasitic drugs

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Core tip: Diarrhea caused by *Cryptosporidium* is a serious and underrecognized cause of diarrhea in solid organ transplant recipients. The most important diagnostic challenge is low index of suspicion, since many new diagnostic methods have improved detection of the parasite. Treatment can be challenging as the disease may cause severe dehydration and antiparasitic drugs have modest activity. Electrolyte and fluid replacement, reduction of immunosuppression and antiparasitic

therapy are the cornerstones of management. Newer antiparasitic drugs and vaccines may help manage the disease in the future.

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INTRODUCTION

Cryptosporidium is a parasitic protozoan causing a gastroenteritis syndrome^[1]. It is a common intestinal pathogen, not detected by routine ova and parasite evaluation. Because testing for *Cryptosporidium* is not routinely sought, the infection is often underdiagnosed, posing important epidemiological problems. In immunocompetent persons, cryptosporidiosis is usually a self-limited disease lasting between just a few days up to 10-14 d^[1,2]. In immunocompromised patients, clinical presentation can vary from asymptomatic to acute gastroenteritis, chronic diarrhea or even extra-intestinal manifestations^[1,3-24]. The parasite binds on the apical surface of the intestinal epithelium fostering its own reproduction and causing direct injury of the epithelial cells and a local inflammatory response, leading to impairment of the absorption and secretory function of the intestine^[1,25]. Several *Cryptosporidium* spp. have been associated with human disease, of which *Cryptosporidium parvum* (*C. parvum*) and *Cryptosporidium hominis* (*C. hominis*) account for > 90% of the cases^[26-28]. In this review, we examine the current epidemiology of *Cryptosporidium* in solid organ transplant (SOT) recipients, review its pathogenesis and clinical manifestations, diagnostic approach, discussion-available treatment options and possible future approaches.

EPIDEMIOLOGY

The incidence and prevalence of cryptosporidiosis varies according to socioeconomic status in both developed and developing countries. In the United States, it is estimated that 748000 cases occur every year^[29], but prevalence in patients with diarrhea can be as high as 12% in developing countries. In SOT recipients are largely unknown (Table 1). Cryptosporidiosis is most likely underreported in SOT, with most of the data being confined to case reports and case series, many of them from endemic areas such as Brazil, India and Middle East^[3,10,30,31]. In a study from Brazil, *Cryptosporidium* infections were more common in renal transplant recipients (35%) and hemodialysis patients (25%) compared to the control group (17.4%)^[30]. Similarly, in a study from Turkey, the prevalence of cryptosporidiosis in kidney transplant recipients was found to be significantly higher than in healthy immunocompetent

patients (21.2% vs 3.0%, $P = 0.01$)^[10]. A recent study from India, shows that cryptosporidiosis accounts for the majority of infectious diarrhea (28.5%) in adult transplant recipients^[3]. Children and immunocompromised patients are disproportionately affected, especially in developing countries^[32]. Between 1.8% and 3.8% of immunocompetent children in child-care settings in the United States, United Kingdom, Spain, and France have been found to be asymptomatic carriers for *C. hominis*^[31,33,34]. This proportion may be underestimated as up to 70% seroprevalence was found in children living in the United States-Mexican border^[35]. Bandin *et al*^[8] reported that *Cryptosporidium* infections were diagnosed in 3.5% of the new pediatric kidney recipients, and was responsible for 18% of the cases of infectious diarrhea over a period of 3 years. This marked heterogeneity in the prevalence of cryptosporidiosis in SOT from different studies (Table 1) is probably the result of different inclusion criteria used in each study, the geographical distribution, the sensitivity and specificity of the diagnostic tests used, type of induction and maintenance immunosuppression regimen^[3,11].

Epidemiological studies, animal models and human case reports show that *Cryptosporidium* is transmitted from person to person spread *via* fecal-oral route, including sexual transmission and possibly *via* respiratory secretions^[28,35-40]. Infectivity depends on the number of oocysts and *Cryptosporidium* species and subtypes^[41,42]. Outbreaks of cryptosporidiosis in developed countries have been described in daycare centers^[43,44] in association with animal petting farms^[45,46] and recreational water use^[47,48]. During the last few decades, several waterborne outbreaks have been reported after ingestion of contaminated recreational water or drinking water, one of these was thought to affect more than 400000 people^[49-58]. Risk factors in SOT recipients reported in the literature are described in Table 2. *Cryptosporidium* oocysts are resistant to chlorine disinfection and can survive for days in treated recreational water despite adequate chlorination^[36,59]. *Cryptosporidium* can be eliminated by boiling the water or just heating it to 62 °C for few seconds and by filtration through < 1 µm filters^[40]. Transmission of cryptosporidiosis *via* respiratory secretions is less common; isolation of *Cryptosporidium* DNA in the sputum of children with intestinal cryptosporidiosis and cough supports the respiratory route of transmission of this organisms^[60]. Even more, all of the life stages of *Cryptosporidium* have been described in the microvillus border of epithelial cells and within the bronchial mucus glands^[61]. Cryptosporidiosis has also been reported as a donor-derived infection after intestinal transplantation^[14].

VIRULENCE IMMUNOPATHOGENICITY

The severity and duration of illness (from asymptomatic shedding of oocysts to severe life-threatening disease)

Table 1 Cases and case series of *Cryptosporidiosis* in solid organ transplant recipients

Ref.	No. of patients	Incidence	Median/mean (range/SD) age (yr)	Allograft	Immuno-suppression regimen	Symptoms	Acute renal failure	Abnormal LFTs
Abdo <i>et al</i> ^[15]	1	NA	40 (NA)	Kidney	TAC + AZA + S	Abdominal pain, D	No	Yes
Acikgoz <i>et al</i> ^[23]	1	NA	6	Kidney	TAC + MMF + S	N, V, D	Yes	No
Arslan <i>et al</i> ^[10]	43	7/43 (16.28%)	32.9 ± 12.2	Kidney (40) ¹ Liver (3) ¹	MMF, TAC, AZA, CsA, S	D	N/A	N/A
Bandin <i>et al</i> ^[8]	38	7/38 (18%)	8.93 (4.5-14)	Kidney	MMF + TAC + S (3) ¹ MMF + TAC (2) ¹ MMF + CsA + S (2) ¹	D (7) ¹ , V (4) ¹ , abdominal pain (7) ¹ , hTN (4) ¹	Yes (7)	No
Bhadoria <i>et al</i> ^[3]	119	34/119 (28.5)	33.96 ± 11.13 (15-52)	Kidney	CsA + MMF + S TAC + MMF + S	D(12), F(11), malaise(25), V(18), abdominal pain (17), weight loss (9), dehydration (15), hypotension (8)	Yes (12)	N/A
Bonatti <i>et al</i> ^[5]	10	NA	51 (34-57)	Kidney (8) ¹ Liver (1) ¹ Lung (1) ¹	TAC + MMF + S (8) ¹ CsA + AZA + S (1) ¹ TAC + S (1) ¹	D (10) ¹ , V (5) ¹ , malaise (4) ¹ , F (1) ¹	Yes	N/A
Campos <i>et al</i> ^[18]	3	NA	3.92 (1.25-7)	Liver	TAC + S (2)	V (1), D (3), F (1), abdominal pain (2)	No	Yes (2)
Chieffi <i>et al</i> ^[30]	23	17.2	N/A	Kidney	N/A	N/A	N/A	N/A
Clifford <i>et al</i> ^[21]	3	3/28 (10.7)	N/A	Kidney	CsA + AZA + S	D(2)	No	No
Delis <i>et al</i> ^[16]	4	NA	20.21 (0.83-34)	Intestine	TAC + P(3) ¹ TAC + MMF + S (1) ¹	D (4) ¹ , abdominal pain (1) ¹ , F (1) ¹	Yes (4) ¹	N/A
Franco <i>et al</i> ^[100]	1	NA	60	Kidney	CsA + MMF + S	D, N, V, malaise, weight loss,	Yes	NA
Frei <i>et al</i> ^[6]	1	NA	34 (NA)	Liver	MMF	D	N/A	N/A
Gerber <i>et al</i> ^[17]	1160	4/1160 (0.34%)	NA	Liver (3) ¹ Intestine (1) ¹	CsA + S (1) TAC + S (3)	D (4) ¹ , lethargy (1) ¹ , weight loss (1) ¹	No	Yes (1) ¹
Hong <i>et al</i> ^[9]	1	NA	7 (NA)	Kidney	TAC + MMF + S	N, V, D	Yes	No
Krause <i>et al</i> ^[4]	6	NA	3.7 (1.1-6.6)	Kidney (4) ¹ Liver-Kidney (1) ¹ Heart (1) ¹	TAC + MMF + S TAC + AZA + S TAC + MMF	D (6) ¹ , F (2) ¹ , V (1) ¹ , abdominal pain (1) ¹ , weight loss (4) ¹	Yes (5/6) ¹	Yes (4/6) ¹
Ok <i>et al</i> ^[19]	69	13/69 (18.8%)	N/A	Kidney	N/A	Asymptomatic, D	N/A	N/A
Pozio <i>et al</i> ^[14]	1	NA	13 (NA)	Intestine	TAC + S	None (1 st episode) D (2 nd episode)	N/A	N/A
Rodríguez Ferrero <i>et al</i> ^[7]	1	NA	78	kidney	MMF + TAC	D, hTN	Yes	No
Tran <i>et al</i> ^[12]	1	NA	59	Kidney	TAC + sirolimus + S	N, V, D, abdominal pain	No	No
Udgiri <i>et al</i> ^[13]	60	NA	35.07 (± 9.22)	Kidney	CsA + AZA + S (47) ¹ CsA + MMF + S (13) ¹	D (2) ¹	N/A	No
Vajro <i>et al</i> ^[24]	2	NA	1.49; 10	Liver	CsA + S	F	No	No
Ziring <i>et al</i> ^[11]	33	2/33 (6.06%)	2.83 (0.83-48.75)	Intestine ± liver	TAC + MMF + S	N/A	N/A	N/A

¹Number of patients; NA: Not applicable; N/A: Not available; N: Nausea; V: Vomiting; D: Diarrhea; F: Fever; hTN: Hypotension; TAC: Tacrolimus; MMF: Mycophenolate mofetil; CsA: Cyclosporine A; AZA: Azathioprine; S: Steroids.

depends on the infecting species, virulence of the parasite and the host immune response (the degree of the immunodeficiency that impacts mainly T cell function), and the incubation period can range from 2 d up to 2 wk^[1,2].

Cryptosporidium significantly affects intestinal cells with consequent alterations in absorptive and secretory functions. This may be either caused by direct cell injury or alternatively by activation of the immune system with release of pro-inflammatory cytokines^[1]. Toll-like receptors (TLR2 and TLR4) play an important part in initiating immune activation following mucosal injury by the parasite^[62-64] and inducing cytokine release

(IL-12, IL-15, IL-18, TNF- α and IFN- α/β) followed by activation of the NF- κ B cells with IFN- γ production, mononuclear cell infiltration in the lamina propria, crypt cell hyperplasia, villous atrophy and blunting^[65-67]. Toll-like receptors also have a role in establishing immunity to infection^[62]. Innate immunity controls infection, but elimination of the parasite seems to require adaptive immunity^[62]. IFN- γ is an important cytokine determining CD4⁺ T cell response to infection, including memory response against *Cryptosporidium* infection in the intestine^[62,68,69] (remove 63, add Pantenburg Infection and immunity). The role of the T cell function is supported by severe and prolonged cryptosporidiosis in

Table 2 Risk factors, diagnosis and co-morbidities in *Cryptosporidium* Infections

Ref.	Exposure	<i>Cryptosporidium</i> spp.	Diagnosis	Co-infection	Tacrolimus levels (early on admission)
Abdo <i>et al</i> ^[15]	N/A	<i>C. parvum</i>	N/A	No	No
Acikgoz <i>et al</i> ^[23]	Petting animals	N/A	ELISA	No	Increased
Arslan <i>et al</i> ^[10]	N/A	N/A	Modified acid fast staining	N/A	N/A
Bandin <i>et al</i> ^[8]	Swimming pool (3) Traveler diarrhea (1) ¹	N/A	Modified acid fast staining Ziehl-Nielsen staining Auramine staining Microscopy Biopsy	No	N/A
Bhadoria <i>et al</i> ^[3]	N/A	N/A	Modified acid fast staining	CMV (8)	Increased
Bonatti <i>et al</i> ^[5]	Travel (water exposure) (4) ¹ Camping (1) ¹ Restaurant (1) ¹ Well water/farm animals (1) ¹	<i>C. jejuni</i> (1/10) ¹	Microscopy Enzyme immunoassay	N/A	Increased
Campos <i>et al</i> ^[18]	N/A	N/A	N/A	No	N/A
Chieffi <i>et al</i> ^[30]	N/A	<i>C. parvum</i>	Carbol-fuchsin staining	N/A	N/A
Clifford <i>et al</i> ^[21]	Public water supply	N/A	N/A	No	No
Delis <i>et al</i> ^[16]	N/A	N/A	Microscopy Biopsy	No	Increased
Franco <i>et al</i> ^[100]	N/A	N/A	Gastric and small bowel biopsies and hematoxylin staining	No	N/A
Frei <i>et al</i> ^[6]	N/A	N/A	Modified Ziehl-Neelsen staining	No	N/A
Gerber <i>et al</i> ^[17]	N/A	N/A	Microscopy (2) ¹ Biopsy (3) ¹	No	N/A
Hong <i>et al</i> ^[9]	Swimming pool	N/A	Modified acid-fast staining DFA	N/A	Increased
Krause <i>et al</i> ^[4]	None	N/A	Immunochromatographic test	No	Increased (5/6)
Ok <i>et al</i> ^[19]	N/A	N/A	N/A	<i>Blastomycosis hominis</i> , <i>Giardia intestinalis</i> , <i>Dientamoeba fragilis</i> , <i>Entamoeba coli</i>	N/A
Pozio <i>et al</i> ^[14]	Allograft	<i>C. hominis</i>	Microscopy	No	N/A
Rodríguez Ferrero <i>et al</i> ^[7]	N/A	<i>C. parvum</i>	Biopsy	No	No
Tran <i>et al</i> ^[12]	N/A	N/A	Modified Kinyoun stain Modified acid fast staining Microscopy Biopsy	No	No
Udgiri <i>et al</i> ^[13]	N/A	N/A	Modified acid fast stain	<i>Giardia</i> spp. (7) ¹ <i>Entamoeba butschlii</i> (1) ¹	N/A
Vajro <i>et al</i> ^[24]	N/A	N/A	Monoclonal antibody fluorescein-conjugated stain	No	NA
Ziring <i>et al</i> ^[11]	Nosocomial (1) ¹	N/A	Direct immunofluorescent assay	N/A	N/A

¹Number of patients; DFA: Direct fluorescent antibody; N/A: Not available. *C. hominis*: *Cryptosporidium hominis*; *C. parvum*: *Cryptosporidium parvum* ; *C. jejuni*: *Cryptosporidium jejuni*.

patients with AIDS and CD4 count < 50 cells/mm³, and improvement of the symptoms after introduction of highly active antiretroviral therapy^[70] (Change reference for more recent one) or after decreasing immunosuppression in transplant recipients that allows recovery of the immune system. Antibodies have a minor role in elimination of the infection, being more an indirect marker of the cellular immune response^[68]. All these changes at the level of the epithelium lead to malabsorption and secretory diarrhea^[12,65].

In SOT the type of immunosuppression might play an important role in development of cryptosporidiosis. A recent study showed that patients on a tacrolimus-based immunosuppressive regimen had a significantly higher risk of *Cryptosporidium* infection compared to the patients on a cyclosporine-based regimen. Being on

cyclosporine seemed to protect against infection (OR = 0.35; 95%CI: 0.17-0.72). Those on tacrolimus who developed cryptosporidium also had graft dysfunction, likely due to dehydration and increased tacrolimus levels^[3].

CLINICAL PRESENTATION

Most of the *Cryptosporidium* infections in the SOT population have been reported in renal transplant recipients (Table 1). *Cryptosporidium* can cause asymptomatic infection in transplant recipients and because of that, many cases may be missed^[30,71]. A relatively high prevalence of oocyst excretion in asymptomatic transplant population might be detected in the stool with random stool screening^[71]. When clinically evident,

SOT recipients typically present with profuse and prolonged watery diarrhea, sometimes associated with nausea, vomiting, abdominal pain and fever^[1,4-10,12-24]. Other nonspecific symptoms have been described in immunocompetent and immunocompromised patients such as malaise, generalized weakness, myalgia, anorexia and headache^[1,5,17]. Persistent vomiting and diarrhea can lead to dehydration and wasting and have been associated with increased morbidity^[4,7,8,17]. Several study described acute renal failure, most likely secondary to dehydration, hypotension and sometimes tacrolimus toxicity^[3-5,7-9,16,23]. Atypical manifestations such as respiratory tract disease, pancreatitis, cholangitis and urinary tract infection, have been reported in patients with immune deficiencies, mainly AIDS^[72-75]. Biliary involvement with *Cryptosporidium* inducing sclerosing cholangitis has been reported in few SOT recipients^[12,15,18]. However, elevated liver enzymes should not be equivalent to the diagnosis of sclerosing cholangitis as they can be abnormal in the settings of hypotension or high tacrolimus levels^[11]. Radiologic findings in support of the diagnosis of sclerosing cholangitis: Abdominal ultrasound can show dilation of the biliary duct; Technetium 99m iminodiacetic scan might show biliary stasis, irregularity of the biliary ducts, focal strictures^[18]; endoscopic retrograde cholangiography or magnetic resonance cholangiopancreatography could demonstrate dilation and/or irregularity of the biliary ducts^[15,76].

Infection of the biliary tree in immunocompromised patients could represent an extra-intestinal reservoir that would allow the organism to avoid certain antiparasitic agents (paromomycin) and would lead to relapses. Drugs with biliary excretion such as nitazoxanide should be preferred in these patients^[2,77]. Relapse rates in cryptosporidiosis are high (up to 40%-60%) due to incomplete eradication of the oocysts, especially from the biliary tree and possibly due to inadequate intestinal drug levels in patients with severe diarrhea^[12,14]. Respiratory cryptosporidiosis can present as an upper or lower respiratory tract infection manifested by nasal discharge, voice change, cough, dyspnea and hypoxemia^[78-81].

DIAGNOSIS

Stool microscopy is the main and cheapest method for diagnosis, however all microscopic methods are labor intensive and have low sensitivity unless a high concentration of oocysts are being released in stool. The size of the oocysts is also important (between 3-7 µm) as they can be confounded with yeast, so modified staining with Ziehl-Neelsen or fluorescent techniques such as auramine-rhodamine can be employed to improve detection. The sensitivity of these stains still remains low^[82,83], requiring about 500000 oocysts/mL in formed stools for detection^[35]. The most commonly used test by microbiology laboratories is currently direct immunofluorescence which may be either a standalone test or a combined *Cryptosporidium*/Giardia diagnostic kit^[35]. There are several enzyme linked immunosorbent

assay (ELISA) kits available with sensitivities ranging from 66%-100% but excellent specificity and have the advantage of being more automated when compared to conventional staining methods^[41,84-89]. Immunochromatographic tests have lower sensitivity compared to other molecular or other antigen tests and are not as sensitive to detect species other than *C. parvum* or *C. hominis* but are easy to perform, correlate well with EIA/ELISA tests and provide results in a matter of minutes^[89,90]. Molecular methods provide the highest diagnostic sensitivity and are the preferred methods for diagnosis given their superior sensitivity and specificity. There are several multiplex polymerase chain reaction (PCR) test that can detect different gastrointestinal pathogens including viruses, parasites and bacteria however, these may not available in all laboratories^[91]. These tests usually have high sensitivity to detect *Cryptosporidium*, although speciation may require further testing and carry a higher cost^[26,41,42,92-94].

Tissue histopathology is a useful method for diagnosis, especially when intestinal biopsies are obtained. Parasites may appear lining epithelial surfaces or in the lumen. When hematoxylin is used to stain the tissue, intracellular parasites appear blue or purple^[2,16,17,20]. Intestinal transplant recipients may have negative stool examinations but the parasite may be readily seen on graft biopsies, highlighting the importance of endoscopic examination even in cases where diarrhea persists and routine stool examinations are negative^[11,16,17].

Detection of *Cryptosporidium* in respiratory sample specimens is usually achieved with acid-fast, modified acid-fast staining or and indirect immunofluorescence^[28,74] although PCR testing may also be possible^[28]. Histopathology may show parasites lining the mucosal epithelium of trachea, bronchi or lung; tissue biopsies may also show intra or extracellular organisms^[28].

TREATMENT

The main treatment approach is oral rehydration whenever possible, however intravenous fluids that include sodium, potassium, glucose and bicarbonate may be required in severe cases. A lactose free diet is recommended since *Cryptosporidium* destroys mature epithelial cells that are located in the villi resulting in loss of enzymes such as lactase. The disease is associated with high intestinal transit that may interfere with fluid, electrolyte, and drug absorption. Antimotility agents may be used once other causes of diarrhea such as *Clostridium difficile* or dysentery are ruled-out.

The first step in SOT patients is an attempt to restore immune function by adjusting or switching immunosuppressive therapy, because severity of disease is likely related to the degree of immunosuppression and CD4 cell counts^[3,10,13,19,37,74,82,95]. This example was illustrated in a renal transplant recipient with enteritis and sclerosing cholangitis, where an accidental reduction of immunosuppression resulted in clearance of the disease^[15]. Mycophenolate, a commonly used

immunosuppressive agent may have some antiparasitic activity against *Cryptosporidium* by inhibiting folate metabolism^[4]. *Cryptosporidium* induced diarrhea may also result in increased tacrolimus levels^[37] as evidenced in two recently published case series^[4,5]. The pathophysiology is not entirely clear but it is likely a combination of factors including reduced cytochrome 3A activity during inflammation^[96], interaction with other drugs, and reduced renal function due to fluid depletion^[4]. Increased tacrolimus may in turn worsen renal function, and prolong immunosuppression^[3]. Cholecystectomy may be indicated for cases with acalculous cholecystitis and sclerosing cholangitis usually needs endoscopic retrograde pancreatography with possible papillotomy and stenting^[97]. To date, there has not been a highly effective agent to treat cryptosporidiosis in immunocompromised individuals^[98]. A meta-analysis of seven trials including 130 patients with AIDS found no evidence for effective agents against cryptosporidiosis, although significant heterogeneity and flaws of individual trials may have influenced the negative results^[95]. Moreover, whether any drug may have partial effect or the use of combination therapy were not investigated in this meta-analysis. To date, no randomized clinical trial with antiparasitic drugs has been conducted in SOT recipients with cryptosporidiosis and most experience is extrapolated either from data in immunocompetent hosts, patients with human immunodeficiency virus (HIV) infection^[37] or case series and case reports (Table 3)^[3-19,21,23,24,30,99,100]. Several antiparasitic drugs such as paromomycin, nitazoxanide or azithromycin have been used with variable success. Nitazoxanide is the only FDA approved drug for treatment of cryptosporidiosis, it is available in tablets and suspension, it has no significant drug-drug interactions or dosing requirements in renal or hepatic failure^[98]. Its activity, including the one of its metabolites has previously been shown *in vitro*^[101] and it is believed to interfere with the pyruvate: Ferredoxin oxidoreductase enzyme-dependent electron transfer reaction, which is essential to anaerobic energy metabolism^[102]. Nitazoxanide has been effective in 3 randomized clinical trials among immunocompetent adults and children, showing reduction in duration of diarrhea and eradication of cysts from stool^[103,104]. Its effectiveness in immunocompromised patients has been variable with some clinical trials showing positive results whereas in other trials the drug was no better than placebo. In a randomized study of nitazoxanide in HIV infected patients with cryptosporidiosis treated with either 500 mg twice a day or 1 g twice a day vs placebo, good responses to nitazoxanide were seen in those with CD4 cell counts > 50/mm³ although no difference to placebo was seen in the subgroup with CD4 < 50/mm³^[105]. Nitazoxanide effectiveness was also questioned in a randomized double-blind trial in children with HIV infection who received the drug for 28 d and there was no difference with placebo for clinical and parasitological cure or mortality^[106]. One difference with patients with HIV infection when compared to SOT recipients is in

many cases the ability to more readily manage and adjust immunosuppression, whereas in HIV infection restoration of the immune system with antiretroviral therapy is key and may take longer time^[98]. The recommended nitazoxanide dose in SOT recipients is 500 mg twice daily for 14 d^[37], however data from randomized trials in SOT recipients is lacking and longer courses of therapy are sometimes employed^[3,4,8].

Paromomycin, a non-absorbable aminoglycoside has limited activity against the parasite, probably the doses used in clinical practice are not enough to achieve the high concentrations needed to inhibit parasitic activity^[97]. It was useful reducing oocyst excretion in a small clinical trial^[107]. Because paromomycin has not been shown to be useful as a standalone agent, combination therapy with other antiparasitic agents such as azithromycin and Nitazoxanide may be an attractive option^[5,7,9,11,14,16,23,108].

Macrolide antibiotics such as azithromycin, clarithromycin or spiramycin also have activity against cryptosporidium^[98], and were shown to reduce duration of symptoms and oocyst shedding in a clinical trial of treatment of children with cryptosporidiosis^[109], but these findings were not replicated on a subsequent randomized trial^[110]. Several clinical trials and case series evaluating the use of azithromycin in immunocompetent and immunocompromised patients with cancer and also HIV infection have shown mixed results in clinical response including duration of symptoms, and oocyst shedding^[110-114]. Several case reports and case series have described successful use of spiramycin and azithromycin either alone, or in combination therapy with paromomycin or Nitazoxanide in SOT patients^[5,7,9,11,13,14,16-18,23]. Drug-drug interactions between macrolides and immunosuppressive agents such as tacrolimus or cyclosporine should be considered before treatment is initiated and may further limit prolonged use of these antibiotics^[99].

Rifamycins also have antiparasitic activity. Rifabutin was shown to decrease cell infection by *Cryptosporidium*^[115] and rifaximin has also been shown to be active *in vitro*^[98]. Interestingly, the incidence of cryptosporidiosis was dramatically decreased in patients with advanced HIV infection who used rifabutin as part of *Mycobacterium avium* chemoprophylaxis^[116,117]. To date, there have been no randomized clinical trials to evaluate its efficacy in SOT recipients or other immunocompromised hosts. Drug-drug interactions with rifabutin may also be an important issue in those who take tacrolimus or cyclosporine^[15,99]. Tacrolimus levels are not affected by rifaximin, however an elevation of rifaximin levels may be seen as a result of P-glycoprotein inhibition.

Because individual drugs lack full activity against the parasite, use of combination therapy may be a more attractive option. Current guidelines recommend starting with nitazoxanide alone as preferred therapy, although combination therapy is listed as an alternative option^[37]. Our review of the literature showed some authors have used nitazoxanide as standard therapy, while others have used this approach in relapsed or refractory cases,

Table 3 Management of *Cryptosporidium* infections

Ref.	Treatment regimen (length)	Changes in immunosuppression	Resolution of symptoms	Graft loss	Death
Abdo <i>et al</i> ^[15]	Rifampin (3 wk)	Temporary lower level of TAC	Resolved	No	No
Acikgoz <i>et al</i> ^[23]	Spiramycin + NTZ + PAR (4 wk)	Switch from MMF to AZA	Resolved	No	No
Arslan <i>et al</i> ^[10]	N/A	N/A	N/A	N/A	N/A
Bandin <i>et al</i> ^[8]	NTZ (4 wk) (2) NTZ (2 wk) (5) ¹	MMF switched to AZA (3) ¹ MMF reduced (3) ¹	Resolved	No	No
Bhadoria <i>et al</i> ^[3]	NTZ (13) (16-60 d) NTZ + fluoroquinolone (21) (16-60 d)	TAC switched to sirolimus (1) ¹ MMF → AZA (3) TAC → CsA (8) Reduction of immunosuppression (11)	Resolved microbiologically (83%)	Yes (3)	
Bonatti <i>et al</i> ^[5]	AZM (14-21 d) (2) ¹ AZM + NTZ (6-18 d) (2) ¹ NTZ (14-16 d) (2) ¹ AZM (5 d) + NTZ + TMP/SMX (14 d) (1) ¹ AZM + PAR(14d) (1) ¹	MMF stopped (4) ¹ MMF reduced (1) ¹	Resolved	No	No
Campos <i>et al</i> ^[18]	Spiramycin → PAR (6 mo) PAR(2)	N/A	Resolved	No	No
Chieffi <i>et al</i> ^[30]	N/A	N/A	N/A	N/A	N/A
Clifford <i>et al</i> ^[21]	N/A	N/A	Resolved	No	No
Delis <i>et al</i> ^[16]	AZM (7 d) + PAR (21 d) (2) ¹ PAR (14 d) (1) ¹ PAR (21 d) (1) ¹	Stopped (1/4) ¹ TAC reduced (1/4) ¹	Resolved	No	No
Franco <i>et al</i> ^[100]	Spiramycin 10 d	MMF → Aza Stopped Aza	Resolved	No	No
Frei <i>et al</i> ^[6]	PAR (4 wk)	No	Resolved	No	No
Gerber <i>et al</i> ^[17]	AZM (3 wk) (1) ¹ PAR (2-3 wk) (2) ¹	No	Resolved	No	No
Hong <i>et al</i> ^[9]	NTZ (4 wk) PAR + AZM (5 wk), oral human immunoglobulin (5 d)	TAC reduced MMF stopped and AZT started	Resolved	No	No
Krause <i>et al</i> ^[4]	NTZ (5-24 d)	No	Resolved	No	No
Ok <i>et al</i> ^[19]	N/A	N/A	N/A	N/A	N/A
Pozio <i>et al</i> ^[14]	AZM (1 wk) + PAR (3 wk) AZM + PAR (1 yr 7 mo)	N/A	Resolved	No	No
Rodríguez Ferrero <i>et al</i> ^[7]	AZM + PAR (14 d) NTZ (6 d)	MMF and TAC reduced	Resolved	No	No
Tran <i>et al</i> ^[12]	PAR (4 wk)	Sirolimus discontinued	Resolved	No	No
Udgori <i>et al</i> ^[13]	Spiramycin (10 d) (2) ¹	No	Resolved	No	No
Vajro <i>et al</i> ^[24]	None	No	Resolved	No	No
Ziring <i>et al</i> ^[11]	PAR + AZM	N/A	Resolved	No	No

¹The number of patients; TAC: Tacrolimus; MMF: Mycophenolate mofetil; AZT: Azathioprine; S: Steroids; AZM: Azithromycin; NTZ: Nitazoxanide; PAR: Paromomycin; N/A: Not available; TMP/SMX: Trimethoprim/sulphamethoxazole.

usually with long courses advocated^[3-5,8,9,23]. Azithromycin has been combined with either nitazoxanide or paromomycin also with reported success^[5,82,115,118]. Caution should be exercised though, because large studies using combination therapy including nitazoxanide have not been carried out to date. Current data on combination therapy is derived from case reports and case series, which may only reflect positive outcomes, while negative results may not be necessarily reported.

PREVENTION

Transplant recipients should be cautious about swimming in streams or lakes and if possible avoid untreated well or lake water. Drinking water should either be treated municipal, filtered by < 1 µm filters or bottled water. Contact with anyone who has diarrhea should be limited,

(food and water may be contaminated by those infected) and hand-washing for everyone, especially all household members is strongly encouraged. Moreover, all surfaces should be cleaned with running water and soap^[37,119]. Safe sexual practice using condoms is also encouraged for anal intercourse, since it increases the risk of transmission as well^[119].

PERSPECTIVE

Oral bovine immunoglobulin (hyperimmune colostrum) seemed an attractive alternative for treatment although it has not been effective at conventional doses and at higher doses oocyst excretion was decreased but diarrhea increased and clinical symptoms were not reduced^[120]. More recently, monoclonal or polyclonal antibodies have shown to reduce oocyst shedding

and improve clinical symptoms^[121]. Thus, although still controversial, using oral bovine immunoglobulin or monoclonal antibodies along with antiparasitic agents may be a strategy to consider in immunocompromised individuals with recurrent or recalcitrant disease^[121].

The genome of both *C. parvum* and *C. hominis* has been decoded and this should also help develop antiparasitic drugs against specific targets such as calcium-dependent protein kinases, microtubule formation inhibitors, hexokinase, lactate dehydrogenase, inosine-5-monophosphate dehydrogenase, and fatty acylCoA binding inhibitors among others^[82,122].

Despite this, the full understanding of *Cryptosporidium* immunopathogenesis remains unclear^[35,68].

Declines in infection rates with increasing age among children in developing countries points to possible acquisition of immunity against the parasite, although immune responses that may lead to protective immunity are not well understood^[35,82]. A study conducted in healthy volunteers who were challenged with *Cryptosporidium*, showed that after second re-challenge episodes of diarrhea were similar but clinical severity was milder and fewer subjects were shedding oocysts^[123]. Both IgG and IgA antibodies increased after exposure, however there was no correlation with infection^[123]. Vaccination may be a viable alternative and vaccine has been evaluated in a mouse model^[124]. The two most common species causing human disease, *C. parvum* and *C. hominis* share > 95% of their genome so it may be possible to have one vaccine for both species (Mead 2015). Several parasitic antigens such as gp15 and gp40 have been evaluated in vaccine development. Both elicit an immune response and production of interferon gamma by mononuclear cells in patients previously infected with cryptosporidium. A vaccine trial in Bangladesh using IgA against gp15 showed the antibody was not species specific and resulted in shorter duration of illness^[82]. There are other targets being investigated including a recombinant DNA vaccine using *Vaccinia*, *Salmonella* or *Lactobacillus* as DNA vectors^[82]. A successful vaccine would first have to be proven effective in immunocompetent hosts before moving on to immunocompromised patients, although the latter are the ones who would most likely benefit from vaccination.

CONCLUSION

Diarrhea caused by *Cryptosporidium* is a serious clinical syndrome in SOT recipients and diagnosis may be delayed if the infection is not routinely suspected or investigated. Advances in diagnostic methodologies has improved the sensitivity of detection, however, treatment remains problematic, especially in immunocompromised patients. Aggressive fluid and electrolyte replacement, reduction in immunosuppression along with antiparasitic therapy are the mainstay of therapy, but few partially active drugs are available and the infection may follow a protracted course with many relapses. Combination therapy with nitaxoxanide and paromomycin or macro-

lides may be the best approach, especially in SOT recipients. New therapies in the horizon such as hyperimmune colostrum, monoclonal antibodies, and vaccination may help increase the armamentarium to manage the disease.

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BK nephropathy in the native kidneys of patients with organ transplants: Clinical spectrum of BK infection

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Abstract

Nephropathy secondary to BK virus, a member of the *Papoviridae* family of viruses, has been recognized for some time as an important cause of allograft dysfunction in renal transplant recipients. In recent times, BK nephropathy (BKN) of the native kidneys has been increasingly recognized as a cause of chronic kidney disease in patients with solid organ transplants, bone marrow transplants and in patients with other clinical entities associated with immunosuppression. In such patients renal dysfunction is often attributed to other factors including nephrotoxicity of medications used to prevent rejection of the transplanted organs. Renal biopsy is required for the diagnosis of BKN. Quantitation of the BK viral load in blood and urine are surrogate diagnostic methods. The treatment of BKN is based on reduction of the immunosuppressive medications. Several compounds have shown antiviral activity, but have not consistently

shown to have beneficial effects in BKN. In addition to BKN, BK viral infection can cause severe urinary bladder cystitis, ureteritis and urinary tract obstruction as well as manifestations in other organ systems including the central nervous system, the respiratory system, the gastrointestinal system and the hematopoietic system. BK viral infection has also been implicated in tumorigenesis. The spectrum of clinical manifestations from BK infection and infection from other members of the Papoviridae family is widening. Prevention and treatment of BK infection and infections from other Papovaviruses are subjects of intense research.

Key words: BK viral infection; BK nephropathy; Cardiac transplant; Bone marrow transplant; Liver transplant; Pancreatic transplant; Lung transplant

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Core tip: BK virus (BKV) is a member of a family of viruses that cause various diseases in animals and humans. Severe disease in transplanted kidneys was the first recognized human disease caused by BKV. In more recent times, BKV was also recognized as a cause of disease in the native kidneys of patients who had received bone marrow, heart, lung, liver and pancreas transplants, as well as in the kidneys of patients with loss of resistance to infection, such as patients with acquired immune deficiency syndrome or patients treated for malignant tumors. In addition to disease of the kidneys, BKV has also caused severe disease of the urinary bladder, the brain, the lungs, the gut and the blood. The diagnosis and particularly the management of infection by BKV present difficulties. Research for new medications specific for treating this infection is imperative.

Vigil D, Konstantinov NK, Barry M, Harford AM, Servilla KS, Kim YH, Sun Y, Ganta K, Tzamaloukas AH. BK nephropathy in the native kidneys of patients with organ transplants: Clinical spectrum of BK infection. *World J Transplant* 2016; 6(3): 472-504 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i3/472.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i3.472>

INTRODUCTION

BK virus (BKV) is a human polyomavirus identified in 1971 when it was isolated from the urine of a Sudanese kidney transplant recipient with renal failure secondary to distal ureteral stenosis^[1]. It belongs to the *Papovaviridae* family of viruses^[2]. BKV along with other papovaviruses, *e.g.*, JC virus (JCV), can cause disease in humans^[3,4]. BKV is ubiquitous in the general population and serologic studies suggest that primary infection occurs in early childhood at a median age of 4-5 years^[5,6]. BKV primary infection usually results in upper respiratory symptoms with rare reports of acute

cystitis^[5,6]. The route of transmission is not conclusively known. It is believed that transmission occurs *via* the respiratory pathway^[5,6].

Latent infection with BKV typically causes clinical disease in the genitourinary tract since the virus has a tropism for renal tubular and transitional epithelial cells. In these cells BKV establishes a life-long latency^[3,4,7]. Viral reactivation usually occurs in patients with immunosuppressed states resulting in viruria. A small percentage of patients with viruria develop an invasive infection of the kidney^[3]. BKV infections involving the urinary tract were the first to be reported in kidney transplant recipients and are the most frequent manifestations of BKV. BKV infection in other organs is less frequent^[2,3,8].

BK nephropathy (BKN) was recognized as an emerging problem in renal transplant recipients with the introduction of improved immunosuppressive treatments such as tacrolimus, mycophenolate, and antilymphocyte globulins^[6,7,9]. Renal transplant failure rates, due to BKN, especially if diagnosed late, can reach as high as 50%-80% within 24 mo^[7]. Therefore screening for BKV in renal transplant recipients has become routine^[2,9]. Costa *et al*^[10] reviewed the clinical and histologic features, diagnosis, monitoring of the virology and immunological picture and treatment of BKN. Their review was based primarily on reports of BKN involving renal allografts^[10].

In recent years, reports of BKN in native kidneys and of BKV infection in other organ systems have emerged with increasing frequency in non-renal solid organ and bone marrow transplant patients^[2,5,7,8,11] as well as in other immunosuppressed patients. The main purpose of this review is to summarize the clinical characteristics, diagnosis, pathophysiology and treatment of BKV infection in patients with solid organ and bone marrow transplantation. The spectra of manifestations of BKV infection and of patient groups developing BKV infection are enlarging. In addition to BKN in native kidneys of transplant recipients, this report will also address manifestations of BKV infection outside the urologic system and in patients without organ transplants. Several aspects of BKV infection, particularly the diagnosis, pathogenesis, and treatment of BKN have been studied extensively in kidney transplant recipients. This review will therefore include relevant studies of renal transplant recipients in these three areas.

The review has three major parts: (1) clinical manifestations of BKV infection; (2) diagnosis of BKN and pathogenesis of BKV infection; and (3) treatment of BKV infection and human diseases secondary to other members of the Papovavirus family. Key points of each major part will be presented at its end.

PART A CLINICAL MANIFESTATIONS OF BKV INFECTION

Two cases will illustrate the clinical features and histology of BKN in native kidneys of transplant recipients.

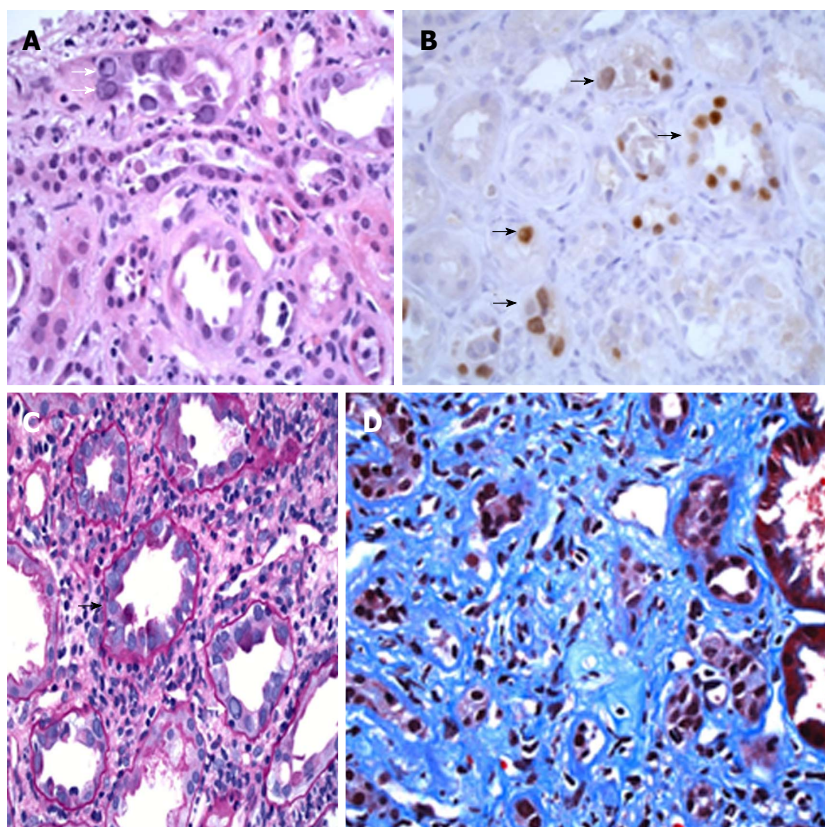


Figure 1 BK nephropathy in the native kidneys of a lung transplant recipient (Patient 2 in this report, A and B) and in the native kidneys of a bone marrow recipient (patient 1 in this report, C and D). Kidney biopsy showing BK nephropathy (BKN), taken from a 70-year-old male with a history of lung transplantation for pulmonary fibrosis. A renal biopsy was performed because of significant worsening in renal function over a one-month period. A: Kidney biopsy showing active BK virus infection of renal tubules, with multiple homogeneous-appearing viral nuclear inclusions (white arrows), and features of associated acute tubular injury, including sloughing of tubular cells (H and E stain, 400 × magnification); B: Multiple renal tubules show positive nuclear staining for the SV40 large T antigen by immunoperoxidase staining (black arrows), confirming infection of tubular cells by polyomavirus (400 × magnification); Kidney biopsy from a 30-year-old male with a history of an allogeneic bone-marrow transplantation for aplastic anemia, who developed sequentially post-transplant Epstein-Barr virus associated large B-cell lymphoma, graft vs host disease and progressive elevation of his serum creatinine. This patient died from pneumococcal pneumonia and invasive aspergillosis two months after the diagnosis of BKN; C: Biopsy of renal cortex showing mononuclear tubulitis (black arrow), intranuclear BK virus inclusions (white arrow), and a prominent interstitial chronic inflammatory infiltrate (PAS stain, 400 × magnification); D: Another area of the biopsy shows extensive interstitial fibrosis and tubular atrophy, consistent with late changes secondary to infection (Trichrome stain, 400 × magnification).

Patient 1

A 30-year-old Hispanic man received a matched allogeneic bone marrow transplant from an unrelated donor approximately two years after the diagnosis of aplastic anemia. Six months after the transplant he developed post-transplant lymphoproliferative disorder (Epstein Barr Virus associated diffuse large B cell lymphoma of the right tonsil). He underwent tonsillectomy, localized radiation, and one cycle of CHOP (cyclophosphamide, adriamycin, vincristine, prednisone) followed by two treatments with rituximab.

Two years after transplantation he developed graft vs host disease of his esophagus and small intestine which required initiation of immunosuppressive therapy. He was placed on tacrolimus. After ten months, tacrolimus was tapered and sirolimus was started because of concern for calcineurin inhibitor toxicity. After three months sirolimus was replaced by mycophenolate mofetil because his graft vs host disease was not improving.

The patient's serum creatinine was 0.7-0.9 mg/dL

pre-transplant and 1.2 mg/dL prior to the initiation of tacrolimus. Renal function worsened while he was on tacrolimus, which was discontinued when the serum creatinine reached 2.0 mg/dL. All blood tacrolimus trough levels were between 2 and 3 ng/mL. Despite discontinuation of tacrolimus, the patient's renal function continued to decline. Approximately four years following the bone marrow transplant, his serum creatinine was 3.15 mg/dL (estimated glomerular filtration rate by CKD-EPI equation of 25 mL/min per 1.73 m²). Urine microscopy was bland and urine protein to creatinine ratio was 0.6 g/g. Renal ultrasound was unremarkable. Serum antinuclear antibodies, antineutrophil cytoplasmic antibodies (ANCA), hepatitis panel, and human immunodeficiency virus (HIV) test were negative. Serum complement levels (C₃, C₄) were normal. Serum BK viral load was 700000 copies/mL.

Percutaneous renal biopsy demonstrated morphologic features consistent with BKN. Light microscopy was notable for lymphocytic tubulitis and viral nuclear inclusions (Figure 1C and D). Immunohistochemical

staining for SV 40 large T antigen showed positivity in tubular nuclei. There were no specific findings on immunofluorescence or electron microscopy.

The patient's BKN was treated with ciprofloxacin only because immunosuppression could not be lowered given his graft vs host disease and leflunomide could not be used due to preexisting leukopenia. During treatment with ciprofloxacin renal function and BKV titer continued to worsen. One month after the start of ciprofloxacin treatment, the patient was hospitalized with pneumococcal pneumonia and invasive aspergillosis. He became progressively septic and died one month later.

Patient 2

A 70-year-old Caucasian male with history of pulmonary fibrosis due to usual interstitial pneumonitis underwent a left sided lung transplant. His immunosuppressive regimen included tacrolimus, mycophenolic acid and prednisone. One year following the lung transplant, he suffered unprovoked pulmonary embolism and has remained on anticoagulation with warfarin since then. Serum creatinine levels were stable at 1.0-1.1 mg/dL until two years following the lung transplant when they began to rise. BK viremia was detected and mycophenolic acid was discontinued. However, renal function continued to decline and serum creatinine reached 3.0 mg/dL. His blood tacrolimus levels were between 5 and 8 ng/mL.

Urine microscopy was bland. Renal ultrasonogram demonstrated normal sized kidneys with multiple bilateral simple cysts. Serum BKV level was 10 million copies/mL. Renal biopsy showed active BKN, with visible viral inclusions, positive tubular nuclear staining for SV-40 large T antigen, and associated tubular cell injury/necrosis and mainly mononuclear tubulitis (Figure 1A and B). There was moderately severe interstitial fibrosis and tubular atrophy (about 40%-45%) and global glomerulosclerosis (13%). There were no specific findings on immunofluorescence microscopy.

Following the renal biopsy, administration of tacrolimus and prednisone was continued and Leflunomide was started at a dose of 10 mg daily and was slowly titrated up to 20 mg daily two months later. He also received three monthly doses of intravenous immunoglobulin (IVIG) at a dose of 1 g/kg. However, despite improved BK viral titers (from 10 million to 3.5 million copies/mL), his serum creatinine continues to range between 2.8 and 3.0 mg/dL.

GENERAL CONCEPTS OF BKV INFECTION IN PATIENTS WITH ORGAN TRANSPLANTS

Evolution of BKN in kidney transplant recipients^[12-22]

An early study by Hogan *et al*^[12] detected polyomavirus excretion in the urine in 20% of renal transplant recipients. Approximately equal numbers of patients

with viruria excreted JCV and BKV. The same study reported a tendency to more frequent complications related to the renal graft in patients with documented viral replication^[12]. Subsequently, Rosen *et al*^[13] described the development of tubulointerstitial nephritis secondary to BKV in a 6-year-old boy with a renal transplant. A few years later Randhawa *et al*^[14] calculated that the incidence of BKN in renal transplant recipients was as high as 5%. Shinohara *et al*^[15], using a BKV-specific antibody, found that the virus was localized in renal calyces, renal pelvis, ureter and the urinary bladder. These findings are consistent with the clinical manifestations of BKV infection in the urinary tract.

Hirsch *et al*^[16] reported associations of BKN with high BK viral loads in the plasma of renal transplant recipients and with treatment for rejection, particularly with corticosteroids. Additionally, Hariharan^[17] computed a high incidence (between 30% and 60%) of irreversible renal transplant failure in patients with BKN. Bohl *et al*^[18] stressed the association between potent immunosuppression and BKN in renal transplant recipients and the need for screening for early detection and prevention of BKN.

In an analysis of a large cohort of renal transplant recipients reported to the Organ Procurement Transplant Network national registry of the United States, Dharnidharka *et al*^[19] found an increasing Kaplan-Maier incidence of BKN with time and a higher risk of BKN with immunosuppressive regimens that included rabbit antithymocyte globulin and tacrolimus/mycophenolate combinations. Subsequently, the same group^[20] stressed the risks of over-immunosuppression in respect to BKV infection and the lack of optimal methods for treating BKN. Nickleleit *et al*^[21] reviewed recent developments in the diagnosis of BKN, including noninvasive diagnostic procedures, and the expanding role of polyomaviruses in oncogenesis in patients with organ transplants. Sawinski *et al*^[22] identified male gender, advanced age of the recipient, previous rejection episodes, severity of leukocyte antigen mismatching, long cold ischemia time, serology for BKV and ureteral stent placement as additional risk factors for BKN.

Evolution of the concepts of BKN in native kidneys and of other manifestations of BKV infection^[2,23-37]

The manifestations of BKV infection from the urinary tract may differ between transplanted organ recipients. BKN and ureteral stenosis were identified as the cardinal manifestations of BKV infection in kidney transplant recipients and hemorrhagic cystitis was recognized as a cardinal manifestation of BKV infection in recipients of bone marrow transplants^[23-25]. The documented sites of BKV-associated disease include the urinary system, the lungs, the eyes, the brain, the retinae and the blood vessels^[24].

Rates of BK viruria and viremia in recipients of organ transplants were reported from several geographical sites. In a study from Madrid^[26], viruria was detected in

26.5% of kidney transplant recipients, 25.5% of heart recipients and 7.8% of liver recipients, while viremia was found in 12.2% of kidney recipients and 7.0% of heart transplant recipients. In Pittsburgh, BK viruria was detected in 8.7% of non-immunosuppressed controls, 34.9% of renal transplant recipients and 15.9% of liver transplant recipients, while BK viremia was detected only in renal transplant recipients (7.7%)^[27]. In the same study, the BK viral load in urine was higher in the kidney transplant patients than in the liver transplant recipients or control patients; interestingly, JC viruria was observed in 34.7% of controls, 22.3% of renal transplant patients and 22.7% of liver transplant recipients. JC viremia was not detected in any group.

In a study from Mayo Clinic, Rochester, Minnesota and University of Toronto, Ontario^[28], combined BK and JC viremia was found in 26% of kidney transplant patients, 7% of heart transplant patients and 4% of liver transplant recipients. In the same study, BK viremia was associated in certain instances with renal transplant rejection. A study combining findings from Philadelphia, Pennsylvania, and Seattle, Washington^[29], found a 15% incidence of BK viruria in 34 recipients of lung, liver, heart, and heart-lung transplants with chronic renal dysfunction. In contrast, a study from Alberta, Edmonton^[32], which also found an incidence of BK viruria in recipients of heart, liver or lung transplants, reported no association between renal dysfunction and BK viruria.

Sharma *et al.*^[34] presented the histological features of BKN, combined in one case with focal medullary JC viral co-infection, in patients with hematologic malignancies, with and without bone marrow transplants, or lung transplants. Several reviews^[2,7,30,31,33,35,36] addressed the manifestations and pathogenesis of BKV infection. Finally, a recent systematic review^[37] analyzed a large number of studies of BKV infection in non-renal solid organ transplant recipients. This study concluded that BK viremia was lower in non-renal than in renal transplant recipients and that BKN is rare in non-renal transplant recipients. In non-renal organ transplant recipients, overall incidence of BK viruria was 20% and incidence of BK viremia was 3%, with the highest incidence of BK viremia and BKN found in heart transplant recipients^[37].

URINARY MANIFESTATIONS OF BKV INFECTION IN PATIENTS WITH BONE MARROW OR STEM CELL TRANSPLANTS AND SOLID TRANSPLANTS OTHER THAN KIDNEY

Table 1 shows the reported organ transplants, other than solitary kidney transplants, in which clinical manifestations of BKV infection have been described. An extensive list of references is attached to each transplanted organ with BKV infection indicating the

Table 1 BK infection studies in organ transplants other than solitary kidney transplants

Transplanted organ	Ref.
Bone marrow, stem cells	[2,5,8,39-81]
Heart	[11,82-96]
Lung	[97-102]
Liver	[103-113]
Pancreas, combined pancreas-kidney	[114-135]

rising interest in this topic.

BK viral infections in the urinary system of recipients with bone marrow or stem cell transplants^[2,5,8,38-81]

Hemorrhagic cystitis has been a frequent and serious complication of bone marrow transplantation. This condition had been attributed to the use of cyclophosphamide. Arthur *et al.*^[38] reported a substantially higher frequency of BK viruria in patients who developed hemorrhagic cystitis compared to those who did not develop hemorrhagic cystitis after bone marrow transplantation. They also identified a temporal association between the development of BK viruria and the onset of hemorrhagic cystitis. Bedi *et al.*^[41] concluded that prophylactic treatment with MESNA and forced diuresis directed at cyclophosphamide toxicity failed to prevent hemorrhagic cystitis in patients with BK viruria.

Subsequently, a large number of publications^[5,39,41,43-45,47-50,52,53,55-57,59,61,62,66-68,71,75] provided firm evidence linking BKV infection and hemorrhagic cystitis in bone marrow or stem cell recipients and addressed various aspects of this syndrome.

Peinemann *et al.*^[45] reported that hemorrhagic cystitis in pediatric bone marrow transplant recipients is characterized by delayed onset, prolonged duration, viral reactivation and use of high doses of the alkylating agent busulfan. Bielorai *et al.*^[46] described patients with BKV-induced hemorrhagic cystitis triggered by cytomegalovirus (CMV) reactivation. Giraud *et al.*^[57] reported that the frequency of BK viruria and hemorrhagic cystitis was reduced in bone marrow recipients with related donors and in those receiving reduced intensity conditioning for the bone marrow transplant. The data analyzed by Koskevu *et al.*^[71] suggest that BKV hemorrhagic cystitis may result from nosocomial transmission in pediatric bone marrow transplant recipients with very low or undetectable BKV antibodies. These authors raised the issues of infection control and prophylactic use of cidofovir.

Various malignancies and aplastic anemia were frequent underlying diseases leading to bone marrow transplantation in the reports of BKV hemorrhagic cystitis. Hereditary hematological diseases, including thalassemia and sickle cell anemia were reported in a few instances^[66]. The severity of BKV hemorrhagic cystitis varies. Patients with life-threatening blood loss from hemorrhagic cystitis require drastic surgical interventions. Sébe *et al.*^[48] reported successful treatment of life-threatening blood loss by subtotal cystectomy in

Table 2 BK nephropathy in recipients of bone marrow or stem cell transplants

Ref.	Gender age	Renal function	Clinical associations
[8]	Female 36 yr	ESRD Dialysis	Relapsed Hodgkin's lymphoma
[8]	Female 43 yr	ESRD Dialysis	Acute myelogenous leukemia
[11]	Male 47 yr	ESRD Dialysis	Non-Hodgkin's lymphoma
[49]	Male 17 yr	ESRD Dialysis	Myelodysplastic syndrome Severe hemorrhagic cystitis No renal biopsy Death from multi-organ failure
[50]	Female 28 yr	ESRD Dialysis	Acute myelogenous leukemia Recurrent CMV reactivation
[51]	NR NR	ARF	Underlying disease NR Adenovirus pneumonia Adenovirus nephritis Death
[58]	Male 14 yr	Rising SCr	Acute myelogenous leukemia Death from multi-organ failure
[60]	Male 10 yr	GFR normalized	Acute myelogenous leukemia No renal biopsy
[63]	Male 51 yr	ESRD Dialysis	Myelodysplastic syndrome Hepatorenal syndrome GVHD Death from <i>Pseudomonas</i> sepsis
[64]	Male 10 yr	Peak SCr 3.5 mg/dL Scr at 1.7 mg/dL post-treatment	Chronic myelogenous leukemia Adenovirus and bacterial infections Severe GVHD
[64]	Male 13 yr	ESRD Declined dialysis	Fanconi's anemia Gram-positive bacteremias Pulmonary aspergillosis Hyperacute GVHD Death
[70]	Female 10 yr	ESRD Dialysis	Recurrent metastatic neuroendocrine tumor Thrombocytopenia, leukopenia, lymphopenia Antineutrophil-antiplatelet antibodies Death from sepsis
[75]	Female 10 yr	Peak SCr 1.58 mg/dL SCr at 1.1-1.4 mg/ dL post-treatment	Myelodysplastic syndrome Acute GVHD
[77]	Male 59 yr	CKD stage 5 not requiring dialysis	Myelodysplastic syndrome
[79]	Male 58 yr	Death due to sepsis eGFR stable at 20 at the time of death	Large B cell lymphoma Acute GVHD

BK nephropathy was manifested at various times post-heart transplantation. Ages reported in this Table are the calculated ages at the time of diagnosis of BK nephropathy. ESRD: End-stage renal disease; ARF: Acute renal failure; SCr: Serum creatinine; GFR: Glomerular filtration rate; GVHD: Graft vs host disease; NR: Not report.

patients with BKV hemorrhagic cystitis.

The level of BK viruria^[40,54,65,72,74,80] and plasma loads of BKV DNA^[76] predict clinical manifestations of BKV infections, including hemorrhagic cystitis. However, BKV infection is not the only, or even the more common, cause of hemorrhagic cystitis^[43]. Use of busulfan^[44] and adenovirus infection^[46] were also identified as other

important causes of this entity.

Other manifestations from the urinary system of BKV infection in bone marrow or stem cell recipients include ureteritis with ureteral stenosis^[78,80] and BKN^[8,11,49,50,51,58,60,63,64,70,75,77,79]. One report^[69] reviewed the features of BKN in bone marrow transplant recipients. Table 2 summarizes reported cases of BKN in recipients of bone marrow or stem cell transplant recipients. The majority of subjects developed end-stage renal disease (ESRD) and were placed on dialysis. Mortality was high in this patient sample. De laCruz *et al*^[73] reviewed the clinical manifestations of BKV infection in hematopoietic stem cell transplantation.

BK viral infections in the urinary system of recipients of heart transplants^[11,82-96]

Table 3 summarizes reported cases of BKN in cardiac transplant recipients^[11,84,85,86,89,90,91,93,94,96]. Rejection episodes of varying severity and frequency requiring increased immunosuppressive medications were reported in nine patients and ESRD in eight. Six patients underwent dialysis and three patients died. Lorica *et al*^[94] describe two additional pediatric heart transplant recipients with BKN. A three-month-old girl was on peritoneal dialysis at the time of the report while a 3-year-old girl on peritoneal dialysis died from BK encephalomyelitis^[94]. Thus, BKN has severe adverse effects on both renal function and overall state of health in cardiac transplant recipients. Persistent detection of the characteristic decoy cells in the urine indicating persistent BKV infection without any evidence of clinical manifestations was reported in one heart transplant recipient^[83].

Puliyanda *et al*^[88] compared the incidence of BK viremia and the risk of BKN in patients with isolated kidney, heart, liver, and combined kidney-heart, kidney-liver, kidney-pancreas and kidney-heart-liver transplant recipients. These authors concluded that the risk of BKN is lower in patients with isolated heart or liver transplants than in those with kidney transplants. High levels of BK viremia were associated with BKN in this study. However, none of the patients with heart transplants exhibited BK viruria and the plasma levels of BKV were low in liver transplant recipients.

Ducharme-Smith *et al*^[95] found BK viruria in approximately one third and BK viremia in only 7% of pediatric heart transplant recipients. One of the viremic patients developed BKN. Multivariate analysis identified history of Epstein-Barr infection as the only predictor of BK viruria in the same study^[95]. In another study, Pendse *et al*^[87] found definitive evidence of BK viruria in 13% of the heart transplant recipients but no signs of BKN. These authors proposed that a second organ-specific insult to the kidneys is needed for patients with BK viruria to develop BKN.

BK viral infections in the urinary system of recipients of lung transplants^[97-102]

A small number of cases of BKN in lung transplant

Table 3 BK Nephropathy in heart transplant recipients

Ref.	Gender age	Renal function	Clinical associations
[11]	Male, 65 yr	ESRD Refused dialysis	No rejection episodes Urothelial transitional cell carcinoma causing bilateral hydronephrosis Death following perforated gastric ulcer
[84]	Female 59 yr	SCr 5.0 mg/dL Awaiting dialysis	Three severe rejection episodes early
[85]	Male 57 yr	ESRD On dialysis	Repeated rejection episodes
[86]	Male 26 yr	ESRD On dialysis	Multiple rejection episodes
[89]	Male 54 yr	ESRD Dialysis	Persistent rejection Death from arrhythmia
[90]	Male 12 yr	Last SCr 2.0 mg/dL	Cardiomyopathy from chemotherapy for Ewing's sarcoma One rejection episode
[91]	Male 8 yr	ESRD On dialysis	8 rejection episodes in 1 st heart transplant BK nephropathy after 2 nd heart transplant
[93]	Female 9 yr	Peak SCr 1.9 mg/dL Last SCr 1.2 mg/dL	Rejection episodes not reported Reduction in BK viral load and improvement in renal function after leflunomide was started
[94]	Male 14 yr	ESRD Dialysis	Multiple rejection episodes Lymphoproliferative disorder in the 12 th year BK nephropathy in the 16 th year Death from multiple organ failure
[96]	Male 60 yr	ESRD On peritoneal dialysis	One rejection episode
[96]	Male 43 yr	eGFR 40 mL/min	Recurrent giant cell myocarditis in the transplanted heart One rejection episode

BK nephropathy was usually manifested several years post-heart transplantation. Ages reported in this Table are the calculated ages at the time of diagnosis of BK nephropathy. ESRD: End-stage renal disease; SCr: Serum creatinine; eGFR: Estimated glomerular filtration rate.

recipients has been reported^[98,101,102]. Pertinent features of these patients are summarized in Table 4. Two of the three patients developed ESRD and were started on dialysis. One of these two patients died. One case of nephropathy secondary to a different polyomavirus (simian virus 40 or SV40) in a 32-year-old man with cystic fibrosis who had received a lung transplant was also reported^[97]. This case progressed to ESRD. Another publication reported a case of BK hemorrhagic cystitis in a lung transplant recipient^[99].

Thomas *et al.*^[100] studied longitudinally the frequency of viruria from three different polyomavirus species (BKV, JCV, SV40) in lung transplant recipients. Viruria, at least in one instance, was found for BKV in 42% of the patients, for JCV in 28% and for SV40 in 7%. Although no definitive evidence of clinical polyomavirus infection was detected in this study, patients with viruria had shorter survival.

BK viral infections in the urinary system of recipients of liver transplants^[32,88,103-113]

We found only one reported case of BKN in a liver transplant recipient^[112]. This case is summarized in Table 4. One man with combined liver-kidney transplants developed IgA nephropathy in his native kidneys and BKN in the transplanted kidney^[110].

Several reports analyzed the frequency of BK viruria and viremia and its relationship with renal disease in liver transplant recipients^[32,88,103-109,111,113]. Low frequencies of BK viruria and viremia and low risk of BKN were commonly reported^[32,88,103,108]. Salama *et al.*^[104] concluded that BKV infection is not associated with a decline in renal function in liver transplant recipients. Rauschenfels *et al.*^[105] concluded that hepatotropic viruses, including BKV, do not have a major role in the pathogenesis of biliary atresia, which is the major condition leading to liver transplantation in pediatric populations.

Higher frequencies of BK viruria and viremia and a risk of kidney disease from BKV infection were reported in a few studies of liver transplant patients. Loeches *et al.*^[106] reported BK viruria in 21% and BK viremia in 18% of the liver recipients, the last one early after transplant, and concluded that persistent BK viremia may be associated with renal dysfunction. Demir-Onder *et al.*^[111] reported similar results. Higher frequency of BK viruria in pediatric than adult liver transplant recipients was described by Brinker *et al.*^[107]. Finally, Mitterhoffer *et al.*^[109] reported a higher frequency of BK viremia (56%) in prospective liver transplant recipients with preexisting chronic kidney disease than in those with normal kidney function (14%).

BK viral infections in the urinary system of recipients of pancreas and kidney-pancreas transplants^[114-135]

We found only one confirmed case of BKN in a recipient of an isolated pancreatic transplant recipient^[114]. This case is summarized in Table 4. BKN has been reported in renal transplants of several recipients of combined kidney-pancreas recipients^[115,117-120,123-125,128,129,132-135]. The prevalence of BKV replication and BK viruria in those with combined kidney-pancreas transplants was high in several studies^[116,126-128]. However, one study^[120] reported a low incidence of BKN (2.9%) in recipients of combined kidney-pancreas transplants. CMV viremia may prevent reactivation of BKV in these patients and in recipients of solitary kidney transplants^[130].

Preservation of pancreatic function was reported uniformly in recipients of combined kidney-pancreas transplants with BKV infection^[115,117,119,120,124,128,129,133]. Preservation of normal kidney transplant function was reported in some studies^[129,132-134], while other studies^[117-119,123] concluded that BKN was an important cause of significant deterioration of the transplanted kidney function. A multivariate analysis performed by Heilman *et al.*^[121] identified BKN and a serum creatinine level at or above 1.6 mg/dL as independent correlates of renal graft fibrosis in kidney-pancreas transplant

Table 4 BK nephropathy in recipients of lung, liver and pancreas transplantation

Ref.	Gender age	Renal function	Clinical associations
Lung [98]	Male 40 yr	ESRD On dialysis	Metastatic seminoma treated successfully Three rejection episodes
[101]	Female 72 yr	Peak SCr 2.6 mg/dL Last SCr 2.2 mg/dL	Prolonged neutropenia post-transplant No rejection episodes
[102]	Male 9 yr	ESRD Dialysis	Collecting duct carcinoma Death from respiratory and cardiac failure
Liver [112]	Male 59 yr	SCr 1.9 mg/dL at diagnosis	Multiple rejection episodes Follow-up after diagnosis not reported
Pancreas [114]	Male 54 yr	SCr 2.2 mg/dL At diagnosis	Follow-up after diagnosis not reported

ESRD: End-stage renal disease; SCr: Serum creatinine.

recipients. A recent study by Schachtner *et al.*^[135] concluded that in comparison to recipients of solitary kidney transplants, recipients of combined pancreas-kidney transplants exhibit a higher incidence and severity of BKN.

The diagnosis of BKN in recipients of combined kidney-pancreas transplants is complicated by the potential absence of decoy cells in the urine. Decoy cells are an important diagnostic clue for BKV infection in the urinary tract. High concentrations of pancreatic enzymes in the urine of transplanted patients may degrade these cells^[122]. Quantitative nucleic acid testing for BKV may assist in the diagnosis of BKN in these subjects^[131]. Kubal *et al.*^[125] reported renal transplant nephrectomies and subsequent successful combined kidney-pancreas transplants in two patients who had developed BKN and ESRD in the initial kidney allograft.

In general, BKN in native kidneys of patients with various transplanted organs is substantially less frequent than in transplanted kidneys, but like BKN in transplanted kidneys tends to lead to ESRD and is associated with significant mortality.

BKV INFECTIONS IN OTHER POPULATIONS

BKV infection with various clinical manifestations has been reported more frequently with diagnostic entities either causing immunosuppression or requiring therapeutic immunosuppression than in individuals without an apparent immunosuppressed state. The manifestations of BKV infection in immunosuppressed and non-immunosuppressed states are briefly discussed below.

BKV infections in patients with HIV infection have

been studied extensively^[136-168]. Both BKN^[139,147,151,154,155,159,164,166] and hemorrhagic cystitis^[157,165] have been reported in HIV patients. A series of studies addressed rates of BK viremia and viruria^[136,137,140,141,143,144,162-163,168], the pathogenesis of BKV infection^[157,165] and various clinical aspects of this infection^[138,140,145,146,149,150,152-154,156,158,160,167] in HIV-positive populations.

A patient under treatment for granulomatosis with polyangiitis developed BK hemorrhagic cystitis^[169]. However, in a series of patients with vasculitis associated with ANCA, only those who had received a kidney transplant exhibited BK viremia^[170]. Manifestations of BKV infection in patients with systemic lupus erythematosus (SLE) include viruria and viremia, and the presence of decoy cells in the urine of a patient with BK viruria, hemorrhagic cystitis and hemophagocytic syndrome^[171,172]. The tendency of experimental animals with BKV infection to form anti-double stranded antibodies (anti-dsDNAs) has led to the hypothesis that BKV infection triggers SLE^[171]. Life threatening hemorrhagic cystitis secondary to polyomavirus JC was reported in an adolescent with ataxia-telangiectasia^[173].

BKV infection in patients with various malignancies has been a major focus of the literature^[174-186]. An early study reported BK viruria in patients receiving chemotherapy for malignancy^[174]. BKN has been diagnosed in patients with chronic lymphocytic leukemia^[176,177,180,183], acute lymphocytic leukemia^[178,180,185] and Hodgkin's lymphoma^[175]. BK cystitis was reported in patients with Hodgkin's lymphoma^[182,184,186]. One other patient with lymphoma^[179] developed neurological manifestations of BKV infection.

The potential role of BKV infection in tumorigenesis has received great attention^[187-248]. Urothelial malignancies in association with BKV infection were described in several recipients of kidney transplants^[200,205,213,214,216,220-223,227,229,233,234,236,238,239,245,247] and one heart transplant recipient^[243]. Malignancies in non-transplanted subjects in which BK infection may play a pathogenetic role include bladder carcinoma^[201,211], renal cell carcinoma^[192,230], prostatic carcinoma^[212,217,235,245], Kaposi's sarcoma^[197], neuroblastoma^[199], leukemia, non-Hodgkin's lymphoma^[205], colorectal carcinoma^[215], gastrointestinal B-cell lymphoma^[240], oral squamous cell carcinoma^[244], cervical carcinoma^[224], breast carcinoma^[226] and melanoma^[206].

The role of BKV in tumorigenesis has been disputed. Several studies^[187,203,208,216,223] failed to find an association of BKV infection with various malignancies and published reviews of the role of BKV in malignancies^[202,209,219,231,232,241,247] reflect the current uncertainty about this topic. However, in animal experiments BKV has been shown to play a role in tumorigenesis^[190,191,193,195,196,198] and several reports^[192,194,199,201,204,209,210,217,218,237,242] have addressed pathogenetic pathways potentially linking BKV infection and tumorigenesis. A commonly discussed mechanism is inactivation of the tumor suppressor proteins p53 and pRB

family by the large T antigen T (T-Ag), which is a major antigen of BKV^[199,204]. Other proposed pathways of tumorigenesis include the role of BKV as a cofactor in various malignancies^[217,237] and BKV integration in the human genome^[242].

Finally it is worth noticing that BKV infections with manifestations from the urinary system have been rarely reported in subjects without other systemic diseases. Examples of these infections include a case of BKN, urothelial ulceration and renal pelvic fibrosis with an imaging picture of a renal mass^[249] and the association of BK, and to a greater extent JC, viruria with asymptomatic hematuria in a small sample of Koreans^[250].

CLINICAL MANIFESTATIONS OF BKV INFECTION OTHER THAN NEPHROPATHY OR HEMORRHAGIC CYSTITIS

Table 5 shows clinical manifestations of BKV infection that have been reported so-far. Manifestations other than BKN and hemorrhagic cystitis^[15,78,81,145,146,251-287] will be reviewed in this section. In addition to the kidneys and urinary bladder, BKV was detected at autopsy in the epithelial cells of the ureters of a patient with non-Hodgkin's lymphoma^[15]. Ureteral involvement by BKV with various degrees of urinary obstruction was reported in patients with bone marrow or hematopoietic stem cell transplants^[78,81,252,255] and renal transplants^[251,253,254]. Fatal BK pneumonia was reported in three patients with hematopoietic stem cell transplants^[257,259,260] and two patients under treatment for chronic lymphocytic leukemia^[258,261]. BKV infection also accounted for 8% of the acute respiratory infections in non-immunocompromized children^[256]. BKV infections in non-immunocompromized patients are probably under represented.

Various neurological syndromes associated with BKV infection have been reported in patients with acquired immune deficiency syndrome (AIDS)^[138,142,145,150,152,158,262,269]. In addition to AIDS patients, BK meningoencephalitis has been reported in non-immunocompromized subjects^[263,264], in patients with malignancies including chronic lymphocytic leukemia^[261], Hodgkin's lymphoma^[266], and in a kidney transplant recipient^[273]. Progressive multifocal leukoencephalopathy, also often diagnosed in AIDS patients, has been associated primarily with the JCV^[261,265], but has also been reported in association with BKV infection in one patient^[270]. However, this last association needs confirmation^[271]. A case of progressive multifocal leukoencephalopathy associated with both JC and BKV infections in a non-immunocompromized patient has also been reported^[272]. BK retinitis associated with other manifestations of BKV infection has been reported in AIDS patients^[145,146]. Progressive outer retinal necrosis developed in a kidney transplant recipient with BKV and varicella zoster virus in the vitreous fluid^[275]. This retinal disease was probably caused by varicella zoster virus. Neurological synd-

Table 5 Clinical manifestations of BK virus infection

Uropoietic system
Nephropathy
Hemorrhagic cystitis
Ureteritis - ureteral obstruction
Respiratory system
Upper respiratory infection
Pneumonia
Central nervous system
Meningoencephalitis
Progressive multifocal leukoencephalopathy (probable)
Retinae
Retinitis
Progressive outer retinal necrosis (questionable)
Blood vessels
Vasculitis
Gastrointestinal system
Intestinal ulcers
Lower gastrointestinal bleeding
Hematopoietic system
Pancytopenia
Neutropenia
Hemophagocytic syndrome
Polyclonal gammopathy
Malignancies
Urothelial tumors
Various other tumors

romes associated with BKV infection were analyzed in two reviews^[268,274].

Deltoid muscle biopsy in a renal transplant recipient who developed progressive weakness and dyspnea, and died after several episodes of life-threatening arrhythmias revealed BK vasculitis^[276]. A detailed description of the glomerular histologic changes in a large study of renal biopsy samples with BKN^[277] failed to identify vascular changes. However, in other reports BKV was found to be localized in endothelial cells of both renal arteries and venules^[278] and venous thrombosis associated with BKN was diagnosed in a renal allograft by ¹¹¹In leukocyte imaging^[279].

BKV is replicating in salivary glands^[280]. High frequency of BKV shedding from the gastrointestinal tract in recipients of hematopoietic stem cell transplants has been reported^[65]. Gastrointestinal bleeding associated with bowel lesions putatively caused by BKV infection was reported in a renal transplant recipient^[281] and a hematopoietic stem cell transplant recipient^[282]. Interestingly, high rates of BK viruria in patients with inflammatory bowel disease have been documented^[283]. However, the clinical significance of this finding will require further study.

Pancytopenia or severe neutropenia associated with BKV infection have been found in kidney transplant recipients^[284-286]. Hemophagocytic syndrome was diagnosed in one of these patients^[286]. Polyclonal gammopathy triggered by BKV infection was reported in a hematopoietic stem cell transplant recipient suffering from B-cell lymphoblastic leukemia^[287]. BKV DNA has been isolated in normal hepatic tissue and elevated hepatic enzymes were reported in bone marrow trans-

plant recipients who had BK viruria^[24].

Key points of part A

Clinical manifestations of BKV infection have been reported in patients with various immunosuppressed states and small numbers of subjects with apparent absence of immunosuppression. Although BKN is much less frequent in the native kidneys of organ transplant recipients than in transplanted kidneys, it is uniformly associated with poor outcomes. BKV infection causes a variety of clinical manifestations in addition to nephropathy and hemorrhagic cystitis.

PART B DIAGNOSIS OF BKN AND PATHOGENESIS OF BKV INFECTION

DIAGNOSIS OF BKN^[10,13,14,16,22,277,288-336]

BKN accounts only for a small fraction of the renal dysfunction encountered in transplant recipients. Its diagnostic features have been extensively studied in renal transplant recipients. Risk factors for the development of BKN including certain immunosuppressive agents, such as mycophenolate, and manifestations of BKV infection in the urinary tract, including BKN, ureteral obstruction, lymphocele, bacterial urinary tract infection, hematuria, and elevated serum creatinine levels have been studied in renal transplant populations^[22,288]. A study from South Korea^[336] identified an accompanying acute rejection episode, in addition to advanced histologic stage of BKN and elevated serum creatinine levels, as factors increasing the risk of renal transplant failure in renal transplant recipients. Reports involving renal transplant recipients constitute the main source of information reviewed in this section.

Renal biopsy constitutes the gold standard for the diagnosis of BKN. Various aspects of the renal biopsy in BKN have been studied^[10,13,14,16,291,294,296,298,299,301-303,307,311,312,314,316,322,324,328]. An early report by Rosen *et al.*^[13] identified tubulo-interstitial nephritis as the main histologic picture of BKN. Viral replication in the tubular epithelial cells, starting in the renal medulla and extended later to the renal cortex, initiates cytopathic changes in the renal tubules that can be confirmed by immunohistochemistry, *in situ* hybridization, electron microscopy or polymerase chain reaction (PCR)^[291,316].

The Maryland classification of BKN^[291,296,298], which is based on the degree of interstitial inflammation and fibrosis, schematically recognizes three histological patterns which are considered to represent successive stages of BKN. The first pattern is characterized by absent or minimal interstitial inflammation and the presence of viral cytopathic changes, including karyomegaly, hyperchromasia, and basophilic nuclear inclusions, in a few tubular cells located primarily in the renal medulla. Cytolytic changes, including cell necrosis, apoptosis, smudged chromatin and hobnail nuclei with desquamation into the tubular lumen and formation of necrotic casts accompany often the cytopathic

changes^[291].

The second pattern of the Maryland classification is characterized by focal or diffuse clusters of tubules with cytopathic and cytosolic changes plus interstitial collections of inflammatory cells, primarily lymphocytes, with tubulitis and tubulo-interstitial atrophy in some cases. The third pattern is characterized by extensive interstitial fibrosis and tubular atrophy, lymphocytic infiltration and paucity of viral cytopathic changes. The course of renal dysfunction roughly correlates with the histological staging^[291].

A key diagnostic feature of BKN is the finding of viral cytopathic changes, which are apparently identical for papovaviruses BKV, JCV and SV40^[296]. The nuclei of the infected cells are large and contain a basophilic inclusion that either replaces the chromatin or displaces it to the periphery of the nucleus (Figure 1A and C). The presence of a halo around the BKV inclusion is used to differentiate between BKV infection and CMV infection. The BKV infected cells have larger nuclei in comparison to cytoplasm and no viral inclusions in their cytoplasm. Immunohistochemical staining for SV40 large T antigen (Figure 1B), which cross-reacts with BKV and JCV, identifies the presence of a papovavirus and allows its differentiation from adenovirus, which can also cause nephritis with intranuclear viral inclusions morphologically identical to those of papovaviruses. Transmission electron microscopy of cells infected with papovavirus shows characteristic intranuclear deposits of polyhedral virions with an average diameter of 40 nm and in some cases fibrillary or microtubular inclusions. Electron microscopy may assist in the differentiation of papovavirus virions from those of CMV, adenovirus and herpesvirus^[296].

The proposed sequence of events leading to the histological changes of BKN is as follows^[296]: Viral infection leads to cell death and disintegration with discharge of virions in the extracellular space. Entrance of virions into adjacent cells leads to spread of the infection. Infected renal tubular cells and virions exfoliate in the urine. If the tubular injury is severe, tubular basement membranes rupture causing spillage of virions and viral proteins into the blood stream. Severe tubular injury also causes an inflammatory response with influx of tubulo-interstitial B cells, T cells, plasma cells and macrophages (Figure 1C). This histological picture can be confused with acute cellular rejection (ACR) in renal transplant recipients. When it is severe or persistent, the tubular injury leads to tubular atrophy and interstitial fibrosis.

The utility of the Maryland staging of BKN, modified by the American Society of Transplantation, has been successfully tested in clinical practice. In one study, the third pattern was associated with higher serum creatinine levels at presentation and greater renal function deterioration in follow-up measurements than the first or second pattern^[299].

The histology of BKN has been reviewed in successive Banff group meetings^[307,312,314,328]. The original

Banff classification also recognizes three histologic patterns, characteristic of the stages of BKN: (1) an early stage without tubular cell necrosis; (2) a stage of active BKN with tubular cell necrosis (Figure 1A); and (3) a late stage characterized by fibrosis (Figure 1D)^[307]. In one study, reasonable agreement between various nephropathologists was reported using this Banff classification^[312]. However, another study failed to demonstrate superiority of the Banff staging of BKN over the Maryland classification^[314]. The latest Banff group meeting stressed the need for improving the reproducibility of large SV40 T antigen immunostaining, which is proposed as an index of the BKV viral load and a potential predictor of the renal graft outcome in patients with BKN^[328]. *In situ* hybridization may offer an alternative to immunohistochemistry in the diagnosis of BKN^[316]. The diagnostic challenges associated with BKN were recently reviewed by Masutani^[324].

In renal transplant biopsies with BKN, the presence of peritubular capillary staining for C4d raises the possibility of coexisting antibody-mediated (humoral) rejection. Some biopsies with BKN show staining of tubular basement membranes for C4d, and this finding is correlated with marked viral cytopathic effect^[303]. Granular immune complex deposits in the tubular basement membranes^[301] and in the subepithelial space of glomerular basement membranes^[302] have been described in patients with BKN. In the latter, BKV was identified ultrastructurally in the immune deposits^[302]. Glomerulonephritis attributed to BKV infection was found in a few renal transplant recipients^[277,321].

The focal lesions of the early stages of BKN may be missed in a renal biopsy^[298,324]. Several diagnostic pathways complementing the renal biopsy have been explored. The value of surveillance renal biopsies in early diagnosis of BKN has been discussed in several reports^[22,310,313]. BK viruria^[22,297,306,308,317,322,332,334] and viremia^[22,289,308,309,317,322,323,332,335] provide another tool for the detection of BKN. High levels of viruria or viremia correlate reasonably with the presence of BKN. Cut-off levels for the diagnosis of BKN have been proposed and tested.

Detection in urinary samples of desquamated tubular or urothelial cells with BKV inclusions provide another tool for the diagnosis of BKV infection in the urinary system^[291]. The cardinal features of these cells, known as "decoy cells", because of their similarity to malignant cells, in a Papanicolaou stain include a greatly enlarged nucleus with a basophilic inclusion next to the chromatin producing a ground-glass or gelatinous look. A halo may surround the basophilic inclusion. Decoy cells may also be detected by phase-contrast microscopy^[292]. Decoy cells led to the diagnosis of BKV infection in an immunocompetent child with otitis media followed by dysuria^[315]. However, decoy cells may be absent from the urine of patients with documented BKN^[333]. In one study, the positive predictive value of decoy cells was low, but improved by immunohistochemical staining of the urine for SV40 large T antigen^[331]. Negative-stain electron

microscopy and semi-quantitative identification of free BKV particles in the urine assists in the identification of patients at high risk of BKN^[300]. Genotyping of BKV by an improved PCR method^[327] and serologic tests^[329] may help in the diagnosis of BKV infection. Ultrasonographic pictures suggesting BKN were recently reported^[330].

In renal transplant recipients, the differentiation between ACR and BKN presents difficulties^[294]. The histologic picture of tubulo-interstitial nephritis is indistinguishable between the two conditions^[319]. Immunophenotyping of the mononuclear cells in the interstitial infiltrates was found to be promising in some studies^[304,318], but could not differentiate between ACR and BKN in others^[305]. Serial monitoring of donor-specific cell-free DNA in the urine may be a sensitive biomarker of acute kidney injury, but does not allow the distinction between ACR and BKN^[320]. Urine analysis methodologies potentially allowing the differentiation of these two conditions are proteomics^[325] and characterization of the percentages and absolute numbers of CD4(+) and CD8(+) effector memory T cells^[326].

Several other questions related to the diagnosis of BK infection in the urinary tract have been investigated. Immunohistochemical analysis of renal biopsies revealed differences in the inflammatory infiltrate between different BKV strains^[290]. Additionally, latent BKV and JCV were found in the urinary tract of immunocompetent subjects in an autopsy study^[295]. One review^[311] analyzed the diagnosis and pathogenesis of BK cystitis in hematopoietic cell transplant recipients. Another study found a high rate of mutations in the coding region VP-1 of BKV in HIV-infected patients with low CD4(+) counts^[330]. The authors of this study postulated that these mutations could affect the clinical manifestations of BKV infection in HIV patients. Whether the diagnosis of BKV infection will require in the future an analysis of the mutations of the virus in various patient groups or individual patients is not clear.

PATHOGENESIS OF BKV

INFECTION^[10,20,35,126,336-436]

BKV is a small, unenveloped icosahedral DNA virus. Its genome sequence contains three functional regions. The early region encodes the large T antigen (T-Ag) and the small T antigen. These antigens are involved in BKV DNA replication and could be treatment targets. As noted earlier, interaction of T-Ag with p53 is believed to be the main pathway of tumorigenesis by BKV. The late region is responsible for the production of the proteins VP1, VP2 and VP3, the role of which in BKV infection will be examined later. Finally, the non-coding control region controls the expression of the viral genes^[423].

The pathogenesis of BKV infection, and specifically of BKN, is a complex process that has not been elucidated completely. Costa *et al.*^[10] listed factors related to the patients, the transplanted organs, and the BKV genotypes as determinants of the development

of BKN. The first contact with BKV occurs early in childhood. Antibodies against BKV are found in 50% of the subjects by age 3 and in 80%-90% by age 20 years, with decrease in the antibody titers in older age^[20]. The incidence of primary infection is similar in immunosuppressed and non-immunosuppressed children^[340].

Age older than 50 years is one of the patient-related risk factors for BKN^[10]. In non-immunocompromised subjects, the rate of BK viruria is low below the age of 30 years and increases progressively after that age^[35]. Older subjects excrete preferentially the BKV viral subtypes I and IV^[35]. In a fraction of the subjects the virus persists without clinical manifestations, but in a state of active asymptomatic replication^[35]. Organs harboring replicating BKV include the kidneys, the bone marrow and the brain^[35]. Persistence of the virus in other tissues, including spleen, normal thyroid glands^[429], pancreas^[342], and lymphocytes in HIV-positive patients^[344], has also been reported. Active BKV disease in various organs is more frequent if another insult to these organs has also occurred. Examples of this sequence include the relatively high frequencies of BKN in kidney transplant recipients and hemorrhagic cystitis in bone marrow or stem cell transplant recipients.

The mode of BKV transmission is not completely understood. Transplacental transmission was described in an early study^[337]. Transmission through the transplanted kidneys has also been documented^[351,430]. Replication of BKV in salivary glands was found *in vitro* suggesting oral transmission^[367]. After the primary infection the virus remains latent in host tissues and is reactivated when an immunosuppressed state supervenes. Following renal transplantation, reactivation of BKV demonstrated by BK viruria is usually noticed after 3-6 mo while reactivation of JCV occurs as early as five days post transplantation^[379]. Early BKV reactivation is associated with viremia^[377] and worse transplant function^[372].

Circulating BKV is taken up by cells. In experimental animals, endothelial cells in hepatic sinusoids and in the kidney were shown to remove rapidly blood-borne BK and JCV-like particles^[409]. Upon contact with the cell membrane BKV is bound to membrane receptors^[381]. The identified specific BKV receptors include polysialated ganglioside GT1b and (2,3)N-linked sialic acid^[351]. Cellular entry of BKV is through caveolar endocytosis^[357,369]. The GT1b receptor, which is involved in caveolar endocytosis^[351], could provide a treatment target in the future.

Differences in cellular entry and trafficking exist between various cell types and viral genotypes^[392]. The capsid proteins VP2 and VP3 have important roles in the nuclear entry of BKV^[414]. BKV genotypes have different potential for pathogenicity^[147,351,356,368,380,389]. The family of transforming-growth factors (TNF) plays a role in BKV gene expression^[359]. BKV infection activates the TNF receptor system in BKN^[432,433]. Monocyte and Th-2 cytokines, including IL-1 RA, IL-3, IL-6 and sIL-6R are

elevated in the urine of renal transplant recipients with BK viruria and may be involved in the pathogenesis of BKN^[370]. In general, BKV infection of renal tubular epithelial cells leads to activation of cellular genes involved in cell cycle regulation and apoptosis and downregulation of a small number of genes^[373].

After entry into the cytoplasm, BKV is transported into the endoplasmic reticulum along the microtubules by a complex mechanism favored by acidic environment^[368]. The ER associated degradation (ERAD) pathway, which is responsible for the transfer to the cytosol of ER secretory proteins that have not attained their proper conformation, where they are degraded by proteasomes, is responsible for transferring BKV into the cytosol, followed by entry of these proteins into the nucleus^[372]. After entry of BKV into the nucleus, BKV genome release takes place^[383]. The Derlin family of the ERAD translocation complex proteins is important for the trafficking of BKV and other polyomaviruses^[370]. Proteasome action is also important in BKV trafficking^[392].

Several reviews have stressed the role of innate immunity in the pathogenesis of BKV infection and the need to monitor both the BK viral load and the state of immunity in populations prone to BKV infection as the first step in the timely management of this infection^[351,395,406]. A recent report reviewed potential preventive and therapeutic approaches for BKV infection related to the mechanisms of innate immunity^[433]. Innate immunity compounds that inhibit BKV infection include lactoferrin^[349], the antimicrobial defensins alpha-defensin human neutrophil protein 1 (HNP1) and human alpha-defensin 5 (HD5) which were shown *in vitro* to aggregate BKV virions thus blocking cellular entry^[363], and the cellular DNA damage response (DDR) which modulates BKV replication^[388,431]. Human leukocyte antigens (HLAs) that are associated with lower risk of BKV infection include HLA-A2, HLA-B44, HLA-DR5^[397] and HLA cw7^[421]. Expression in BKV-infected cells of p53, binding of which to the BKV large T-Ag is proposed as a mechanism of tumorigenesis, may provide a therapeutic target in the future^[353]. In renal tissues, large T-Ag is expressed in decreasing frequency in medullary collecting ducts, distal and proximal convoluted tubules and Bowman's capsule^[350]. Viral replication pathways which could form the basis of therapeutic approaches in the future include agnoprotein, a viral phospho-protein^[364], viral microRNA (miRNA)^[394,410], and autophagy in host cells^[401].

Disruption of adaptive immunity plays a major role in the pathogenesis of BKV infection. Both cellular and humoral aspects of adaptive immunity in BKV infection have been extensively studied. Age affects both the cellular and humoral immune responses to BKV infection^[407]. BKV-specific cellular immunity is vital for control of viral replication and prevention of chronic viral disease^[383]. Low levels of cytotoxic T cell (CTL) response correlate with high BKV loads and high anti-BKV antibody titers, while a high CTL response correlates with low viral loads and low anti-BKV

antibody titers^[347]. The finding that viral capsid epitopes of BKV share homology with other polyomaviruses, including JCV and SV40 suggests that infection with one of the viruses could establish cross-immunity against the other viruses *via* a cellular-immune response^[348].

Loss of BKV-specific T cell immunity in the post-transplant period identifies kidney transplant recipients at high risk for BKV infection^[427]. In patients with BKV infection, recovery of cellular immune responses to large T-Ag correlates with improvement of BKN^[365,384]. However, in one study the percent of activated T cells correlated with the degree of BK viremia^[396]. In the same study, patients with decreased renal function exhibited high levels of activated T cells and BK viremia.

Monitoring of both non-virus specific and virus-specific T cell responses in transplant patients has been advocated^[405,417]. Monitoring these responses post-transplantation may have a role in the detection of BKV reactivation^[423]. T cells respond to different BKV antigens^[419]. The nuclear factor of activated T cells (NFAT) binds to the viral promoter and regulates viral transcription. This factor is involved in a complex regulatory pathway that can affect the course of BKV infection^[375]. The genetic variation of BKV strains is limited^[425]. In HLA-A*0201 individuals, cytotoxic T-cell lymphocyte (CTL) responses are elicited towards two of the VP1 epitopes, VP1(p44) and VP1(p108)^[347]. CTLs directed against VP1(p44) are more abundant than VP1(p108) in healthy individuals, while the opposite is true in kidney transplant patients who present with BKN. This suggests a shift in the epitope immunodominance in the setting of active BKV infection^[347]. Flow-cytometry analysis of BKV-specific T cells also showed that VP3 is an important target of cellular immunity^[386].

CD4 T cells have a role in BKV clearance^[387,412]. Though the pattern of cellular response to BKV antigens has not been fully clarified, it has been discovered that in kidney allograft recipients, VP1-specific interferon-gamma producing T cells were more likely to be CD4⁺, while CD8⁺ lymphocytes are more frequently directed against the large T antigen^[361]. Stimulation of CD28 in T cells is one of the rejection mechanisms blocked by belatacept. Subpopulations of human T cells exposed to antigens may be activated by mechanisms different than CD28 and cause rejection resistant to belatacept. In mice models polyomavirus exposure leads to reduced expression of CD28 in T cells and was proposed as a mechanism of resistant rejection^[422]. Activated CD4 T cells upregulate CD30, another cell marker of B and T cells, causing an increase in serum soluble CD30 (sCD30), which plays a role in the pathogenesis of rejection^[366]. Levels of sCD30 are associated with BK viremia and may be of use in the management of the immunosuppressive regimen for renal transplant patients as well as a prognostic factor for graft rejection^[436].

The role of dendritic cells in antigen presentation to T cells is well known. Dendritic cell deficiency was shown to be a risk factor for reactivation of BKV infection after renal transplantation^[382]. A genotypic analysis in renal

transplant recipients found that low frequencies of the activating receptor KIR3DS1 are associated with the development of BKV infection and that there appears to be a genetic predisposition for BKN linked to natural killer cells^[402].

The interplay between genetics and immunology is reflected in the finding that the NFAT can transcriptionally regulate BKV. During T-cell activation, NFAT translocates to the nucleus where it regulates the expression of various genes^[341]. NFAT regulates BKV transcription, while NFAT inhibition with an NFAT inhibitor peptide, 11R-VIVIT, reduces BKV replication^[375]. In addition there is growing evidence that epigenetic factors may contribute to the regulation of BKV and its tissue propagation. Viral microRNAs (miRNAs) are playing a crucial role in viral replication. BKV-encoded miRNAs (miR-B1) have been studied in patients with BKN. After BKV infection, miR-B1 levels are significantly increased and these miRNAs suppress T-ag-mediated autoregulation of BKV replication. Thus, miR-B1 offers a potential treatment strategy for controlling BKV infection^[410].

In addition to cellular immune response, humoral immunity also plays an important role in BKV infection. Antibodies to various BKV antigens were detected in normal controls and patients suffering from various diseases; patients with urinary bladder carcinoma exhibited the highest frequency and titers of anti-BKV antibodies^[338]. HIV patients with BK viremia and JC viremia have a low frequency of antibodies against these two viruses^[343]. In renal transplant recipients, BKV-specific IgG levels were in the pre-transplant period lower in those who developed active BKV infection than in those who did not develop BKV infection; the rise in the antibody titers post-transplant, however, was higher in patients who developed BKV infection^[360]. In this last group, antibody titers correlated with the intensity of BKV infection. This suggested that specific antiBKV IgG response is not associated with viral clearance^[360]. A prospective study concluded that determination of the serostatus of prospective kidney transplants and recipients allows stratification of the risk for BKV infection post-transplant^[398].

Pre-kidney transplant levels of anti-BKV antibodies did not clearly predict post-transplant BK viremia in pediatric renal transplant recipients^[374]. However, there is considerable evidence pointing to a link between antibody titers and BKV disease progression in the post-transplant period. Pediatric patients with hemagglutination-inhibition titers < 40 were found to be at greater risk of disease progression, and seronegative recipients were found to be at greater risk of developing BKN if seronegativity was demonstrated by the VP1 enzyme immunoassay^[351]. In patients at different stages of BKN, BKV-specific IgG levels were higher in those who had recovered from BKN than in patients with acute infection. Interestingly, the density of plasma cells in the interstitial infiltrates of BKN was found to correlate with the levels of circulating anti-BKV IgM

in one study^[378]. BKV infection was fatal in a patient with hyper-IgM deficiency. This patient, whose class switching from IgM to IgG was impaired, was not able to produce the protective IgG antibodies against the virus^[351]. This case suggests that immunoglobulin response has an important role in controlling BKV infection.

Measurement of the anti-BKV titers is an important tool to detect the onset of viral replication^[352]. Further research is needed to determine the extent to which these antibodies can neutralize the virus or its active viral components, though some suggest that there are BKV neutralizing antibodies that target VP1^[351,361,378]. *In vitro* coinubation of BKV with human intravenous IgG preparations caused 90% inhibition of viral DNA after 7 d in culture, a finding consistent with a direct neutralizing mechanism. This suggests a mechanism of protection against viral reactivation in an immunocompetent person by virus-specific antibodies^[378].

Other aspects of antibody formation in BKV infection are of importance. In experimental animals, BKV infection induces the formation of anti-double stranded DNA antibodies^[362]. This finding has led to the suggestion that BKV is implicated in the pathogenesis of SLE, as noted earlier in this report^[171,172]. In a recent report, preemptive reduction of immunosuppression for BK viremia was found to be associated with high incidence of formation of HLA-specific antibodies (dnDSA)^[420]. The authors of this report proposed that in order to prevent the consequences of rejection dnDSA levels should be monitored in renal transplant recipients subjected to reduction of immunosuppression for BK viremia.

The effects of interferon on BKV infection have also attracted attention. Exposure of interferon-sensitive cells infected with BKV to high concentrations of interferon resulted in significant reduction of the BKV load in an early study^[339]. However administration of interferon to a renal transplant recipient with BK viremia and viruria had no appreciable effect in the same study. *In vitro*, interferon- γ inhibits the expression of the BKV T-ag and VP1^[353]. Polymorphisms in the interferon- γ gene appear to affect the development of BKV infection in Hispanics^[408].

A review of subversion mechanisms of several viruses causing kidney disease^[354] stressed the role of immunosuppressed state in the pathogenesis of the viral kidney diseases, included BKN. The state of immunosuppression is the major mechanism of BKV reactivation and has been stressed in numerous reports^[10,351,372,393,422]. Immunosuppressive medications that may increase the risk of BKV infection include tacrolimus^[372,393,403], and mycophenolate^[393]. ABO incompatible kidney transplantation is a risk factor for BKV infection^[413,415]. Although immunoglobulin preparations inhibited BKV replication *in vitro*^[378] and administration of intravenous immunoglobulin was found to be effective and safe in treating BK viremia in one study^[385], desensitization of ABO and HLA incompatible kidney transplant

recipients with IVIG and rituximab was associated with higher incidence of BKV infection^[126,391].

Other factors associated with increased risks for BKV infection and BKN include recipient age exceeding 50 years^[10], male gender, comorbidities (diabetes mellitus)^[10], negative recipient BKV serology prior to transplantation^[10], prior rejection episodes^[10,424], renal dysfunction^[10], large BKV loads^[10], deceased donor^[403], positive CMV serology in donor and recipient^[424], more than one transplant^[424] and hypoxia^[428]. In allogeneic stem cell transplant recipients, severe graft vs host reaction and oral mucositis are risk factors for BKV reactivation^[434]. Mathematical modeling of the immune responses to BKV infection^[432] could provide in the future new developments in the prevention and management of this disease.

Key points of part B

Renal biopsy is required for confirmation of the diagnosis and staging of BKN; BK viral loads in blood and urine and the presence of decoy cells in the use assist in the diagnosis of BKV replication; elucidation of the mechanism of BKV entry into cells and nuclei, factors affecting BKV replication and of the roles of cellular and humoral immunity in KBV infection have the potential of leading to novel prevention and treatment strategies.

PART C TREATMENT OF BKV INFECTION AND DISEASES CAUSED BY OTHER PAPOVAVIRUSES. TREATMENT AND PREVENTION OF BKV INFECTION^[10,19,20,79,92,385,437-493]

Current practices in the prevention and management of BKV infection are based on information obtained primarily from renal transplant recipients. In this patient group, reduction in immune responses to infection as a result of immunosuppression has been recognized as the universal risk factor for symptomatic BKV infection^[10]. A large retrospective study of treatment of BKN in United States renal transplant recipients concluded that the incidence of BKN has been on the rise and is associated with increased risk of graft loss^[19]. The same study reported that certain antirejection medications, including rabbit antithymocytic globulin and tacrolimus/mycophenolate combination, are risk factors for BKN.

Reducing the total immunosuppressive dose and converting to medications less prone to be associated with BKV infection has been reported to have beneficial effects on BK viremia and viruria in various renal transplant cohorts^[447,451,455,471,480,481,484]. In a study from China, monitoring renal transplant recipients for BK viremia and preemptive reduction of immunosuppression was associated with resolution of the viremia and good graft function over five years of follow-up^[481]. Reduction of

immunosuppression, with careful monitoring for signs of rejection of the transplanted organ, and discontinuation of immunosuppressives that are associated with higher risk of BKV infection, *e.g.*, mycophenolate, is currently the mainstay of management of BKV infection in transplant recipients.

Prevention and management of BKV infection in vulnerable populations is hampered by the absence of medications specific for papovaviruses. Certain drug classes have demonstrated antiviral properties *in vitro* and have been tried for preventing or treating BKV infection. The antiviral activity of cidofovir, an acyclic nucleoside phosphonate nucleotide analog, is linked to inhibition of viral DNA polymerases. The drug, which is approved for the treatment of CMV retinitis, was found to inhibit *in vitro* BKV replication in human cell series^[439,483], although one study found modest antiviral activity and low selectivity of this compound^[445]. Beneficial effects of cidofovir in transplant recipients with BKV infections, including BKN and hemorrhagic cystitis, have been reported in case reports and case series^[438,443,446,473].

Cidofovir is administered parenterally. A review concluded that intravesicular administration of cidofovir is effective in cases of severe hemorrhagic BK cystitis^[461]. The use of cidofovir in the management or prevention of BKN is limited by nephrotoxicity, which is the main adverse effect of the drug. Mitochondrial changes in renal tubular epithelial cells^[458] and renal dysfunction may develop in patients receiving the drug. Hydration prior to the injection and concomitant administration of probenecid reduce the risk of nephrotoxicity. Reduction of the dose of cidofovir without probenecid administration was reported to have beneficial effects on the renal function of a patient with BKN^[443]. However, renal dysfunction has led to the discontinuation of the medication in several reports.

The issues raised by cidofovir have led to the search for compounds related to it, but with less toxicity and higher selectivity. A systematic *in vitro* study found several acyclic nucleoside phosphonates, including cidofovir, with inhibitory activity on BKV replication^[459]. Brincidofovir, a compound derived by conjugating cidofovir with a lipid and designed to lead to intracellular release of cidofovir, has antiviral activities against several DNA viruses and was shown *in vitro* to inhibit BKV replication in human urothelial cells^[489]. This compound was recently reported to reduce the viremia and stabilize the renal function without reduction of immunosuppression, which included mycophenolate, in a recipient of allogeneic hematopoietic stem cell transplant with BKN^[79]. Despite the stabilization of the renal function, this patient, who had graft vs host disease, died from sepsis six months after the initiation of brincidofovir treatment. Treatment of BKV infection by brincidofovir will need further evaluation.

Leflunomide is a pyrimidine synthesis inhibitor used in the treatment of rheumatoid arthritis and has been shown to inhibit BKV replication *in vitro* in human

tubular epithelial cells^[452] and human salivary gland cells^[483]. However, only modest antiviral activity and low selectivity of the drug were found in one *in vitro* study^[439], while no antiviral activity of the compound was found in another *in vitro* study^[459]. In case reports and case series, beneficial effects of leflunomide were reported for BK viremia^[93,478], BKN^[442,444,448] and hemorrhagic cystitis^[465] in organ transplant recipients. In resistant cases, administration of cidofovir concomitantly with leflunomide^[442] or ciprofloxacin followed by leflunomide^[478] had apparent beneficial effects. The side effects of leflunomide include hepatotoxicity and neutropenia. Leflunomide treatment requires monitoring of its active metabolite in the blood to ensure therapeutic levels as well as monitoring of hepatic function tests and hematological parameters. A systematic review did not find any kidney transplant survival benefit by the use of leflunomide or cidofovir^[455]. The need for prospective randomized studies was stressed even in studies reporting beneficial effects of leflunomide^[465].

Fluoroquinolones inhibit *in vitro* the DNA topoisomerase of BKV. Levofloxacin and ofloxacin were reported to inhibit BKV replication in human renal tubular epithelial cells *in vitro*^[457]. This effect of this class of antibiotics was criticized because of its low selectivity index^[441]. Ciprofloxacin failed to inhibit BKV replication in another *in vitro* study^[483]. Two retrospective studies in renal transplant recipients reported beneficial effects of fluoroquinolones on BKV infection. Reduction of BK viremia followed ciprofloxacin or levofloxacin administration in one study^[440] and sequential treatment with ciprofloxacin and leflunomide in another study^[478]. However, one retrospective study failed to show any benefit of ciprofloxacin or levofloxacin in the prevention of BKV infection in recipients of allogeneic hematopoietic stem cell transplants^[469] and two randomized studies failed to show any effectiveness of levofloxacin in the prevention of BKV infection^[472] or the treatment of BK viremia^[474] in kidney transplant recipients.

HMG-CoA reductase inhibitors are another class of drugs that has been tried unsuccessfully for the treatment of BKV infection. After the original *in vitro* observation that pravastatin blocks BKV cellular entry^[449], a retrospective multicenter study failed to show any effect of statin doses that maximize their cholesterol-lowering effect on BK viruria or the development of BKN in renal transplant recipients^[479]. Intravenous (*i.v.*) immunoglobulin administration without reduction of the immunosuppression had beneficial effect in a pediatric case of BKN^[450] and, in association with reduction of the immunosuppression, was associated with clearing of the BK viremia and good graft survival in a retrospective study of renal transplant recipients^[385]. Issues associated with IVIG treatment were discussed in the section on pathogenesis. Following immunoglobulin infusion one kidney transplant recipient developed increase in BK viremia and BKN^[464] and a second kidney transplant recipient with BKN developed severe antibody-mediated rejection^[468]. A retrospective study found no difference in

1-year renal transplant outcomes between patients with BKN treated with reduction of the immunosuppression alone or with active treatment including administration of IVIG, leflunomide and ciprofloxacin^[471]. Plasma exchange, along with intravenous immunoglobulin and cidofovir, has also been used for the treatment of BKV infection in renal transplant recipients^[455]. A recent review concluded that reduction of the immunosuppression is the only proven effective treatment of BKN in renal transplant recipients, while cidofovir, leflunomide, fluoroquinolones and *i.v.* immunoglobulin have not been shown to offer any benefits^[480].

The search for immunosuppressive agents lowering the risk of BKV infection has been the topic of several studies. Induction by alemtuzimab was associated with a higher risk of severe rejection and BKN than induction by antithymocytic globulin^[487], even though antithymocytic globulin has been recognized as a risk factor for BKN^[19]. Beneficial effects on BKV infection were reported with the use of the mTOR inhibitors everolimus^[486,493], or sirolimus^[488] instead of mycophenolate and tacrolimus in transplant recipients.

One report reviewed the conservative and surgical approaches to BK hemorrhagic cystitis in bone marrow transplant recipients^[437]. Hyperhydration is sufficient for mild cases. Severe cases may require blood transfusions, suprapubic catheters, permanent bladder irrigation, or various surgical procedures^[437]. Limited experience exists with certain other treatments. Successful combined kidney-liver transplant was reported in a patient with high grade BK viremia, fulminant hepatic failure and loss of his first kidney transplant to BKN^[482]. The first kidney transplant was not removed in this case. Administration of the protease inhibitor bortezomib, which is used as a chemotherapeutic agent in multiple myeloma and mantle cell lymphoma, to a patient with severe BKN and plasma cell-rich infiltrates in the renal interstitium was associated with substantial improvement of the renal function and renal histology^[491]. Treatment by hyperbaric oxygen was associated with resolution of the hematuria in 94% of a series of patients with BK hemorrhagic cystitis^[462].

In a survey of European transplant centers, 66% of the responders stressed the need for new antiviral agents for BKV infection^[485]. Agents that have been tested with some promise in experimental animals or *in vitro* include cyclosporine A^[456], gamma interferon^[460], two inhibitors of the ATPase of the large T BKV antigen, bithionol and hexachlorophene^[463], the small molecule Retro-2(cycl) which inhibits host retrograde viral trafficking^[470], an expression plasmid for the Large BKV T antigen shRNA delivered by virus-like particles^[475], gallic acid-based compounds^[476] and the anti-malarial artesunate^[477]. In a retrospective study in renal transplant recipients with BK viremia, switching the immunosuppressive regime to a combination of low-dose cyclosporine plus an mTOR inhibitor was well tolerated and was associated with better short-term graft function

than reduction of the immunosuppression alone^[466].

The management of BKV infection in transplant recipients is currently based on reduction of the immunosuppression and, in some cases, substitution of mTOR inhibitors for mycophenolate and calcineurin inhibitors. The induction scheme that is best for prevention of BKV infection is not known. Systematic surveillance for BK viremia and viruria^[335,400,451,492] will assist in the early detection and could benefit the outcome of BKV infections.

HUMAN DISEASE ASSOCIATED WITH OTHER PAPOVAVIRUSES^[2-4,12,27,28,97,107,177,250,262,266,342,344,494-502]

BKV belongs to the *Polyomaviridae* family of viruses. Similar structure and animal species as natural hosts are the common features of the members of this family. Other human viruses in the same family that have been associated with human disease include the JCV, the Merkel virus and, probably, the SV40. The natural hosts of SV40 are monkeys and its role in human disease is disputed. The role of *Polyomaviridae* in human disease has been reviewed in several reports^[2-4].

The structure of JCV has the closest association with BKV among all the known human *Polyomaviridae*. A 75% sequence homology between BKV and JCV has been found^[500]. JCV infection has been studied extensively. Substantial rates of JC viremia, viruria and persistence in tissues of transplant recipients and other populations, including non-immunosuppressed subjects, have been reported^[12,27,28,107,343,345,494,495,499,501]. Renal manifestations associated with JCV infection include a case of nephropathy in a patient with malignancy^[177] and decreased renal function in kidney and liver transplant recipients with JC viruria^[497]. The pathogenetic role of JCV in HIV-positive patients with progressive multifocal leukoencephalopathy has been established^[261,265]. JCV is oncogenic in animal species, including primates. In humans JCV infection has been associated with brain tumors and carcinomas of the gastrointestinal tract, breast and cervix, but this association has not been found universally^[496].

Merkel virus is oncogenic in humans. It is linked to Merkel carcinoma, a rare aggressive skin tumor affecting primarily older individuals^[498,499]. Nephropathy associated with SV40 infection was reported in a lung transplant recipient^[97].

The number of *Polyomaviridae* diseases attributed to this viral family is expanding. A recent revision of the taxonomy of the family recognized 76 viral species, 13 of which have humans as their natural hosts^[502]. In this taxonomy, BKV is listed as human polyomavirus 1, abbreviated as BKVvV, JCV is listed as human polyomavirus 2, abbreviated as JCPyV, and Merkel virus is listed as human polyomavirus 8, abbreviated as MCPyV. No doubt this virus family will have a center

stage in organ transplantation and probably in other immunocompromised states in the years to come.

Key points of part C

Reduction of immunosuppression is the first step in the treatment of symptomatic BKV infection; certain classes of anti-rejection medications are less prone to facilitate BKV replication; the clinical usefulness of drugs putatively inhibiting BKV replication is disputed. The toxicities of these drugs are important; the lists of papovaviruses and of human diseases attributed to them are expanding. Papovavirus-related diseases will be a major study topic in the future.

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Recent insights in the pathogenesis of post-transplantation lymphoproliferative disorders

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Abstract

Post-transplant lymphoproliferative disorder (PTLD) is an aggressive complication of solid organ and

hematopoietic stem cell transplantation that arises in up to 20% of transplant recipients. Infection or reactivation of the Epstein-Barr virus (EBV), a ubiquitous human herpesvirus, in combination with chronic immunosuppression are considered as the main predisposing factors, however insight in PTLD biology is fragmentary. The study of PTLD is complicated by its morphological heterogeneity and the lack of prospective trials, which also impede treatment optimization. Furthermore, the broad spectrum of underlying disorders and the graft type represent important confounding factors. PTLD encompasses different malignant subtypes that resemble histologically similar lymphomas in the general population. Post-transplant diffuse large B-cell lymphoma (PT-DLBCL), Burkitt lymphoma (PT-BL) and plasmablastic lymphoma (PT-PBL) occur most frequently. However, in many studies various EBV⁺ and EBV⁻ PTLD subtypes are pooled, complicating the interpretation of the results. In this review, studies of the gene expression pattern, the microenvironment and the genetic profile of PT-DLBCL, PT-BL and PT-PBL are summarized to better understand the mechanisms underlying post-transplantation lymphomagenesis. Based on the available findings we propose stratification of PTLD according to the histological subtype and the EBV status to facilitate the interpretation of future studies and the establishment of clinical trials.

Key words: Epstein-Barr virus; Post-transplant lymphoproliferative disorder; Immunodeficiency; Diffuse large B-cell lymphoma; Burkitt lymphoma; Plasmablastic lymphoma

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Core tip: At the moment different post-transplant lymphoproliferative disorders (PTLD) are grouped in broad categories (early, polymorphic, monomorphic and Hodgkin-like PTLD) and the Epstein-Barr virus (EBV) status is not taken into account. However, increasing

evidence demonstrates that different malignant PTLD and EBV⁺ and EBV⁻ lesions are clinically and biologically distinct, stressing the need for subtype-specific management. We propose that in future studies patients should be stratified according to the histological lymphoma subtype and the EBV status to minimize bias and to simplify the establishment and analysis of clinical trials.

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INTRODUCTION

Despite the increasing incidence of cancer worldwide, only a limited number of cancer-causing factors have been identified. Viruses are amongst them: An estimated 15% of cancers are attributed to viral infections. One of the most widely spread oncogenic viruses is the Epstein-Barr virus (EBV), a gamma human herpesvirus with a seroprevalence of 90%-95% in adults. EBV, discovered in 1964^[1], is best known as the cause of infectious mononucleosis (or kissing disease)^[2]. EBV-driven lymphoproliferative disorders (LPD) are characterized by an EBV-driven immortalization of B-cells. In an otherwise healthy individual, development of such LPD is countered by a strong immune response [mainly of cytotoxic T-cells (CTL)], which ultimately resolves the infection. However, when the immune system is compromised [e.g., in acquired immunodeficiency syndrome (AIDS) patients or in organ transplant recipients under chronic immunosuppression] EBV-driven LPD may eventually progress to overt lymphoma.

During the last decades, the number of solid organ (e.g., kidney, heart, liver, etc.) and stem cell transplantations has increased significantly. In parallel, the risk of graft rejection has dropped thanks to the development of more potent immunosuppressive agents resulting in longer survival of transplant recipients. However, a major drawback of the chronically immunosuppressed status of these individuals is the development of a potentially fatal post-transplant lymphoproliferative disorder (PTLD) in up to 20% of transplant recipients^[3]. PTLD is a relatively new disease entity that is now widely recognized. The first cases were described in renal transplant patients, shortly after the introduction of chronic immunosuppressive drugs in the 1960s^[4]. Despite the strong association between EBV and PTLD (about 70% of PTLD are EBV-positive, EBV⁺), disease biology is not well understood^[3]. The pathological presentation of PTLD is variable, ranging from a localized benign LPD to lymphoma associated with poor survival^[5]. Treatment of PTLD patients is largely based on insights in lymphomagenesis in immunocompetent

patients, in which there is no evident role for EBV in the majority of cases. For application of more adequate therapy it is indispensable to characterize PTLD more thoroughly.

The most common malignant PTLD subtype is post-transplant diffuse large B-cell lymphoma (PT-DLBCL), followed by Burkitt lymphoma (PT-BL) and plasmablastic lymphoma (PT-PBL). PT-BL and PT-PBL are aggressive, but poorly studied malignant PTLD subtypes. The number of reported cases is limited and most studies mainly focus on patient management^[6-9].

In this review we summarize the available data on the genetic profile, the gene expression pattern and the microenvironment of these malignancies to better understand the mechanisms underlying post-transplantation lymphomagenesis. A literature search was performed for "PTLD" or "post-transplant lymphoproliferative disorder" with or without "diffuse large B-cell lymphoma", "Burkitt lymphoma" or "plasmablastic lymphoma" and the available literature regarding PTLD pathogenesis was collected. For a review of the diagnosis and management of PTLD we refer the reader to^[3,10].

DISCUSSION

EBV exploits the germinal center route of B-cell activation

During a normal humoral immune response, a circulating B-cell that encounters its cognate antigen becomes an activated blast with two possible fates.

The B-cell can mature into a short-lived plasma cell that quickly produces IgM class antibodies with limited specificity (T-cell independent pathway). Alternatively, the B-cell may form a germinal center (GC) in a lymph node, mucosa-associated lymphoid tissue or spleen (T-cell dependent pathway). In the GC, the specificity of the B-cell's antibody is enhanced by somatic hypermutation (SHM, random mutation of the antibody's variable chain, IgV) and its functional versatility is altered by class switch recombination from IgM to IgG, IgE or IgA. Eventually, the B-cell matures into a plasma cell or a memory B-cell^[11]. B-cells transiting the GC are germinal center B-cells (GCB). B-cells that have completed the GC reaction are called activated B-cells, non-GCB or post-germinal B-cells (Figure 1).

According to the classic model, EBV infects naive B-cells and promotes formation of a GC. During GC transition, EBV proteins provide a selective advantage and stimulate differentiation to memory B-cells, the presumed reservoir of EBV. This process is enabled by coordinate expression of EBV proteins, primarily latent membrane proteins (LMP1, 2A-B) and EBV nuclear antigens (EBNA1, 2, 3A-C). Based on the pattern of expression, three different latency expression profiles are recognized^[12]. These latency programs are associated with different stages of EBV B-cell infection and with particular lymphoproliferative disorders (Table 1 and

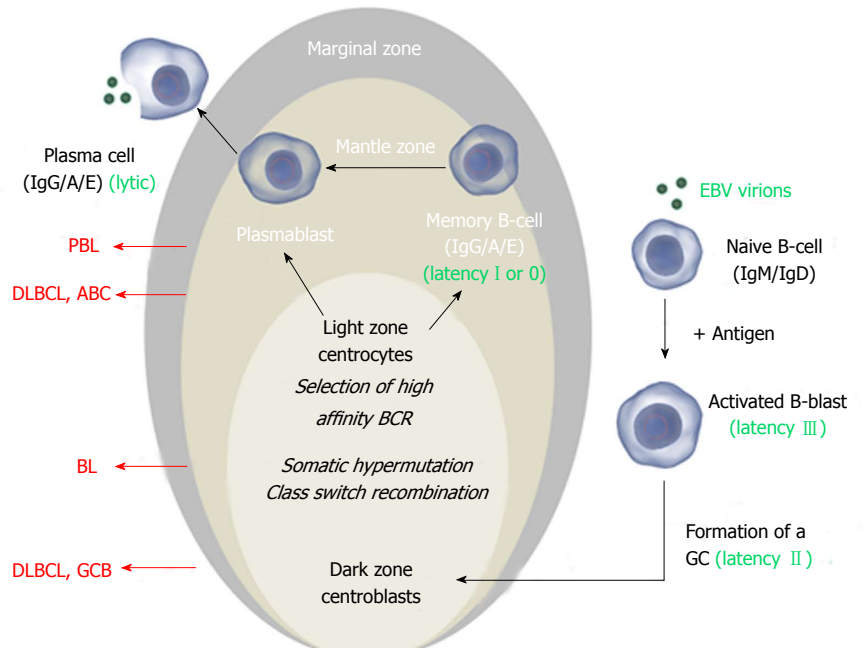


Figure 1 The Epstein-Barr virus exploits normal B-cell activation pathways. Activation of a naive B-cell (that expresses IgM and IgD on its surface) by its cognate antigen results in B-cell activation and differentiation into a memory B-cell or a plasma cell, most commonly via T-cell dependent activation. The antigen-activated B-cell enters a primary follicle in lymph node or spleen and forms a germinal center (GC), transforming the primary follicle into a secondary follicle. This structure is composed of three distinct regions. The marginal zone^[1], which consists mainly of activated B-cells and GC-matured IgM+ B-cells, the mantle zone or corona^[2], which comprises naïve and memory B-cells surrounds the GC^[3]. The GC consists of a dark zone and a light zone. In the dark zone, the activated B-cells (centroblasts) proliferate and downregulate expression of IgM and IgD to allow somatic hypermutation (SHM) and class switch recombination (CSR), increasing the antibody's affinity, specificity and functional versatility. In the light zone of the GC, the B-cells (centrocytes) with the best antibody are selected and ultimately mature into memory B-cells or plasma cells. Instead of IgM and IgD, these express high affinity IgG, IgA or IgE antibodies. Classically, Epstein-Barr virus (EBV) infects naïve B-cells that are stimulated to form a GC. In the activated blast, viral latency III (LMP1+/EBNA2+) is expressed and induces proliferation. In the GC, latency II (LMP1+/EBNA2-) is expressed and infected centroblasts presumably undergo SHM and CSR, involved in antibody maturation. After leaving the GC, they differentiate into plasma cells or (mainly) memory cells (latency I, EBNA1+ or latency 0, no expression of viral proteins). *In vitro* and *in vivo*, plasma cell differentiation results in activation of the EBV lytic cycle. In all stages, the viral DNA (circle in the nucleus) is maintained as an episome. Different stages of this process can give rise to malignancy resulting in different lymphoma subtypes that have features of their normal counterpart. Here the stages at which EBV+ and EBV- B-cell lymphoma may arise are shown for the most common subtypes. Images from www.somersault1824.com were used in this figure. PBL: Plasmablastic lymphoma; DLBCL: Diffuse large B-cell lymphoma; ABC: Activated B-cell; GCB: Germinal center B-cell.

Table 1 Epstein-Barr virus-driven lymphoproliferative disorders are linked with particular Epstein-Barr virus latency programs

Latency	Expressed <i>EBV</i> gene products	Normal B-cell stage	Associated disease
III (growth)	EBER1-2, EBNA1-6, LMP1, LMP2A-B	Activated B lymphoblast	PT-DLBCL AIDS-related lymphoma Acute infectious mononucleosis
II (default)	EBER 1-2, EBNA1, LMP1- 2A	B-cell undergoing the GC reaction	PT-DLBCL Classical Hodgkin lymphoma
I	EBER 1-2, EBNA1	Memory B-cell	(PT-) Burkitt lymphoma (PT-) PBL

EBER: Epstein-Barr virus-encoded RNA; EBNA: Epstein-Barr virus nuclear antigen; LMP: Latent membrane protein; PT-DLBCL: Post-transplant diffuse large B-cell lymphoma; PBL: Plasmablastic lymphoma; EBV: Epstein-Barr virus.

Figure 1). EBV⁺ PT-DLBCL is classically associated with the most elaborate viral expression pattern, latency III. EBV⁺ PT-BL and PT-PBL on the other hand most

frequently express the more restricted latency patterns I or II^[13,14].

LMP1, a constitutively active mimic of CD40 (a crucial costimulatory factor in T-cell mediated B-cell activation), is regarded as the major oncogenic protein of EBV. LMP2A is a functional mimic of a B-cell receptor and provides survival signals to the B-cells. EBNA1 ensures replication of the viral genome during cell division. EBNA2 acts as a master transcriptional regulator of both viral and cellular genes^[12]. Two viral miRNA clusters (BART-miRNAs and BHRF1 miRNAs) are differentially expressed depending on the particular viral latency program^[15]. EBV-encoded RNA (EBER) 1 and 2 are the only gene products that are expressed throughout all latency and lytic phases of the viral cycle and represent the most reliable markers to determine EBV infection^[16].

Key features of EBV latent proteins are shown in Figure 2A. For more details about the viral gene products we refer the reader to other reviews^[17,18].

In vitro and *in vivo*, plasma cell differentiation of an EBV-infected B-cell is associated with activation of EBV lytic replication resulting in production of new viral

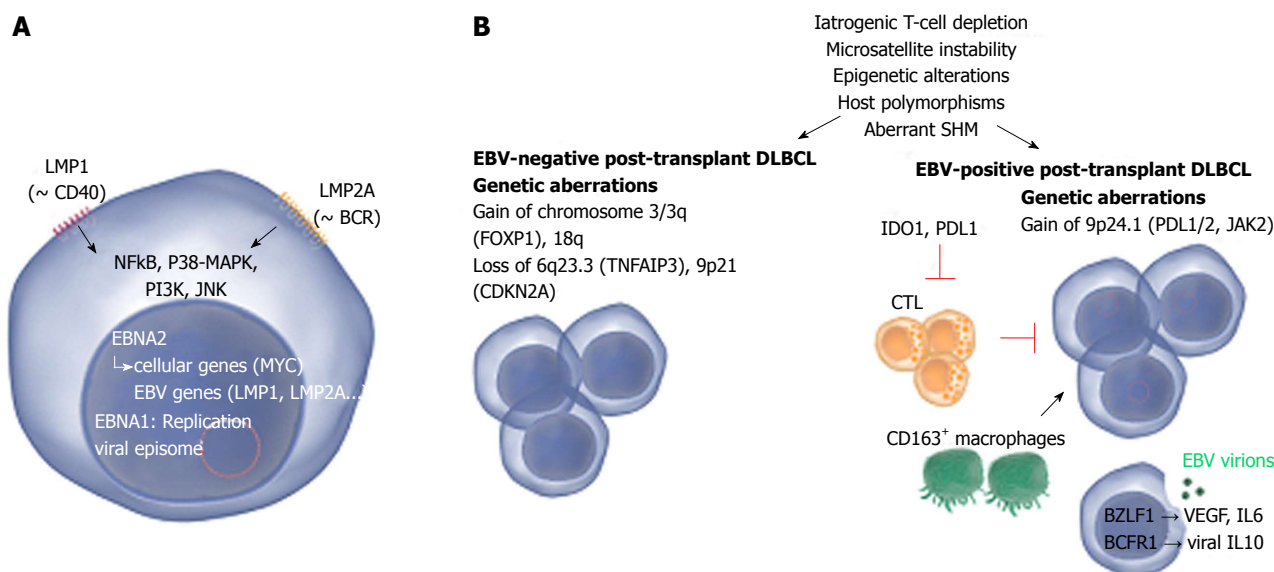


Figure 2 Common and distinct pathogenetic mechanisms in Epstein-Barr virus-positive and -negative post-transplant diffuse large B-cell lymphoma.

A: Two Epstein-Barr virus (EBV) proteins that are thought to play a role in EBV-driven lymphomagenesis are LMP1 and LMP2A. LMP1 is analogous to CD40 and promotes cell transformation by inducing NF- κ B, that in turn upregulates BCL-2, A20 and C-FLIP, all involved in blocking apoptosis. LMP2A mimics a chronically active B-cell receptor (BCR) and prevents BCR-mediated activation of EBV lytic replication. LMP2A also provides the necessary survival signals which can compensate for the loss of a functional BCR. Other pathways that are induced comprise janus kinase, p38-MAPK and PI3K signaling. Nuclear EBNA1 and EBNA2 are involved in replication of the viral episome and induction of viral as well as cellular genes respectively; **B:** The pathogenesis of EBV-positive and -negative lymphoma is marked by a number of common as well as distinct pathogenetic mechanisms. Mechanisms that contribute to both EBV-positive and -negative lymphoma involve iatrogenic T-cell suppression, microsatellite instability (resulting in accumulation of mutations), epigenetic alterations (mainly hypermethylation), host polymorphisms (in particular in genes encoding proteins involved in immunity), aberrant somatic hypermutation (SHM, resulting in accumulation of point mutations) and aberrant up- or down-regulation of host miRNAs which may substantially impact gene expression. EBV-negative PT-DLBCL is characterized by genetic aberrations found in EBV-negative DLBCL arising in the general population, e.g., alterations involving FOXP1. EBV-positive PT-DLBCL on the other hand harbors fewer genetic lesions. Gain of 9p24.1 (harboring PDL1/2, JAK2) has been detected and may contribute to tumor immune evasion. A minority of the EBV-positive cells actively produce viral particles. This lytic replication may promote lymphoma growth by expression of IL-6 and VEGF. Also viral IL-10 (vIL10) is expressed which contributes to suppression of anti-tumor responses by antagonizing IFN- γ . The expression of EBV proteins attracts cytotoxic T-cells (CTLs) to the site of the tumor however the question remains whether effective anti-tumor responses can be produced as also tolerant immune responses are induced. IDO1 (expressed in tumor cells and dendritic cells) and PDL1 (expressed in tumor cells and macrophages) suppress T-cells and may substantially impair the activity of CTLs. Also CD163⁺ macrophages (thought to be immunotolerant M2 macrophages) may play a role in immune evasion. Images from www.somersault1824.com were used in this figure. PT-DLBCL: Post-transplant diffuse large B-cell lymphoma; IL: Interleukin; IFN: Interferon; VEGF: Vascular endothelial growth factor; BCR: B-cell Receptor.

particles^[19]. The main activators of this process are viral ZEBRA/BZLF1 and BRLF1 proteins^[20].

Although still highly debated, increasing evidence indicates that also the lytic program of EBV is of importance for B-cell transformation, the early stages in particular^[21-23]. EBV lacking ZEBRA/BZLF1 and BRLF1 has significantly decreased transforming potential *in vivo*, associated with reduced expression of proliferation-promoting factors (IL-6, IL-10 and viral IL-10) (Figure 2B)^[24]. Intriguingly, particular genetic variants of ZEBRA/BZLF1 and BRLF1 have been associated with lymphoma^[25]. So far, few studies have examined lytic replication in human lymphoma biopsies^[26]. In a recent report, EBV lytic replication in PTLD was associated with tumoral XBP-1 expression, early onset and short survival^[27].

In the following sections, the pathogenesis of PT-DLBCL (Figure 2B), PT-BL and PT-PBL, the most common malignant PTLD subtypes, is discussed.

DLBCL

Cell of origin: DLBCL in the general population comprises at least two molecular subtypes: GCB derived

and non-GCB derived DLBCL^[28], thought to arise from normal GC and non-GC B-cells respectively (Figure 1). Both subtypes have been reported in the transplant setting^[29]. The cell of origin is classically determined using a microarray-based surrogate set of three immunostainings (CD10, BCL6, MUM1)^[30] and has prognostic implications: In the general population, GCB DLBCL has a better prognosis than non-GCB DLBCL^[28]. Whether the same is true for post-transplant DLBCL is difficult to determine since the vast majority of EBV-associated cases are of non-GCB origin^[26,31,32] (Figure 1). The induction of pathways like NF- κ B signaling by EBV, which is highly characteristic for non-GCB DLBCL could explain this observation^[33] (Figure 2A).

Another way to define the cell of origin is provided by genotypic analysis of SHM. A naïve pre-GC B-cell carries unmutated IgV, intracлонаl heterogeneity reflects ongoing IgV SHM in GC centroblasts and a centrocyte/post-GC B-cell carries stable IgV mutations. Using this method the vast majority of EBV⁺ as well as EBV PT-DLBCL were shown to carry IgV mutations indicating that PT-DLBCL derive mainly from GC and post-GC B-cells^[26,29]. The few PT-DLBCL that do lack SHM are

consistently EBV⁺ and arise early after transplantation. They may derive from naïve pre-GC B-cells or from B-cells that have transited the GC without completing the GC program^[34-36].

Genetics: Genetic studies have demonstrated that PT-DLBCL has genomic aberrations in common with DLBCL arising in immunocompetent individuals (gains of 8q24 harboring *MYC*, 3q27 harboring *BCL6*, 18q21 harboring *BCL2*, 7q harboring *CDK6*; loss of 17p13 harboring *TP53*) but also bears distinct alterations (gain of 5p, loss of 4q, 17q, Xp)^[37,38]. EBV⁺ and EBV⁻ PTLD are rarely distinguished, but in one study EBV⁺ PT-DLBCL was associated with gains of 7p, 7q and 11q24-q25 and del(4q25-q35)^[39]. EBV⁺ PT-DLBCL on the other hand frequently harbored trisomies of chromosomes 9 and 11. It has been suggested that overall, EBV⁺ PT-DLBCL carries fewer (recurrent) genetic lesions than EBV⁻ cases^[37].

An aCGH study on a series of 21 non-GCB PT-DLBCL validated these findings^[40]. Overall, EBV⁺ PT-DLBCL harbored fewer copy number alterations than EBV⁻ cases. EBV⁺ and EBV⁻ PT-DLBCL shared only one recurrent aberration (gain 12q21q21); the significance of this lesion is unclear. The most frequent genetic aberration detected in the EBV⁺ cases was gain of 9p24.1 that harbors *PDL1*, *PDL2* and *JAK2* and could contribute to *PDL1* overexpression (Figure 2B). Notably, also in EBV⁺ DLBCL in elderly individuals (DLBCL-E) gain of 9p24.1 was among the most frequently detected lesions^[41] suggesting that overlapping processes underlie the pathogenesis of EBV-driven lymphomas.

In contrast, EBV⁻ PT- and IC-DLBCL shared many common aberrations (gain of chromosome 3/3q and 18q, and loss of 6q23.3/*TNFAIP3* and 9p21/*CDKN2A*) characteristic for non-GCB DLBCL^[42] suggesting EBV-PT-DLBCL and IC-DLBCL are biologically similar (Figure 2B).

SHM may also contribute to oncogenesis when it misfires and results in mutation of proto-oncogenes, like *PIM1*, *PAX5*, *RhoH/TTF* and *MYC*. Because primarily the 5' regulatory region is targeted, aberrant SHM may alter the expression profile of the affected gene(s)^[29]. In one study, aberrant SHM of *PIM1*, *PAX5*, *RhoH/TTF* and/or *MYC* was detected in 40% of PT-DLBCL, independently of the EBV status^[29,43].

Microsatellite instability (MSI) is induced by loss of a gene involved in DNA mismatch repair accelerating the accumulation of mutations (mainly in microsatellite sequences). Interestingly, MSI seems restricted to immunodeficiency-related lymphomas and has been reported in a fraction of PTLD, unrelated to EBV status (in a series of 72 PT-DLBCL, 7% was microsatellite instable^[44]). In colon carcinoma, MSI has been associated with an increased number of tumor-infiltrating lymphocytes (presumably because of the formation of neo-antigens which are then presented in MHC I on the surface of the tumor cell) suggesting that MSI

lymphomas are more immunogenic than microsatellite stable tumors^[45,46]. It is feasible that such immunogenic lymphomas are only tolerated in an immunocompromised host, accounting for the lack of MSI lymphomas in immunocompetent individuals.

Gene expression profile: Two early gene expression profiling studies of PTLD produced partly contradictory results, probably because of the small sample size and the different composition of the case series. Segregation of eight PT-DLBCL cases based on the EBV status in a study by Craig *et al.*^[32] could not be confirmed by a report of Vakiani *et al.*^[26], who suggested that PTLD was distinct from non-Hodgkin lymphoma in immunocompetent individuals. As a result, a number of key questions remained unresolved until recently. Are EBV⁺ and EBV⁻ PTLD different or not? And how do these disease states relate to lymphoma in the general population?

Consistent with the study of Craig *et al.*^[32] a GEP study of 21 PT-DLBCL by our group pointed to a dominant role for cytotoxic antiviral immune signaling in EBV⁺ vs EBV⁻ cases, implying that the presence of EBV in the tumor cells greatly affects the microenvironment^[47].

Cytokines upregulated in EBV⁺ PT-DLBCL and associated with viral infection included *CCL3*, *CCL4* and *CCL8* involved in chemotaxis and/or activation of monocytes (*CCL3*, *CCL4*) and T-cells (*CCL3*, *CCL8*). Notably, *CCL3* and *CLL4* could also be part of an autocrine loop: *In vitro*, these cytokines were highly expressed by EBV⁺ lymphoblastoid cell lines (LCL) and promoted LCL proliferation and survival^[48].

In contrast to Craig *et al.*^[32] we also detected enhanced immunotolerant signaling (*PDL1*, *IDO1*) in EBV⁺ vs EBV⁻ PT-DLBCL (Figure 2B). These networks are likely induced to counter pro-inflammatory signaling. Upregulation of *PDL1* is in line with *in vitro* studies that demonstrated a functional link between EBV and *PDL1* expression in tumor cells^[49], confirmed by histological studies of human EBV⁺ tumor biopsies^[50]. *IDO1* is involved in suppression of T-cells by degradation of tryptophan and was previously found overexpressed in EBV⁺ gastric carcinoma^[51].

Notably, blockade of immune checkpoints (*IDO1* or the *PDL-PD1* axis) results in boosting of the immune response and has already shown promising results in clinical cancer trials^[52]. This approach may be useful also in PTLD where it may increase the efficacy of adoptive T-cell therapy. However, because of the associated increased risk of graft rejection, the safety of checkpoint inhibitors in PTLD treatment requires further investigation.

EBV⁺ PT-DLBCL represents the minority of PT-DLBCL cases, however there is some evidence that its incidence is increasing^[53], potentially (partly) because of the overall longer survival of transplant recipients. The etiology of EBV⁺ PTLD is unknown and therefore a major

question is how these tumors relate to EBV lymphomas in the general population.

A number of hypotheses have been raised to explain the etiology of EBV PTLD.

The hit-and-run theory, based on *in vitro* data^[54], states that after transformation EBV-infected B-cells may eventually lose (part of) the viral genome. However so far, there is no *in vivo* evidence supporting this theory^[55,56].

Given the strong association between EBV and PTLD other infectious agents, *e.g.*, HHV8 or cytomegalovirus (CMV) may be implicated in EBV PTLD. However, PTLD cases in which HHV8 is detected are extremely rare^[57,58] and because CMV does not infect B-cells it can only play an indirect role^[59]. A study of AIDS-related lymphoma found only EBV to be significantly associated with pathogenesis, suggesting that also EBV PTLD is probably not caused by an infectious agent^[60].

Craig *et al.*^[32] suggested that EBV⁺ and EBV monomorphic PTLD are biologically distinct and the results of our GEP analysis support this hypothesis. In the comparison of GEP data of EBV⁺ and EBV PT-DLBCL, BCR signaling was upregulated in EBV⁺ cases. As suggested by the authors, this finding could be the result of mimicked BCR signaling by LMP2A in EBV⁺ PT-DLBCL^[32], however it could also be an artifact: Because of dominant immune signaling in EBV⁺ cases tumoral BCR signaling is seemingly upregulated in EBV⁺ cases.

To gain more insight in the biology of EBV PT-DLBCL, GEP profiles of EBV PT and IC cases were compared. Only pathways involved in T-cell signaling were significantly differentially expressed and downregulated in PT compared to IC-DLBCL suggesting that the tumoral expression profiles are overall similar. Notably, decreased T-cell signaling explains why some cases of EBV PT-DLBCL respond to RIS^[61,62], which is generally more effective for EBV⁺ lesions. Therefore, restoration of the immune response in EBV PTLD patients should remain one of the cornerstones of treatment.

Notably, gain of chromosome 3/3q (encoding *FOXP1*) in EBV IC/PT-DLBCL had the strongest impact on gene expression (Figure 2B). Bio-informatics analysis of the gene set upregulated in this subgroup predicted that *FOXP1*, a master transcriptional regulator, regulates the expression of the majority of the genes (unreported data), suggesting *FOXP1* is a major network hub in the pathogenesis of these cases. Because several studies support a central role of *FOXP1* in non-GCB DLBCL pathogenesis^[63] the downregulation of *FOXP1* in EBV⁺ non-GCB PT-DLBCL is striking. Also following *in vitro* EBV infection of peripheral blood mononuclear cells *FOXP1* is downregulated^[64], indicating that *FOXP1* expression is incompatible with EBV signaling. An interesting question is whether forced expression of *FOXP1* in EBV⁺ non-GCB DLBCL cells is toxic for the tumor cells.

Microenvironment: The tumor microenvironment

consists of the collection of stromal and immune cells that make up the cellular environment in which the tumor cells reside and has been shown to significantly influence prognosis in different lymphoma subtypes^[65,66], also in PTLD. Particularly the infiltration of CTL has been associated with favorable prognosis (the EBV status was not taken into account). In the same study, the infiltration of regulatory T-cells (Treg), immune response modulators that prevent excessive immune activation, was limited in all PTLD cases^[67]. This may be attributed to obstruction of Treg cell development by immunosuppressive agents. Analysis of the normal intestinal mucosa showed that liver transplant patients on a long-term combination regimen had significantly lower levels of Treg cells compared to healthy controls^[68]. Although the scarcity of Treg cells in PTLD lesions may impede suppression of anti-tumor immune responses, also inhibition of B-cell proliferation by Treg cells is alleviated, potentially contributing to PTLD development^[67]. A thorough review of the microenvironment of PTLD has not been performed but a study of AIDS-related DLBCL may give clues: Increased tumor vascularization and a higher number of infiltrating CTL were detected in EBV⁺ compared to EBV⁻ cases^[69].

Cell counts for different immune markers (manuscript submitted) performed on a series of PT-DLBCL showed increased infiltration of CD8⁺ CTL in part of the EBV⁺ compared to EBV⁻ cases. CTL, probably attracted to the tumor site by the presence of EBV, expressed granzyme B suggesting they were activated (Figure 2B). In contrast, NK cells, critical cytotoxic effector cells in the early response to viral infection and tumor cells, were virtually absent in all biopsies, based on staining for NCAM1/CD56. However, this does not exclude a role for NK cells in PTLD. In a study involving pediatric transplant recipients, CD56^{high} NK cells were abundant only in asymptomatic transplant recipients whereas in PTLD patients, the functionally impaired CD56^{dim/negative} NK population was increased^[70].

Tumor immune evasion is a major challenge for effective cancer treatment^[71] and several reports have shown that such mechanisms also play a role in PTLD. Tumoral expression of PDL1, involved in T-cell suppression^[72], as well as galectin-1, involved in apoptosis-induction of CTL among others^[73], has been reported^[51]. Also immunoregulatory M2 macrophages (marked by CD163 expression) may be part of a negative feedback loop to prevent excessive CTL-induced tissue damage^[74]. M2 macrophages, which were significantly more abundant in EBV⁺ vs EBV⁻ PT-DLBCL (manuscript submitted), are thought to contribute to tissue remodeling and tumor progression in contrast to classical pro-inflammatory M1 macrophages^[75] (Figure 2B). These data are consistent with studies of EBV⁺ DLBCL-E and EBV⁺ Hodgkin lymphoma. Also in these malignancies, the presence of EBV has been associated with upregulation of CD163 expression^[41,74].

It is not clear whether these cells are recruited to the

tumor site or develop *in situ*. Studies have shown that the M2 phenotype can be induced by particular cytokines, among which IL-4 and IL-10^[76]. We speculate that also EBV-encoded IL-10 contributes to M2 macrophage polarization in EBV⁺ PT-DLBCL^[75]. Interestingly, M2 macrophages are themselves producers of IL-10 and may be the source of the high levels of IL-10 detected in PTLD patients^[77].

In a prospective trial of Hodgkin lymphoma, increased tumor-associated macrophage infiltration was associated with inferior outcome^[78]. An interesting question to be resolved is whether also in PTLD macrophages influence prognosis.

BL

Cell of origin: BL is a highly aggressive lymphoma characterized by a high mitotic rate and numerous tingible body macrophages (loaded with debris from apoptotic cells). Three clinical variants of BL are recognized: Endemic BL (with a high prevalence in equatorial Africa), sporadic BL (prevalent in Western countries) and immunodeficiency-associated BL [primarily affecting human immunodeficiency virus (HIV) infected patients, but also reported in transplant recipients]. The association with EBV is different for the three subtypes and strongest in the endemic variant (nearly 100% EBV⁺), followed by the immunodeficiency-associated variant (30%-80% EBV⁺) and sporadic BL (15%-20% EBV⁺). Notably, EBV⁺ Burkitt lymphoma is the EBV transformed tumor with the most limited expression of viral proteins (typically only EBNA1 is expressed)^[79].

BL is classically thought to arise from a GCB cell however analysis of the SHM patterns in a series of endemic, sporadic and AIDS-related BL suggested that BL may arise from different stages of B-cell differentiation, associated with the EBV status. EBV⁺ BL were highly mutated and may derive from a late antigen-selected GC B-cell or memory B-cell for EBV⁺ BL. EBV⁻ BL on the other hand harbored only a limited number of mutations and may arise from an early centroblast^[80].

Genetics: The hallmark of BL is the presence of translocations involving MYC [with IgH: t(8;14)(q24;q32)] which are also found in PTLD with Burkitt morphology^[37]. It is highly debated whether MYC-translocation-negative BL is a form of true molecular BL^[81]. In a recent study, an 11q aberration was detected in MYC-negative high-grade B-cell lymphomas resembling BL (both at the morphological as well as the molecular level, but without MYC rearrangement)^[82]. In our series of IC- and PT-BL this peculiar 11q gain/loss was particularly frequent in PT cases lacking MYC translocation, suggesting a different pathogenesis of BL in different immune settings. However, a recent study demonstrated that 11q gain/loss and MYC translocation are not mutually exclusive^[83]. It is possible that both aberrations

have complementary effects: Integrated analysis of genomic and transcriptomic data of our series of MYC translocation-positive and -negative cases suggested that the 11q-gain/loss is a molecular variant of MYC rearrangement, affecting similar pathways.

Gene expression profile and microenvironment:

In contrast to PT-DLBCL, the gene expression profile of EBV⁺ and EBV⁻ BL is not significantly different, indicating that MYC signaling rather than the EBV status has the major impact on the expression profile^[84]. BL lesions are composed of very little stromal infiltrate indicating that BL tumor cells are poorly immunogenic. Remarkably, even when BL cells express highly immunogenic EBV antigens EBNA3A, -3B, and -3C^[85] or foreign antigens are introduced by a recombinant virus^[86] they are not recognized by antigen-specific CTL clones. An *in vitro* study pointed to a crucial role of MYC. It was demonstrated that this oncogene negatively regulates NF- κ B and interferon signaling by suppression of STAT1 resulting in decreased immunogenicity^[87].

PBL

Cell of origin: PBL is an aggressive terminally differentiated variant of DLBCL that has many morphological and immunophenotypic characteristics in common with a plasmablast (a B-cell in the final stages of plasma cell differentiation). PBL typically arises in the oral cavity of HIV⁺ patients^[88] but has also been reported in immunocompetent individuals^[89] and transplant recipients^[8].

In a series of AIDS-related PBL (10/12 were EBV⁺), evidence of somatic hypermutation was found in only 4/10 analyzed cases suggesting histogenetic heterogeneity of PBL^[90].

Genetics: Currently, very little is known about the molecular-genetic basis that drives PBL. One study showed that up to 47% of EBV⁺ AIDS-related PBLs are marked by MYC translocations^[91]. Array-comparative genomic hybridization involving 16 PBL demonstrated that, despite the high degree of immunophenotypical similarity between PBL and plasma cell myeloma (PCM)^[92], the genomic aberration pattern of PBL is more similar to DLBCL than to PCM^[93].

Gene expression profile and microenvironment:

A gene expression profiling study reported that PBL was more similar to extraosseous plasmacytoma than to DLBCL^[94] reflecting the plasma cell immunophenotypical features of these malignancies. No significant differences were found between EBV⁺ and EBV⁻ PBL, however this may be related to the small sample size.

Reanalysis of our gene expression data (3 EBV⁺ PT-PBL vs 20 EBV⁺ PT-DLBCL, fold change 2, FDR < 0.05^[95]) confirmed enhanced MYC signaling and demonstrated unfolded protein response endoplasmic reticulum stress signaling in PBL (unreported data). These findings

provide an explanation for the success of bortezomib treatment in PBL case reports^[96,97] and suggest that BET bromodomain inhibitors may represent a potential new therapeutic strategy, as has been successfully demonstrated in experimental models of multiple myeloma^[98].

As for EBV⁺ DLBCL, EBV⁺ PBL may be associated with a tolerant microenvironment. In a recent clinicopathological analysis of 82 PBL arising in HIV⁺ and HIV patients particularly EBV⁺ tumors highly expressed PD1-PD1 in both malignant cells and microenvironment^[99].

CONCLUSION

The findings presented in this review underscore the heterogeneous nature of PTLD and could serve as a basis to revise the current PTLD classification. We propose that within the group of monomorphic PTLD, the different histological lymphoma entities (DLBCL, BL, PBL) should be distinguished. We suggest that also the EBV status should be included to further stratify PTLD patients in future studies and clinical trials.

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Physical rehabilitation for lung transplant candidates and recipients: An evidence-informed clinical approach

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Abstract

Physical rehabilitation of lung transplant candidates and recipients plays an important in optimizing physical function prior to transplant and facilitating recovery of function post-transplant. As medical and surgical interventions in lung transplantation have evolved over time, there has been a demographic shift of individuals undergoing lung transplantation including older individuals, those with multiple co-morbidities, and

candidates with respiratory failure requiring bridging to transplantation. These changes have an impact on the rehabilitation needs of lung transplant candidates and recipients. This review provides a practical approach to rehabilitation based on research and clinical practice at our transplant centre. It focuses on functional assessment and exercise prescription during an uncomplicated and complicated clinical course in the pre-transplant, early and late post-transplant periods. The target audience includes clinicians involved in pre- and post-transplant patient care and rehabilitation researchers.

Key words: Lung transplantation; Rehabilitation; Physical therapy; Exercise training; Physical activity

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Core tip: This expert review brings together clinical experience and research evidence on physical rehabilitation for lung transplant candidates and recipients. The evaluation of exercise capacity, muscle function, mobility, activities of daily living and physical activity is discussed. Rehabilitation training guidelines for pre-transplant, acute care, early and late post-transplant phases are provided with special attention to complicated and uncomplicated clinical courses. Special populations such as heart-lung transplant and paediatric lung transplant are also included.

Wickerson L, Rozenberg D, Janaudis-Ferreira T, Deliva R, Lo V, Beauchamp G, Helm D, Gottesman C, Mendes P, Vieira L, Herridge M, Singer LG, Mathur S. Physical rehabilitation for lung transplant candidates and recipients: An evidence-informed clinical approach. *World J Transplant* 2016; 6(3): 517-531 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i3/517.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i3.517>

INTRODUCTION

Lung transplantation is performed for a variety of advanced lung diseases, with primary indications including interstitial lung disease (ILD), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and pulmonary vascular disease^[1]. Since the world's first successful single lung transplant in Toronto, Canada^[2] physical rehabilitation has played an integral role in preparing individuals for lung transplantation and facilitating their recovery^[3,4].

Although pre- and post-transplant rehabilitation is recommended in the majority of lung transplant centers in Canada^[5], there are currently no clinical practice guidelines for rehabilitation in lung transplant candidates and recipients. Several narrative reviews have been published on rehabilitation^[6,7], however they have focused on guidelines for individuals with a relatively uncomplicated pre- and post-transplant course. As the selection of lung transplant candidates

has evolved over time due to surgical and medical advancements, the demographics of transplant candidates has shifted from only the youngest and fittest candidates to adults of older age and those with increased co-morbidities and functional limitations^[1]. This shift in demographics may have important implications for rehabilitation approaches and functional expectations pre- and post-transplant. In addition, lung transplant candidates can present with acute respiratory decompensation, and several medical strategies are being used to "bridge" candidates to transplantation using mechanical ventilation and/or Extra Corporeal Life Support (ECLS)^[8-11]. These technologies can have a significant impact on the degree of deconditioning that these individuals experience prior to transplant, as their capacity to participate in active rehabilitation is limited. The rehabilitation needs of individuals who have high oxygen requirements, require hospitalization pre-transplant due to respiratory failure, and/or require extensive rehabilitation post-transplant due to a prolonged and complicated clinical course are not well described.

The overall purpose of this review is to provide an evidence-informed clinical approach to rehabilitation based on over 30 years of clinical rehabilitation experience at our center, integrating the research evidence for rehabilitation in lung transplantation. The specific aims of this review are to: (1) provide a practical approach to functional assessment and exercise training pre- and post-lung transplant, including the peri-operative and long-term follow-up periods; (2) describe and contrast exercise training and mobility for lung transplant candidates and recipients with an uncomplicated and complicated clinical course; and (3) discuss rehabilitative approaches for special populations within lung transplantation such as re-transplant, heart-lung transplant and pediatrics.

FUNCTIONAL ASSESSMENT OF LUNG TRANSPLANT CANDIDATES AND RECIPIENTS

The mechanisms of exercise limitation pre- and post-lung transplant are multifactorial, including alterations in lung mechanics and gas exchange, cardiovascular limitations and peripheral muscle dysfunction, and have been described in detail elsewhere^[12,13]. In order to evaluate exercise capacity and function in lung transplant candidates and recipients, a combination of aerobic testing, muscle function, mobility testing and assessment of physical activity is utilized. Measures that may be used in clinical practice for physical assessment in the lung transplant population have been summarized in Table 1. The Rehabilitation Measures Database^[14] provides information on the psychometric properties, normative data, instrument description and equipment, minimally clinically important difference and considerations for a number of rehabilitation

Table 1 Physical assessment of lung transplant candidates and recipients

Measured construct	Clinical tests	Clinical utility
Exercise capacity	Lab-based test:	Cause of exercise limitation
	Cardiopulmonary exercise test on cycle or treadmill	Assess need for oxygen
	Field-based walk tests: 6MWT, ISWT ^[19,27]	Assess functional capacity
	Upper extremity endurance capacity: UULEX ^[28]	Outcome measure pre-post rehab and pre-post transplant Exercise prescription
Muscle function (strength, endurance)	Peripheral muscles:	Assess muscle strength and/or muscle endurance
	Manual muscle testing or hand held dynamometry	
	Handgrip force	Outcome measure
	1-repetition maximum	Exercise prescription (1-RM for peripheral muscles, MIP for IMT)
Physical performance and mobility	Respiratory muscles: MIP/MEP	Assess mobility, balance and physical function
	Gait speed (over 4 m) ^[110]	Assess need for gait aid
	Sit-stand tests (e.g., 30 s sit to stand; 5 times sit to stand) ^[111,112]	Outcome measure
	Short Physical Performance Battery ^[113]	Exercise prescription
	Timed Up and Go ^[114]	Discharge planning
	Balance tests (e.g., Berg balance scale, BESTest) ^[115,116]	
	FIM ^[117]	
	Tests specifically for ICU/ inpatients:	
	Egress test ^[118]	
	Various ICU physical function tests ^[119-121]	
Physical activity	Physical Activity questionnaires, e.g., PASE ^[122] ; IPAQ ^[123] ; DASI ^[124]	Assess physical activity Outcome measure
	Pedometers or accelerometers	Set activity goals (e.g., target daily step count)

CPET: Cardiopulmonary exercise test; 6MWT: Six-minute walk test; ISWT: Incremental shuttle walk test; UULEX: Unsupported upper limb exercise test; MMT: Manual muscle testing; 1RM: One repetition maximum; HGF: Handgrip force; HHD: Hand-held dynamometry; MIP: Maximal inspiratory pressure; MEP: Maximal expiratory pressure; IMT: Inspiratory muscle testing; SPPB: Short physical performance battery; TUG: Timed Up and Go; FIM: Functional independence measure; PASE: Physical activity scale for the elderly; IPAQ: International physical activity questionnaire; DASI: Duke activity status questionnaire.

assessment instruments included in Table 1.

Aerobic exercise capacity

Exercise capacity is a major predictor of waiting list survival pre-transplant across disease categories^[15,16], and is also associated with post-transplant health outcomes including days on mechanical ventilation, length of hospital stay and survival^[4,17,18]. The six-minute walk test (6MWT)^[19] is the most common functional test of exercise capacity for lung transplant candidates and recipients in Canada^[5], and is used widely internationally. It is a global marker of health status reflecting severity of disease and level of functional impairment, and has been found to correlate with VO_{2max} in lung transplant candidates^[20]. The six-minute walk distance (6MWD) is incorporated into several composite scores

that can determine the urgency for lung transplant including the BODE and Lung Allocation Score^[21,22]. A 6MWD of less than 400 m or a predicted distance of between 45%-55% is common in lung transplant candidates^[4,15,23,24]. The 6MWD improves significantly following transplant reaching 65%-85% predicted, with the largest gains reported in the first three to four months^[23-26]. Other field-based walking tests that have been used in chronic lung disease such as the incremental and endurance shuttle walk tests, (ISWT and ESWT) may also be used to quantify exercise capacity in lung transplant candidates and recipients^[27].

Upper extremity exercise capacity plays an important role in many basic and instrumental activities of daily living and may provide unique information about upper extremity endurance not reflected in the field-based walking tests. In individuals with COPD, arm exercise capacity has been measured using the Unsupported Upper Limb Exercise Test (UULEX)^[28]. A small group of lung transplant candidates with ILD at our center demonstrated reduced arm exercise capacity compared to controls using the UULEX^[29], however this test has not been used in routine clinical evaluation.

Muscle function

Peripheral muscle function can be tested through multiple techniques, some of which are more applicable to the clinical setting due to lower costs and fewer requirements for specialized equipment, training and personnel such as manual muscle testing, hand held dynamometry (HHD), handgrip dynamometry and one-repetition maximum (1-RM; Table 1). The quadriceps is the most common muscle tested in the research literature and lung transplant candidates exhibit quadriceps weakness of 49%-86% predicted^[30]. An immediate drop in quadriceps strength from pre-transplant to post-transplant at the time of hospital discharge of 15%-32% has been reported with a gradual recovery to pre-transplant levels by three to four months post-transplant^[23-26]. Lower extremity muscles (e.g., quadriceps, ankle plantar flexors) show more pronounced weakness than upper extremity muscles (e.g., biceps)^[29-31].

Body composition (muscle and fat mass) can be measured as part of a physical or nutritional assessment using bioelectrical impedance analysis, dual X-ray absorptiometry or skinfolds. More specific measures of muscle size (e.g., cross-sectional area and muscle layer thickness) can be obtained from ultrasound, computerized tomography, or magnetic resonance imaging, however these are not typically performed for clinical assessment. Muscle atrophy has been reported in research studies of lung transplant candidates and recipients using several measures such as low fat free mass, reduced muscle volume and cross-sectional area^[29,30].

Short tests of physical performance and mobility may be a useful addition to the functional assessment in the pre-transplant phase (Table 1). Lung transplant

candidates have shown reduced functional performance on the Short Physical Performance Battery (SPPB) and Timed Up and Go (TUG) compared with controls^[29,30]. The SPPB has recently been used as a marker for frailty pre-lung transplant and shown to be a predictor of disability, delisting and waitlist mortality^[32].

Physical activity

Level of physical activity can be evaluated using questionnaires, however there is no specifically validated scale for lung transplant candidates or recipients. Commercially available pedometers or accelerometers may also be used to obtain daily step counts and activity level. Measurement of physical activity can be an important adjunct to exercise capacity testing, since it is reduced pre- and post-transplant and can be used for physical activity counseling and setting targets for daily activity.

Low levels of physical activity with a reported mean of 1400-3200 daily steps, reduced time spent in moderate intensity activity, walking and standing, and greater time in sedentary activities has been reported in lung transplant candidates^[23,24,33,34]. A research study conducted in our center demonstrated that lung transplant candidates with ILD had increased physical activity levels on days they participated in pulmonary rehabilitation, and the 90 min rehabilitation session accounted for 58% of the total daily steps^[33]. Levels of daily physical activity improve following lung transplant however remain below predicted levels in terms of daily steps, walking time and movement intensity compared to healthy controls; and show great variability^[23,24,34-37].

GENERAL PRINCIPLES OF EXERCISE TRAINING

Exercise prescription should be individualized, include both aerobic and resistance training, and follow general exercise training principles of specificity, overload and progression^[38]. Based on our clinical experience, respiratory and cardiovascular reserve, stability and clinical course of lung disease, muscle strength and muscle endurance can have a significant impact on the frequency, intensity, type and duration of exercise that is prescribed and the rate of progression. Figure 1 outlines general rehabilitation guidelines used at our center during the pre- and post-transplant phases.

Pre-transplant rehabilitation

Pre-transplant exercise training is recommended in Canadian lung transplant centers for a specified duration or during the entire waiting period prior to transplant to optimize fitness and prevent the cycle of inactivity and deconditioning that can occur with advanced lung disease^[5]. There are few randomized controlled trials that examine the effect of exercise training pre-transplant^[39,40], however retrospective and pre-post studies of exercise training in lung transplant candidates have

shown that 6MWD can be maintained or even increased in spite of progressive lung disease^[4,41-43]. Predictors of rehabilitation success pre-transplant (e.g., improved 6MWD) have not been identified in lung transplant candidates^[43].

Pulmonary rehabilitation guidelines for exercise training can be applied to lung transplant candidates with modifications to account for increased severity of lung disease and multiple underlying disease states^[44,45]. If disease progression and functional deterioration occurs during the waiting period, physical function needs to be reassessed on an ongoing basis and exercise prescription modified as needed. Alternative modes of training including high intensity interval training^[39] and Nordic pole walking^[42] have been described in lung transplant candidates. Inspiratory muscle training has been utilized in chronic lung disease, primarily COPD, to improve inspiratory muscle strength and endurance; however studies have not been specific to lung transplant candidates^[46]. Although supervised outpatient pulmonary rehabilitation in a hospital or community setting are common^[5], alternative modes of delivery such as tele-rehabilitation may be an important alternative for individuals living far from a transplant center, however pre-transplant tele-rehabilitation has not yet been studied in lung transplant candidates^[47].

Guidelines for pre-transplant exercise prescription have been summarized in Table 2 from protocols used in research studies and our current clinical guidelines. Exercise intensity and duration are prescribed and progressed according to exertional oxygen saturation, heart rate and symptoms of dyspnea and leg fatigue using the modified 0-10 Borg scale^[48]. A percentage of the 6MWT speed can be used for lung transplant candidates to prescribe walking speed on the treadmill^[49].

Special considerations for pre-transplant rehabilitation

Supplemental oxygen for exercise training: As lung transplant candidates often require supplemental oxygen for rest and/or exertion^[4], oxygen titration is an important component of exercise training. Guidelines for oxygen supplementation for exercise are not clearly defined^[50], so oxygen titration orders, institutional policies and delegation practices may vary between facilities. At our center, all lung transplant candidates have a prescribed oxygen titration range provided by a physician, which is often to maintain an oxygen saturation (% SpO₂) of at least 88% with exercise, however, oxygen prescription may be modified based on patient diagnosis, medical co-morbidities, arterial blood gases, functional capacity and symptoms. Lung transplant candidates are supported with sufficient oxygen to maintain the prescribed oxygen saturation in an attempt to increase aerobic exercise intensity and duration to obtain a greater physiological benefit with training. In our clinical experience, oxygen requirements for exertion may increase during the waiting

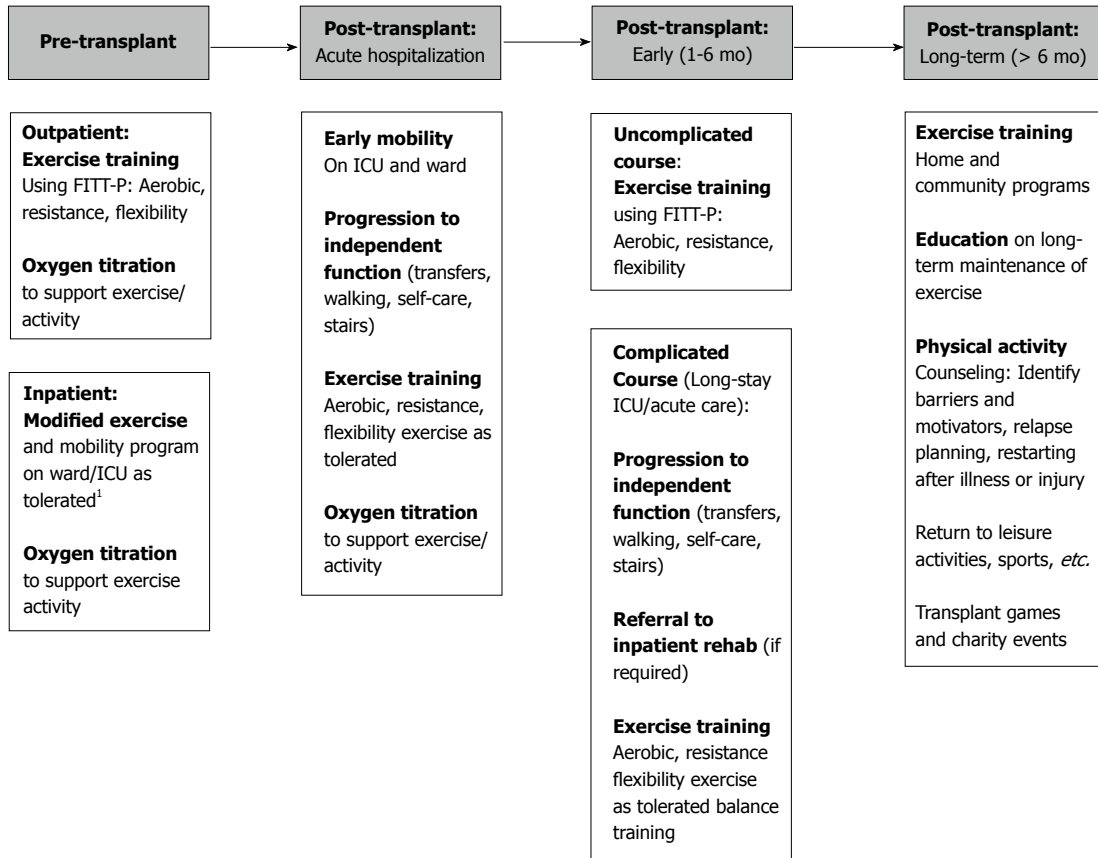


Figure 1 Overview of rehabilitation during the pre- and post-transplant phases. At each phase, monitoring and re-assessment are needed to modify/progress the exercise program. ¹Some hospitalized lung transplant candidates and recipients may require mechanical ventilation and/or extracorporeal life support (ECLS) and can be mobilized on these devices. FITT-P: Frequency, intensity, type, time, progression; ICU: Intensive care unit.

period pre-transplant with some individuals (such as those with ILD) requiring very high levels of oxygen supplementation, high flow oxygen delivery devices and/or non-invasive ventilation. There is a lack of literature on the safety guidelines and hazards of high flow oxygen for exercise training^[51], and our clinical practice is to communicate closely with the medical team regarding arterial blood gases and/or other medical concerns.

Exercise training in pulmonary hypertension:

Historically, individuals with pulmonary arterial hypertension (PH) were excluded from exercise training, however alongside changes in medical management, a number of studies over the past decade have shown efficacy and safety of carefully prescribed exercise in stable, medically optimized individuals with PH^[52]. For individuals with moderate to significant primary or secondary PH who are not symptomatic at rest, our clinical practice is to avoid exertional hypoxemia, symptoms of chest pain, dizziness, pre-syncope, nausea and visual changes during exercise training. We prescribe exercise intensity and duration as guided by lower dyspnea scores (e.g., Borg score 2-3 or slight to moderate). High intensity aerobic and resistance training and Valsalva maneuvers are avoided. Changes in weight, abdominal circumference, lower leg edema

and other evidence of worsening right heart failure are monitored with close communication with the medical team, and care is taken to avoid interruption of continual intravenous vasodilators (e.g., prostaglandins).

Infection control: Infection control procedures are essential for preventing spread of certain infections such as methicillin-resistant staphylococcus aureus, mycobacterium abscessus or CF-related infections during group exercise programs. At our center, individuals with CF are physically separated by three meters during group exercise training and individuals with Burkholderia cepacia exercise separately at the end of the day. Guidelines on cleaning equipment, hand-washing, gown and mask use and isolation practices may vary at different institutions.

Team approach to rehabilitation: Education is an important component of rehabilitation, specifically on issues related to safe and effective exercise, exertional oxygen use, home exercise, assistive devices and energy conservation techniques^[53]. Psychosocial support to address stress and expectations during the waiting period and concerns regarding surgery is also beneficial^[54]. Collaboration with the registered dietitian to ensure that nutritional needs are being met and balancing exercise participation with nutritional needs

Table 2 Guidelines for pre-transplant exercise prescription in stable outpatients

	Aerobic	Resistance	Flexibility
Frequency	2-5 d/wk	2-3 d/wk	3-5 d/wk
Intensity	50%-80% HR reserve Dyspnea > leg fatigue: Moderate to hard (3-5 on modified Borg scale) ^[48] SpO ₂ > 85%-90% Continuous or intermittent training ¹ : 60%-80% 6MWT speed for walking ^[41,49] 60% peak workload for cycling ^[39,43] or just above anaerobic threshold ^[40] Interval training ² : 100%: 0% peak work rate (cycle) ^[39]	30%-80% 1-RM or use 8-15-RM ^[125]	
Type	Walking (treadmill, corridor, Nordic poles) ^[42] Cycling (leg and/or arm ergometer)	Major muscle groups of upper and lower body (quadriceps, hamstrings, plantar flexors, gluteals, biceps, triceps, pectorals, latissimus dorsi) Training modalities: Free weights/ dumbbells Elastic bands Pulleys Gym equipment Body weight (stairs, squats, heel raises, wall push-ups)	Major muscle groups of upper and lower body Thoracic cage and chest wall mobility
Time/ Training	Continuous: 15-30 min	1-2 sets × 8-15 reps	Hold up to 10-30 s
Volume	Intermittent: 5-10 min × 2-3 bouts Interval ² : 30 s exercise: 30 s rest (12-36 min) ^[39]		each, repeat 2-4 times
Progression	Progress time up to 20-30 min continuous Perform regular 6MWTs and adjust speed accordingly for treadmill training; and increase Watts on cycle Higher level patients may tolerate a treadmill incline of 1%-4%	Increase weights based on tolerance; (approximately 0.5 kg or 1 lb. per week, as tolerated) ^[41] Body weight exercises: Can add hand or ankle weights	Hold stretches to point of tightness/ slight discomfort

¹Intermittent training is regular or irregular intervals of the same low to moderate intensity *vs* interval training, which involves pre-set, alternating, short intervals of high intensity to intervals of rest or lower intensity;

²There are several different interval training protocols described in chronic lung disease^[126]. SpO₂: Oxygen saturation measured by pulse oximetry; HRR: Heart rate reserve; 6MWT: Six-minute walk test; ISWT: Incremental shuttle walk test; HR: Heart rate; BP: Blood pressure; RR: Respiratory rate; ESWT: Endurance shuttle walk test; reps: Repetitions; RM: Repetition maximum.

with close monitoring of weight are performed at our

center. Some individuals are required to lose weight pre-transplant and may benefit from nutritional counseling in addition to aerobic exercise training. A palliative care referral for opioid administration may be beneficial to assist with symptom control of dyspnea, cough and other symptoms that may impact on exercise ability and quality of life. A study at our center observed a trend towards increased caloric expenditure during exercise training in 64 lung transplant candidates referred to palliative care post opioid initiation^[55].

Considerations for a complicated pre-transplant clinical course:

In cases of a prolonged waiting period prior to transplant, we find that exercise intensity and duration may not be progressed if there is significant disease progression, respiratory exacerbations and infections, medical instability and hospital admission for respiratory failure. Maintenance of physical function or slowing the rate of physical deterioration can become important functional goals. Increased dyspnea, decreased function or acute worsening of gas exchange should be investigated as they can indicate underlying infection, respiratory exacerbation or pulmonary embolism. Some lung transplant candidates experience profound respiratory deterioration and need to await lung transplantation on the hospital ward or in the intensive care unit (ICU). Although there is no research evidence on inpatient rehabilitation for lung transplant candidates hospitalized with respiratory deterioration and failure, we provide a modified exercise program based on patient tolerance to help offset functional decline. Corridor ambulation and bedside cycling are encouraged as tolerated, but may not be tolerable by some individuals due to severe gas exchange abnormalities that are not corrected with high levels of supplemental oxygen. Resistance exercises, which do not confer the same degree of exertional desaturation should be continued as tolerated, with a focus on maintaining proximal muscle strength (e.g., shoulder and hip) and lower limb strength in anticipation of early ambulation and return to self-care activities post-transplant^[56]. Neuromuscular electrical stimulation (NMES) has been shown to enhance muscle mass and function in individuals with severe COPD and incapacitating dyspnea, and may be a useful adjunct for individuals unable to participate in a traditional outpatient pulmonary rehabilitation program^[57].

Selected lung transplant candidates require bridging to transplant due to respiratory failure. Mechanical ventilation and ECLS can be associated with significant deconditioning due to increased sedation time limiting mobility and active participation in rehabilitation, and in some cases, irreversible muscle damage from persistent critical illness polyneuropathy and myopathy^[58]. Facilities with an experienced critical care mobility team can mobilize individuals on mechanical ventilation and/or ECLS who are medically stable and cognitively capable^[59], although guidelines for mobility prescription

Table 3 Exercise and mobility for hospitalized lung transplant candidates and recipients

Setting	Interventions/prescription	Considerations for a complicated hospital course
Intensive care unit	Upright positioning AROM for upper extremities	PROM, A/AROM for those who are sedated/not actively moving
	Acupuncture for incisional pain	Trunk control and sitting balance prior to standing and walking
	Progressive mobility program, consisting of: Bed mobility > dangling > transfer to chair > standing > marching on spot > ambulation with HWW up to 100-200 m with or without MV	Specialized equipment to facilitate mobility, such as: Standing frames, sit-stand lifts or mechanical lifts, standing and walking slings, portable treadmills, portable ventilators for ambulation in ICU (with appropriate settings to facilitate exercise), manual resuscitation bag with PEEP valve
	In sitting or lying: Resistance training using light weights, elastic resistance bands	Bedside cycle ergometer or treadmill for aerobic training Video gaming system (e.g., Nintendo Wii™) for balance and strengthening exercises ^[127]
Step-down unit/ward	AROM upper extremities	Transfer training
	Progressive mobility program: Up to chair 1-3 ×/day; supervised walking 1 ×/day building up to 100 m; progress to 4-5 ×/day for 10-15 min bouts and increase distance > 100 m Stair climbing Resistance training: Up to 5 lbs. (1 set × 10 reps) Education re: Lifting restrictions Postural correction/re-education Oxygen titration: Maintain SpO ₂ > 88% on exertion	Gait training Gait aids: Progress from HWW > rollator > no gait aids, if able Specialized seating Referral to inpatient rehabilitation for those who are not independent for discharge home

ROM: Range of motion; HWW: High-wheeled walker; MV: Mechanical ventilation; AROM: Active range of motion; PROM: Passive range of motion; A/AROM: Active/assisted range of motion; PEEP: Positive end expiratory pressure.

in critically ill individuals are not clearly defined^[60]. A recent systematic review presented evidence that early mobilization and ambulation is safe even in patients awake on veno-venous Extra Corporeal Membrane Oxygenation (ECMO) support^[61]. Physiotherapists at our center undergo specialized training in managing ECMO circuits, and with the support of an early mobility team, close communication with the medical team and a positive ICU culture towards the safe mobilization of selectively assessed critically ill patients^[62].

Post-transplant rehabilitation

Immediate post-transplant rehabilitation in the ICU: The rehabilitation goals in the early phase post-

transplant are to increase general mobility, functional capacity, muscle strength and endurance, and facilitate discharge from hospital. Reduced ICU length of stay has been associated with increased quadriceps muscle strength at hospital discharge in lung transplant recipients^[26]. One study identified factors that contribute to an extended hospital stay which included high urgency listing status, bridging to transplant with mechanical ventilation and/or ECLS, diagnosis of pulmonary hypertension, prolonged intubation post-transplant and colonization with multidrug resistant pathogens^[63]. The functional consequences of a prolonged ICU stay can be profound and long-term^[64].

Physical rehabilitation should begin as early as possible post-operatively and should prioritize upright positioning (e.g., sitting) and mobilization (e.g., out of the bed)^[65,66]. Early mobilization in the ICU has not yet been studied specifically in lung transplant patients, but the same treatment approaches reported for other critically ill patients are likely applicable. Table 3 Muscle wasting related to critical illness is early and impactful^[67,68], highlighting the need for rapid and effective interventions to protect the muscle from atrophy and weakness. To date, several systematic reviews support safety, feasibility and beneficial impact of early physical therapy and mobilization in mechanically ventilated patients^[69-76]. There is evidence that early physical therapy and mobility training can result in improved quality of life^[71], physical function^[71,72], muscle strength^[71,73] and functional outcomes^[69]. Further research is needed to determine whether these improvements translate into decreased hospital and ICU length of stay^[77,78] and better long-term physical function^[60].

Rehabilitation in the ICU should take into consideration pre-transplant function, cardiorespiratory function, muscle strength, range of motion (ROM), balance, cognitive impairments, pain control and medical stability. Early active muscle training and cardiopulmonary conditioning should begin as soon as feasible within the hospital setting (e.g., turning in bed, sitting at the edge of bed, sitting in a chair, standing, and walking). In addition, self-care and activities of daily living should be encouraged as soon as possible^[79]. Low levels of exercise (e.g., with elastic therapy bands or unloaded pedaling on the bicycle) with subsequent increases in the duration and workload can be made as the patient progresses^[79,80]. In critically ill patients, even passive or active exercise training sessions for 20 min/d using a bedside ergometer is able to increase short-term functional recovery^[75].

The emerging literature using NMES has shown that it may be a safe, low cost treatment for early intervention in critically ill patients who may not be able to participate in active exercise^[75,81] since it can passively activate the muscles^[75,81,82]. However, studies to date have included a general, mixed population of ICU patients and the evidence is not specific to lung transplant recipients. Furthermore, the ability to deliver NMES effectively in the context of underlying ICU

acquired myopathy and polyneuropathy^[83] has not been substantiated.

Post-transplant rehabilitation in the hospital step-down unit and ward: At our center functional reassessment and exercise are resumed following ICU transfer until discharged home or to inpatient rehabilitation, with oxygen titration orders to maintain oxygen saturation at least 88% on exertion. Most lung transplant recipients at our center are weaned off supplemental oxygen prior to hospital discharge, but a few may still require low flow oxygen for exertion for several weeks to months, especially single lung transplant recipients.

Rehabilitation interventions provided at our center during the hospital stay post-transplant are summarized in Table 3. Medical issues that may be encountered in this early post-transplant phase that can impact exercise include infection, acute rejection, anxiety, depression, post-surgical pain at the thoracotomy tube site and chest wall, arrhythmias, veno-thrombotic events, infections requiring isolation, postural hypotension, skin ulcers and poor wound healing. Side effects of medications include fluid retention, anemia, nausea, tremors, decreased visual acuity, hyperglycemia and hypertension^[65], which need to be considered when prescribing exercise so that appropriate modifications should be made.

Outpatient rehabilitation: Structured outpatient rehabilitation within the first three months following lung transplant is available at Canadian transplant centers^[5]. Functional goals in the outpatient phase may include ambulation without gait aids, liberation from supplemental oxygen, return to pre-transplant muscle strength and 6MWD of 65%-85% predicted levels^[23-26,84]. Large functional gains are reported during this period of rehabilitation in individuals with a relatively uncomplicated post-operative course^[23-26]. Lung transplant recipients indicate that exercise training is a valuable part of their post-transplant care and essential to improve physical function^[85]. A greater improvement in 6MWD post-transplant is predicted by greater recovery of muscle strength and a lower pre-transplant 6MWD^[25,84]. Studies examining exercise training following lung transplantation show significant increases in exercise capacity, muscle strength and bone mineral density^[24,86-88] (Table 4).

Considerations for a complicated post-transplant clinical course: There are a multitude of complications that can significantly increase the length of hospital stay and impact rehabilitation including: Major bleeding, infections, prior multi-drug resistant infections and colonization, difficulty weaning with prolonged mechanical ventilation, pre- and post-transplant ECLS, diaphragmatic paralysis, severe agitation, delirium, depression, acute neurological events, critical illness polyneuropathy, hemodynamic instability, primary

graft dysfunction and acute renal failure requiring hemodialysis^[65,66].

An assessment of functional goals can help inform discharge planning and recommendations for inpatient transplant rehabilitation, complex continuing care or homecare services. A retrospective study from our center showed that lung transplant candidates who were older, had a lower pre-transplant 6MWD, were mechanically ventilated prior to transplant and had a longer total length of hospital stay were more likely to be discharged to an inpatient rehabilitation facility vs home^[89]. Compared to other inpatient rehabilitation patients (e.g., stroke, joint surgery) lung transplant recipients are more likely to require transfer back to acute care for medical management related to complications such as infection, rejection and cardiac events^[90,91].

In our clinical practice, individuals who experienced a complicated post-transplant course may require a referral to a multidisciplinary inpatient rehabilitation program to regain basic mobility (e.g., independent transfers, walking, and the ability to engage in activities of daily living such as self care) prior to discharge home. Upon discharge, these individuals are encouraged to enroll in an outpatient pulmonary rehabilitation program, or be prescribed a program that can be done in the community or home setting to work on improving endurance and strength. These individuals often require a mobility aid (e.g., rollator walker or cane) and their 6MWD is well below predicted values, showing a slow improvement over 12 to 18 mo. Specific exercises to target balance and coordination impairments are sometimes needed to be included in the outpatient or home exercise program. Individuals with a complicated post-transplant clinical course may experience persisting myopathies and/or neuropathies, and not all critically ill survivors recover to the same extent as there may be significant differences in recovery of muscle function and rehabilitation potential^[58]. This remains an area of active research.

Late/ongoing post-transplant maintenance

The 6MWT is reassessed regularly post-transplant^[5], to monitor changes in exercise capacity and exertional oxygen saturation, which may change over time. Although the majority of exercise training programs occur in the first three to four months following transplant, longer-term exercise training may provide additional benefits to exercise capacity and the management of long-term co-morbidities of hypertension, hyperlipidemia and diabetes are prevalent at one, three and five years post-transplant^[1,24]. A randomized trial found that lung transplant recipients who underwent rehabilitation in the first three months following transplant had higher physical activity levels, improved fitness and lower 24-h blood pressure one year post-transplant compared to recipients who did not participate in rehabilitation^[24]. Daily physical activity has been reported to be significantly reduced one year following transplantation as

Table 4 Guidelines for early post-transplant exercise prescription in stable outpatients

	Aerobic	Resistance	Flexibility
Frequency	3-5 d/wk	2-3 d/wk	3-5 d/wk
Intensity	50%-80% HR reserve or < 85% age-predicted HRmax ^[4,23] Leg fatigue > dyspnea: Moderate to hard (3-4 on Borg scale) SpO ₂ > 88% Continuous training: 75%-100% 6MWT speed for walking ^[24,25] 50%-80% peak workload for cycling ^[24,59,128]	60%-80% 1RM ^[24,26] 10-RM No upper extremity lifting/pulling/pushing > 10 lbs. first 3 month Extra restrictions if sternal instability	Hold stretches to point of tightness/slight discomfort
Type	Walking (treadmill, corridor) Cycling (leg); avoid arm ergometry in first 3 month to allow for incision healing	See pre-transplant Avoid abdominal muscle exercises for first 3 month	Major muscle groups of upper and lower body Thoracic cage and chest wall mobility Postural re-education
Time/ Training Volume	Continuous: 20-30 min	1-3 sets × 8-15 reps	Hold up to 10-30 s each, repeat 2-4 times
Progression	Progress time to 30 min, then progress speed on treadmill; increase incline after approximately 6 wk post-transplant (if tolerated) Increase Watts on cycle Walk: Run program for some high level patients (at least 6 wk post-transplant) 30-60 s running bouts interspersed with walking for 20-30 min	Start with sit-stands and when able to perform without arm support progress to squats with hand weights Weekly increase weights based on tolerance; (approximately 0.5 kg or 1 lb. per week, as tolerated) within lifting guidelines (e.g., < 10 lbs. for upper extremities for first 3 month) Body weight exercises: Can add hand or ankle weights (e.g., squats and stair climbing)	Hold stretches to point of tightness/slight discomfort Extra restrictions if sternal instability (e.g., avoid chest expansion stretches)

6MWT: Six-minute walk test; CPET: Cardiopulmonary exercise test; HR: Heart rate; HRR: Heart rate reserve; SpO₂: Oxygen saturation measured by pulse oximetry; RR: Respiratory rate; BP: Blood pressure; ISWT: Incremental shuttle walk test; ESWT: Endurance shuttle walk test.

compared to healthy controls^[35]. Physical activity levels varied in long-term recipients and have been found to be inversely associated with body weight^[37].

Exercise training in lung transplant recipients in the long-term phase (> 6 mo) has been shown to have beneficial effects on endurance capacity and muscle

strength^[87,88,92]. Long-term adherence to exercise may be greater if individuals participate or resume activities they enjoy. Thinking beyond a traditional gym protocol and exploring individuals' interests, access and resources can be helpful when counseling individuals about increasing and maintaining physical activity in their home community. National and World Transplant Games^[93,94] and charity events are excellent opportunities for setting fitness and performance goals and staying active while raising awareness of lung disease and transplantation. As an example of the benefits of this training, lung and heart-lung transplant recipients (> 6 mo post-transplant) who participated in ten weeks of upper extremity training through Dragon boat racing showed improved aerobic and anaerobic fitness^[95].

Inexpensive pedometers, activity watches, fitness monitors and smart phone applications can be used to track daily steps and activity levels, and set targets to increase physical activity. Additional activities such as yoga, Tai Chi, dance and seasonal activities such as swimming, paddling, outdoor cycling, hiking, skating and snowshoeing can be done in a social setting with family and friends. A gradual introduction to new activities should be emphasized, and we counsel transplant recipients to avoid activities with an increased theoretical risk of injury such as contact sports, skydiving, bungee jumping and scuba diving. Episodic medical issues such as illness, infection or injury can interrupt an exercise regimen, so physical activity counseling on how to modify and resume exercise after an episode of illness is important and can be addressed at reassessment.

SPECIAL POPULATIONS

Heart-lung, multi-organ and re-transplantation

At our center individuals who have undergone heart-lung transplantation, multi-organ transplantation (e.g., lung-liver) and re-transplantation participate in a similar pre- and post-transplant rehabilitation program as lung transplant candidates and recipients. Individuals awaiting a heart-lung transplantation may have congenital heart disease with cardiac shunts that can lead to right heart shunting and severe hypoxemia that may not be responsive to supplemental oxygen^[96]. This may necessitate lower training intensity^[97] (e.g., using heart rate and/or Borg dyspnea and fatigue scores) and lower oxygen saturation guidelines for exercise training. Following heart-lung transplantation, a longer warm-up and cool down is recommended to allow for the slower changes in heart rate due to disrupted cardiac innervation^[98]. The modified Borg scale is used to guide exercise training instead of heart rate. There is a lack of information on exercise training for individuals listed for re-transplant^[99], but based on clinical experience at our center, individuals often have a lower functional capacity compared to listing for their first transplant.

Pediatrics

Children (from birth to 18 years of age) are typically followed in specialized pediatric healthcare centers. Clinical assessment of the pediatric lung transplant candidate should include posture, ROM, muscle strength and gross motor function appropriately for the age of the child. The 6MWT has been shown to be a valid measure in children^[100] and is utilized by a majority of pediatric centers in North America^[101]. There are published normative values for 6MWD across various ages^[102], however interpretation of the 6MWT data is sometimes difficult to differentiate from growth and development of the child, so it should be used as part of a thorough clinical assessment to identify issues amenable to rehabilitation. While pre-transplant physical functioning and its relationship to post-transplant outcomes has not been studied extensively, one study in pediatric patients found a correlation between 6MWD and short term transplant outcomes including length of ICU stay, days of mechanical ventilation and time until discharge^[18].

Pre-transplant rehabilitation: There are no studies examining the impact of exercise training in pediatric lung transplant candidates, however clinical experience indicates that it can be of significant benefit for these children and helps to prevent deterioration in function. Due to the limitations of available programs for children, families must often commute to the transplant center. However, older teens may be referred to adult pulmonary rehabilitation programs with support from pediatric specialists. Children may also have exacerbations of their underlying condition requiring hospitalization and modification of their exercise programs. Exercise prescription with slow progression can be approached similarly as for adults including both aerobic and resistance training^[103,104]. Strength training is unlikely to increase muscle bulk for pre-pubertal children, but can improve function. Exercise training for younger children should include activities encouraging gross motor skill development, such as integration of physical education activities and incorporate growth and developmental factors of the child's maturing system. Physical therapists should also encourage regular school attendance, participation in physical education curriculums (within medical restrictions) with appropriate modifications to help ensure adequate levels of physical activity. Collaboration with school professionals, teachers, and physical education instructors may be needed to ensure safe follow through of these recommendations.

Post-transplant rehabilitation: Exercise capacity and general fitness improves for children following lung transplant but remains reduced compared to age-predicted values^[104-106]. Opportunities and access to rehabilitation post-transplant are often limited. A study examining the impact of an early semi-individualized physiotherapy prescribed exercise program early (within the first three months) after hospital discharge found

similar improvements in 6MWD, strength and flexibility in children who attended the hospital three times a week compared to children who performed the exercise at home with parents^[105], suggesting that home-based training may be a way to bridge the gap in accessibility. A study at our center with children who were attending the World Transplant Games showed the positive effects of home-based training, which included general exercise programs and event-specific, skill-based training done independently for three months prior to the Games. The children showed short term benefits in levels of physical activity and each subject demonstrated an increase in at least one parameter of fitness on the Fitness-GRAM[®]^[106]. Taken together, these studies suggest that home-based intervention or exercise prescription can be of benefit for these children when provided with appropriate education regarding safe exercise. The transition of adolescents and young adults to adult care is an increasingly important area of focus since this has been recognized as a vulnerable time for adolescent transplant recipients^[107]. Research on strategies to optimize successful transition highlights the importance of an inter-professional approach with involvement from both the pediatric and adult care centers.

CONCLUSION AND FUTURE DIRECTIONS

Medical and surgical advances continue to improve the availability of lung transplantation^[108]. Exercise training provides an essential role in optimizing functional capacity and fitness pre-transplant, as well as improving outcomes and quality of life post-transplant. Physiotherapists and clinical exercise specialists working with lung transplant candidates and recipients require expertise in general exercise training principles and specialized knowledge of pre- and post-transplant complications, oxygen titration, side effects of medications and a sound understanding of how to modify exercise programs during episodic illnesses/ exacerbations and/or change in lung function pre- and post-transplant. Although studies have been conducted on exercise training in lung transplantation, there is a need for larger studies examining long-term outcomes^[109]. Individuals with a complicated pre- and post-transplant course pose a particular challenge for clinicians, and further research on rehabilitation for this population is needed. The development of standardized physical function measures that can help predict post-transplant outcomes, and the investigation of alternative modes of exercise training are also warranted.

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Genetic barriers in transplantation medicine

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shared human leukocyte antigens (HLAs) and ABO blood group antigens between donor and recipient. In recent years, killer cell receptor [*i.e.*, killer cell immunoglobulin-like receptor (KIR)] and major histocompatibility complex (MHC) class I chain-related gene molecule (*i.e.*, MICA) were also reported as important determinants of transplant compatibility. At present, several different genotyping techniques (*e.g.*, sequence specific primer and sequence based typing) can be used to characterize blood group, HLA, MICA and KIR and loci. These molecular techniques have several advantages because they do not depend on the availability of anti-sera, cellular expression and have greater specificity and accuracy compared with the antibody-antigen based typing. Nonetheless, these molecular techniques have limited capability to capture increasing number of markers which have been demonstrated to determine donor and recipient compatibility. It is now possible to genotype multiple markers and to the extent of a complete sequencing of the human genome using next generation sequencer (NGS). This high throughput genotyping platform has been tested for HLA, and it is expected that NGS will be used to simultaneously genotype a large number of clinically relevant transplantation genes in near future. This is not far from reality due to the bioinformatics support given by the immunogenetics community and the rigorous improvement in NGS methodology. In addition, new developments in immune tolerance based therapy, donor recruitment strategies and bioengineering are expected to provide significant advances in the field of transplantation medicine.

Key words: Transplantation; ABO blood group; Human leukocyte antigen; MICA; Killer cell immunoglobulin-like receptor; Graft rejection; Graft vs host disease

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Abstract

The successful of transplantation is determined by the

Core tip: Transplantation is a systematic medical procedure for patients with organ failure and haematological disorders. Immunologically compatible donor

and recipient are determined by several genetic markers which include matching for ABO blood group, human leukocyte antigen, MICA and killer cell immunoglobulin-like receptors. The elucidation of genes code for these markers of tissue identity reviewed here and significant advancement in the field of transplant immunology are expected to have a positive impact on transplantation medicine. These include both the waitlisted and transplanted patients.

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INTRODUCTION

Transplantation is a systematic medical procedure for patients with organ failure and haematological disorders^[1,2]. Transplantation can be classified into four categories: Autograft, isograft, allograft and xenograft based on the origins and the recipients of the grafts (cells, tissues or organs). In autograft transplantation (also known as autologous transplantation), a graft is taken and transplanted from different parts of the same individual. The processes of transferring grafts between genetically identical and non-identical individuals of the same species are known as isograft and allograft transplantation, respectively. In contrast, xenograft refers to the transplantation of grafts between two different species such as from baboon to human. Implantation of human cancer cells in mice for tumour study is also assumed to be xenograft transplantation^[3,4].

The current practice of allograft transplantation is to have as many match for ABO and human leukocyte antigen (HLA) loci as possible between the donor and recipient. However, this is not the case for isograft and autograft as the transplanted graft originated from the genetically identical resources. Incompatibility between donor and recipient will cause rejection since the graft will be considered as non-self by the recipient's immune surveillance and the rate of graft rejection will vary depending on time courses, types of tissue or organ grafted and the immune responses involved.

REJECTION AND GRAFT VS HOST DISEASE

In general, there are three types of graft rejections, *i.e.*, hyperacute, acute and chronic rejection^[4]. These types of rejections are categorized based on the speed that the rejection occurs. For hyperacute rejection, this process may occur within minutes or hours, and is usually not longer than 24 h. Sometimes, hyperacute rejection may occur immediately during the surgery process. This type

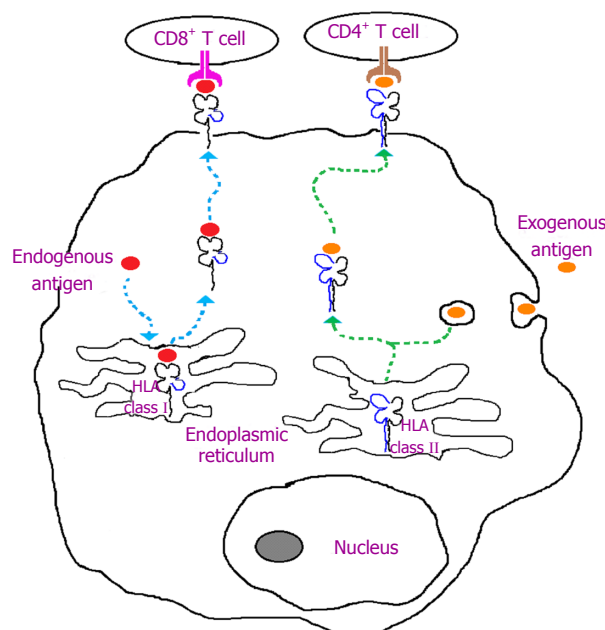


Figure 1 Schematic diagram of human leukocyte antigen class I and II antigenic peptide presentation to CD8⁺ T and CD4⁺ T cells, respectively. HLA: Human leukocyte antigen.

of rejection is due to preformed alloantibodies against the mismatched ABO and HLA antigens between patient and donor. The alloantibodies may exist due to previous transplantation or transfusion, pregnancy or infections^[5]. This pre-existing antibody can activate the complement system and cause injury to the endothelial cells which will then lead to platelet adhesion and thrombosis. Therefore, the graft will never be vascularised and the organ must be removed immediately. The hyperacute rejection may be managed with systematic antibody screening and cross matching between donor and recipient^[6].

The most common type of graft rejection is acute rejection. The onset of rejection varies from weeks to months and is largely attributed to HLA incompatibility. This type of rejection involves both cellular- and humoral-mediated immunity. However, the cellular-mediated immune responses are more significant through either direct recognition of non-self HLA molecules on the surface of the graft or indirect antigenic peptide presentation by self HLA molecules to T cells^[7-9] (Figure 1). The CD4⁺ T cells will also secrete several types of cytokines such as interleukin-4 (IL-4) and IL-2. These cytokines will then lead to several mechanisms including inflammation, recruitment of other inflammatory cells and may also induce T and B cell proliferations^[9]. The major histocompatibility complex (MHC) class I chain-related gene A (*MICA*) molecules are also important markers of tissue identity and have been implicated in transplant immunology^[10,11]. The stress-induced *MICA* has previously known as PERB11.1 glycoproteins and are coded for by the gene located on the classical class I subregion of MHC^[12] (Figure 2) and incompatibility between the donor and recipient for the *MICA* antigen

Table 1 List of killer cell immunoglobulin-like receptors and their human leukocyte antigen ligands

KIR	Alleles	Protein variants	HLA ligands
2DL1	43	24	C2
2DL2	28	11	C1, C2
2DL3	34	17	C1, C2
2DL4	46	22	G
2DL5	41	17	Unknown
2DS1	15	7	C2
2DS2	22	8	Unknown
2DS3	14	5	Unknown
2DS4	30	13	A*11, some C
2DS5	16	11	Unknown
3DL1	73	58	Bw4
3DS1	16	12	Unknown
3DL2	84	61	A*03,-11
3DL3	107	55	Unknown
3DP1	22	0	0
2DP1	23	0	0

The C1 are HLA-C allotypes with serine and asparagines at position 77 and 80 of $\alpha 1$ domain, respectively. The C2 are HLA-C allotypes with asparagines and lysine at position 77 and 80 of $\alpha 1$ domain, respectively. The Bw4 are HLA-B allotypes with isoleucine or threonine at position 80 of $\alpha 1$ domain. This table is adapted from Robinson *et al*^[99] and Parham *et al*^[104]. KIRs: Killer cell immunoglobulin-like receptors; HLA: Human leukocyte antigen.

will trigger cytotoxic activity of lymphocytes (CD8⁺ and $\gamma\delta$ T cells) and natural killer (NK) cells^[11,13-15] (see the following sub-sections). The role of MICA in graft rejection and donor specific antibodies to MICA antigens have been reported by several others^[11,16-18].

The third type of rejection is chronic rejection which takes place months to years following transplantation procedure. It induces chronic damage *via* the production of cytokines and alloantibodies which activate the classical pathway of complement system^[19,20]. However, the actual mechanism of this rejection is not very well understood. It is usually characterized by fibrosis and arteriosclerosis, due to extensive proliferation of smooth muscle cells. Repairing process of damaged tissues and macrophages activation in chronic rejection can lead to fibrosis formation^[21-23].

The transplanted allograft can also trigger immune reactions [*i.e.*, graft vs host disease (GVHD)] against mismatched antigens possessed by the recipients. The GVHD is predominantly occurs in bone marrow transplantation which involves alloreactivity of donor's lymphocytes against the incompatible tissues of the immune-suppressed host^[8]. However, improved outcomes were observed in haplo-identical (*i.e.*, a single HLA haplotype-mismatched) stem cell transplantation^[24-26]. In this context, donor's NK cells will recognize leukaemia cells as non-self and initiate alloreactivity (*i.e.*, graft vs leukaemia effect) against the cancerous cells after haplo-identical stem cell transplantation^[27-29]. The inhibitory and alloreactivity of NK cells are determined by HLA molecules which acting as ligands (Table 1) for their immunoglobulin-like receptors [*i.e.*, killer cell immunoglobulin-like receptors (KIRs)]^[29,30] (see the

following sub-sections). Thus, this receptor-ligand incompatible might lead to either NK alloreactivity against transplanted graft or GVHD. Our understanding of this immune surveillance has provided the basis for the adoptive infusion of NK cells as part of immunological based modality in transplantation and ultimately reduce the potential toxicity effects of other immunosuppression agents^[29,31,32] (see later).

MANAGEMENT OF GRAFT REJECTION

The immunosuppressive therapy is used to increase the survival rate of the graft, especially during acute rejection. However, this therapy cannot be used for chronic rejection since it is difficult to manage. This therapy does not only involve drugs but also antibodies^[33,34]. Examples of the drugs that have been used in immunosuppressive therapy are like mycophenolate mofetil, cyclosporine, tacrolimus and sirolimus^[35-38]. Each of these drugs has their own mechanism of action which will result in immune cells suppression. For example, mycophenolate mofetil is administered to block proliferation of lymphocytes by inhibiting the key enzyme that is important for purine synthesis and DNA replication^[36] while cyclosporine is given to inhibit transcription factor for T-cell activation^[39,40]. For antibodies, a number of monoclonal and polyclonal antibodies have been given to the patients in preventing graft rejection. Most of these antibodies are specific for T cells or T cell sub-populations and they are very effective for blocking T cells activation and binding^[41,42].

However, most of the immunosuppressive agents can cause various side effects to the recipient on their long term use. Besides that, the immunosuppression effects of the agents are not specific only on the graft, but also attack the overall body systems including the lymphocyte maturation. Hence, this will put the recipient at a high risk of getting other infections, cancer, cardiovascular diseases and metabolic bone diseases^[33,43-45]. Additionally, the recipient will have a chance of getting transplant rejection once they stop taking these immunosuppression agents. As an alternative, researchers are working on finding a new therapy that maintains the health of the graft without compromising the immune system. This new method involves inducing immune tolerance and mainly focus on T cell depletion in thymus (*i.e.*, central tolerance) and suppression of mature T cells in lymph nodes (*i.e.*, peripheral tolerance)^[20,46].

The key element in tolerance induction is specificity, which means the recipient immune system is not completely paralyzed. For example, the traditional antithymocyte globulin (TGA) was used as immunosuppressive agent drugs to prevent an acute rejection in organ transplantation^[47-49]. As an alternative, this treatment is replaced with another antibody known as anti-IL-2R α receptor antibodies. This type of antibody is widely used to replace TGA as it does not cause chronic expression of cytokines and improves the development of immune

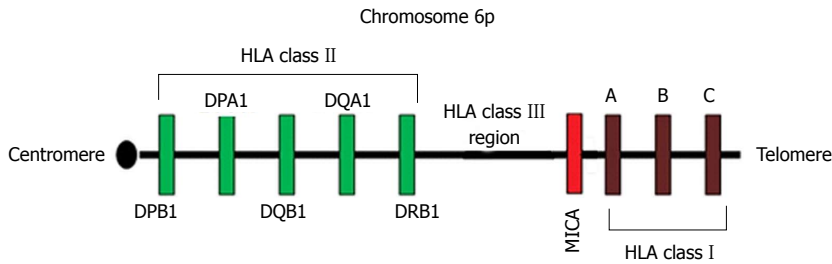


Figure 2 Approximate locations of human leukocyte antigen class I and II and major histocompatibility complex class I chain-related gene A loci on the short arm of chromosome 6. HLA: Human leukocyte antigen; MICA: Major histocompatibility complex class I chain-related gene A.

tolerance^[50-53]. Besides anti-IL-2R α , the combination of costimulatory molecule blockage with inhibitory of signal activation also appear to be effective in inducing tolerance in a few animal studies. Interaction between T cell receptor and costimulatory signals such as CD28 is required for T cell activation. Thus, blockage of the CD28 and its ligands (*i.e.*, B7 family molecules) resulted in transplantation tolerance^[46,54,55] and induction of anergic state in T cells activation^[56]. In addition, another molecule that binds to ligand for T cell activation (*e.g.*, CD152 or also known CTLA-4) also has a potential in inducing tolerance. For example, treatment with CTLA-4 immunoglobulin (Ig) during bone marrow transplantation in murine models was able to induce long-term survival rate of allograft^[57]. Similarly, Ig treatment of other ligand for T cell receptor (*e.g.*, PD-1) and costimulatory molecule (*e.g.*, CD40) have also been shown to limit T cell proliferation and activation^[58-60]. Acute rejection in non-human primates is also preventable by anti-CD40L treatment with or without CTLA-Ig^[61,62].

Besides using inhibitory molecules, Treg (CD4⁺CD25⁺) and NK cells can also be used to suppress CD4⁺ and CD8⁺ T cell proliferation^[63-67] and reduced rejection and GVHD^[68-74]. Other than post-transplant, infusion of Treg cells before a transplant procedure is found to promote immune reconstitution and improve immunity to opportunistic infection, hence, preventing GVHD^[75]. By increasing NK cells by total lymphoid irradiation, the immune tolerance is induced after organ and HSC transplantation^[76]. A study suggests that the interaction of NK cells and Treg cells can promote immune tolerance. IL-4, which is secreted by NK cells, induces the expression of negative costimulatory molecules on the Treg cells^[77]. The purification of NK cells in allogeneic transplantation may be achieved by depleting CD3⁺ cells followed by CD56⁺ cell enrichment^[78]. Donors are also reported safe in completed clinical trials of NK cells infusion^[79-81]. Stimulated NK cells with IFN- γ , IL-2 and anti-CD3 show MHC-independent cytotoxicity effect and NK cells infusion is proven safe to use after autologous HSCT^[82]. The strategies of using immune cell infusion therapy have significantly increased the level of immune tolerance against allogeneic graft. New discoveries on Treg and NK cells administration posit that they appear to be effective in inducing transplant tolerance and rapid

immune reconstitution. This may help to induce a better protection of infection or cancer relapse and consequently reducing GVHD incidence.

GENETIC MARKERS

Immunologically compatible donor and recipient are determined by several genetic markers which include matching for ABO blood groups, HLA, MICA and KIRs (see preceding sections). These antigens are encoded by highly polymorphic and independent loci in our genome and are distributed differently between individuals and populations. Incompatibility between the donor and recipient for these antigens will lead to either allograft lost or GVHD. In the following sub-sections, we discuss the molecular bases for the genes encoded for the determinants of transplant compatibility.

ABO

The ABO is important blood group in transfusion and transplantation and consists of three antigens; A, B and O. These red cell antigens are determined by the ABO allelic variants (*A*, *B* and *O* alleles) on the long arm of chromosome 9. The co-dominant *A* and *B* alleles differ by four nucleotide substitutions (C526G, G703A, C796A and G803C) while the Δ 261G deletion differentiates between the recessive *O* and *A* alleles^[83-85]. The α 1,3-N-acetylgalactosaminyltransferase encoded by *A* allele and α 1,3-D-galactosyltransferase encoded by *B* alleles then convert H antigens, the products of *H* gene located on human chromosome 19 to either A or B antigens, respectively^[86]. In contrast, there is no enzymatic activity on H antigen for those bearing the *O* allele due to the Δ 261G deletion on the background of *O* allele. Thus, the A, B, O and AB phenotypes are determined by the three ABO allelic variants; *A*, *B* and *O* alleles.

HLA

The HLA class I molecules consist of a non-polymorphic β 2-microglobulin and a highly polymorphic α -chain glycoprotein encoded by the genes within MHC on the chromosome 6^[87-89]. There are three types of HLA class I molecules (*A*, *C* and *B*) with their specificities depend on the polymorphic α -chain encoded by *HLA-A*,

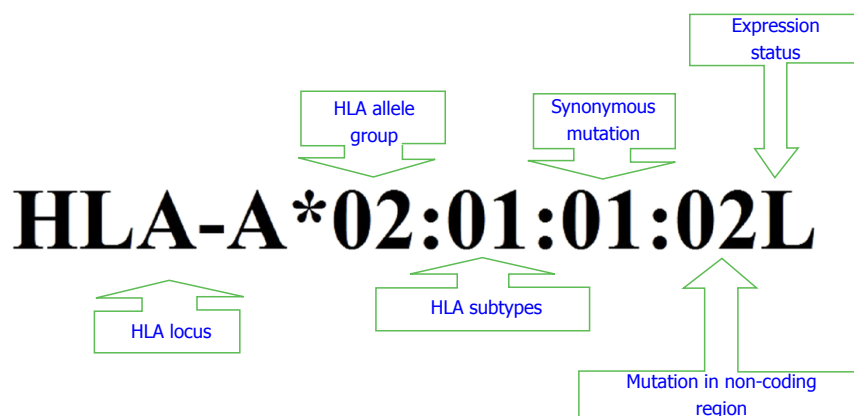


Figure 3 Systematic human leukocyte antigen nomenclature developed by the World Health Organization Nomenclature Committee for Factors of the human leukocyte antigen system. HLA: Human leukocyte antigen.

-B and -C genes in the classical class I sub-region of MHC^[90]. In contrast, both α - and β -chains of class II HLA molecules (DP, DQ and DR) are encoded by genes in the classical class II sub-region of MHC^[12] (Figure 2). The *HLA* class I and II gene clusters within MHC are separated by the class III sub-region which codes for complement components and not part of endogenous and exogenous peptide presentation to CD8⁺ and CD4⁺ cells, respectively^[91-93] (Figure 1).

The World Health Organization has developed an alphanumeric nomenclature to name *HLA* antigens, genes and alleles (Figure 3). This systematic alphanumeric nomenclature begins with letters representing specific *HLA* gene and followed by an asterisk and two sets of digits specific for *HLA* allele group and glycoprotein. Two additional sets of digits are then used to specify synonymous nucleotide changes and mutation outside the non-coding region, respectively. Suffixes (e.g., L: low cell surface expression, N: Null, C: Allele is expressed in cytoplasm but not on the cell surface and A: Aberrant expression) may be added to the end of this string of numbering system to indicate expression status of particular *HLA* alleles^[12,94].

MICA

The MICA molecules are stress induced antigens encoded by a gene within MHC region (Figure 2) and are expressed by a wide range of cells including monocytes, keratinocytes and fibroblasts^[14,87,95-97]. Unlike HLA class I molecule, MICA is not linked to β_2 -microglobulin and NK cells and CD8⁺ T ($\alpha\beta$ and $\gamma\delta$) cells reactivity are stimulated through interaction of MICA and its ligand, the NKG2D receptor^[13-15,98]. Variants of *MICA* gene are largely due to single nucleotide polymorphism and repeated units of alanine (i.e., 4 to 10 Ala residues) in exons 2, 3 and 4 and exon 5, respectively^[99-102] (see González-Galarza *et al.*^[100] for the list of populations characterized for MICA). The diversity within *MICA* gene reflect its role in immunity and as a marker of tissue identity^[96,97].

KIR

The NK cells recognize healthy and unhealthy cells through either their lectin-like or immunoglobulin-like receptors encoded by NK and leukocyte receptor complexes located on human chromosome 12 and 19, respectively^[103,104]. The leukocyte receptor complex also code for KIRs, one of the highly polymorphic transmembrane glycoprotein receptors expressed by NK cells^[105,106]. Currently there are 16 *KIR* genes and more than 570 genotypes (combinations of haplotype A and B *KIR* genes - Table 2) and 600 alleles were documented in public databases^[99,100].

Each KIR is classified according to the number of their extracellular immunoglobulin (two and three domains and assigned as 2D and 3D, respectively) and the length of cytoplasmic (short and long and assigned as S and L, respectively) domains, respectively^[107]. The KIRs with short and long cytoplasmic domains are activating and inhibitory receptors and transduce their signals through DAP-12 and tyrosine-based motifs, respectively. The only exception is for KIR2DL4 which transmits both, inhibitory and stimulatory signals^[99]. The highly diverse and complex of KIRs were also reported for their ligands, the HLA class I molecules (Table 1) and both have significant influences in transplantation and pathogenesis of various diseases^[108].

COMPATIBILITY TESTING BETWEEN DONOR AND RECIPIENT

Typing of ABO and HLA, antibody screening and cross matching are three important procedures in determining the compatibility between donors and recipients. These procedures have been largely conducted using serological approaches (e.g., complement dependent cytotoxicity test, ELISA, Luminex and flow cytometric assays; see Howell *et al.*^[8] for details). Alloantibodies against the transplanted organs/cells are usually developed in highly transfused patients or due to previous transplantation and pregnancy. These are the three main

Table 2 Here are the examples of both, gene content and allelic variations of the genes code for killer cell immunoglobulin-like receptors

KIR gene	KIR haplotype					
	A	A	A	B	B	B
¹ KIR3DL1	*015	*086	*005	*007	*086	X
¹ KIR2DL1	*003	*003	*003	*010	*004	X
¹ KIR2DL3	*001	*001	*001	X	X	X
¹ KIR2DS4	*001	*001	*010	*003	*001	X
² KIR2DL2	X	X	X	*003	*001	*001
² KIR2DL5	X	X	X	*B002	*B002	A*001
² KIR3DS1	X	X	X	X	X	*013
² KIR2DS1	X	X	X	X	X	*002
² KIR2DS2	X	X	X	*001	*001	X
² KIR2DS3	X	X	X	*001	*003	X
² KIR2DS5	X	X	X	X	X	*001
³ KIR2DL4	*001	*028	*011	*006	*028	*005
³ KIR3DL2	*002	*002	*010	*002	*002	*007
³ KIR3DL3	*013	*002	*009	*014	*013	*003
¹ KIR2DP1	*009	*001	*001	*004	*007	*007
³ KIR3DP1	*001	*001	*003	*001	*003	*003

^{1,2,3}The haplotype A and B and framework KIR genes, respectively. The X indicates the absent of KIR genes/alleles.

events where individuals might be exposed to non-self antigens including the clinically important transplant antigens such as ABO antigens, HLA and MICA. Thus, antibody screening and cross matching are crucial to avoid allograft lost. Nowadays, molecular typing techniques such as those using sequence specific oligonucleotide primer, and Sanger sequencing have largely been used for genotyping of ABO, HLA and MICA and KIR genes. These molecular techniques have several advantages as they are not dependent on the availability of anti-sera, cellular expression and have greater specificity and accuracy as compared with the antibody-antigen based typing (recently reviewed by Howell *et al.*^[8], Dunn^[109] and Edinur *et al.*^[110]).

FUTURE DEVELOPMENTS AND CONCLUDING REMARKS

Advances in the field of molecular biology and genetics have contributed immense benefits to the medical field including in transplantation medicine. A number of molecular techniques have been developed following the elucidation of molecular bases of the genes encoding for transplant determinants. Currently, several different genotyping platforms can be used to screen blood group, HLA, MICA, and KIR loci (see Howell *et al.*^[8], Dunn^[109], Edinur *et al.*^[110] and Finning *et al.*^[111]). It is now possible to genotype multiple markers and to the extent of complete sequencing of human genome using the next generation sequencer (NGS). This high throughput genotyping platform has been tested for HLA (e.g., see Bentley *et al.*^[112], Holcomb *et al.*^[113], Wang *et al.*^[114] and Skibola *et al.*^[115]) and it is expected that NGS will be used to simultaneously genotype large number of clinically relevant transplantation genes in near

future. This is not far from reality due to bioinformatics support given by the immunogenetics community and the rigorous improvement in NGS methodology (see Robinson *et al.*^[94] and Grada *et al.*^[116]). In addition, new developments in immune tolerance based therapy, donor recruitment strategies and bioengineering (tissue engineering and regenerative medicine) will provide significant advances in the field of transplantation medicine. This paper provides only brief discussions of these new developments, while others^[20,46,110,117,118] have conducted systematic reviews of them.

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Hemodynamic monitoring in heart failure and pulmonary hypertension: From analog tracings to the digital age

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Abstract

Hemodynamic monitoring has long formed the corners-

tone of heart failure (HF) and pulmonary hypertension diagnosis and management. We review the long history of invasive hemodynamic monitors initially using pulmonary artery (PA) pressure catheters in the hospital setting, to evaluating the utility of a number of implantable devices that can allow for ambulatory determination of intracardiac pressures. Although the use of indwelling PA catheters has fallen out of favor in a number of settings, implantable devices have afforded clinicians an opportunity for objective determination of a patient's volume status and pulmonary pressures. Some devices, such as the CardioMEMS and thoracic impedance monitors present as part of implantable cardiac defibrillators, are supported by a body of evidence which show the potential to reduce HF related morbidity and have received regulatory approval, whereas other devices have failed to show benefit and, in some cases, harm. Clearly these devices can convey a considerable amount of information and clinicians should start to familiarize themselves with their use and expect further development and refinement in the future.

Key words: Hemodynamic monitoring; Right heart catheterization; Pulmonary hypertension; Heart failure; Left ventricular assist device; Transplant; Outcomes

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Core tip: Hemodynamic monitoring forms the cornerstone of heart failure (HF) and pulmonary hypertension diagnosis and management. We review invasive hemodynamic monitors including a number of implantable devices that can allow for ambulatory determination of a variety of intracardiac pressures. These implantable devices have afforded clinicians an opportunity for objective determination of a patient's volume status and pulmonary pressures. Devices such as the CardioMEMS and thoracic impedance monitors are supported by a body of evidence that show the potential to reduce HF related morbidity. Clinicians

should start to familiarize themselves with their use and expect further development and refinement in the future.

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INTRODUCTION

Heart failure (HF) is an increasingly prevalent disease affecting, by some estimates, over 23 million people worldwide^[1]. It is a clinical syndrome characterized by the inability of the heart to adequately provide effective net forward blood flow, either due to left ventricular systolic dysfunction (heart failure with reduced ejection fraction or HFrEF), as a result of left ventricular diastolic dysfunction and/or valvular disease (heart failure with preserved ejection fraction or HFpEF), or due to right sided HF related to pulmonary arterial hypertension (PAH) or primary right ventricular (RV) dysfunction. This may result in both acute and chronic volume overloaded states, poor end organ perfusion and significant morbidity and mortality. In the United States, HF is the most common cause for hospitalizations in those over age 65 with over 1 million admissions per year^[2]. Despite improvements in contemporary medical therapy for HFrEF, long term morbidity and mortality remain unacceptable and 30-d rehospitalization rates remain roughly 23%^[3]. For HFpEF patients, there is still no disease modifying therapy which has been shown to improve survival in randomized clinical trials^[4].

PAH is a far rarer condition affecting perhaps 52 out of one million in the population at any given time^[5]. However, it is a progressive and insidious disease characterized by remodeling of the pulmonary arterial tree, associated with endothelial dysfunction, vascular smooth muscle hypertrophy, and vasoconstriction^[6]. The gradual rise in RV afterload leads to compensatory RV hypertrophy and dilatation, but if left untreated, culminates in RV dysfunction, fall in cardiac output and clinical symptoms^[7].

The right heart catheter (RHC) has long been considered the gold standard for the diagnosis of PAH and also for monitoring disease progression. It has also been shown to be effective in determining the etiology of patients in shock, and hemodynamic parameters obtained from RHC have prognostic utility in HF patients. Moreover, in selected patients with advanced HF, there may be a role for hemodynamically tailored HF therapy with use of an indwelling Swan-Ganz catheter in the intensive care unit, though this approach has not been associated with superior survival^[8,9].

Standard RHC does, however, have significant limi-

tations and over the past two decades, a number of newer implantable hemodynamic monitors (IHMs) have been developed for use in HF patients. The increasing adoption of IHM in HF and PAH patients may afford new opportunities for improving clinical outcomes in these disease states and thus forms the subject of this review.

HISTORY AND LIMITATIONS OF THE RHC

The RHC was first developed by Forssmann *et al*^[10] in 1929 after experimenting on himself to find a way to both measure intra-cardiac pressures and deliver therapies^[10]. After further pulmonary artery (PA) catheter development and refinement by Drs. Swan and Ganz in 1970, it gained widespread use in the management of advanced HF and shock despite relatively limited evidence regarding its efficacy in reducing deleterious clinical outcomes.

Though it is an invasive procedure, RHC has since become recognized as safe with a relatively low rate of complications, especially when performed in referral centers^[11]. However RHC has a variety of limitations, many of which are inherent to the RHC procedure and its associated technology.

In general, at most centers RHC is performed in the supine position at rest, and the catheter does not lend itself well to either ambulatory or frequent measurements outside of an inpatient setting. Indeed, even in-hospital readings must be taken in a meticulous fashion to avoid the issues inherent to the procedure such as respiratory variation in pressures and inappropriate pressure transducer placement and zeroing.

In an effort to limit variation and standardize measurements from a PA catheter, many centers take readings at end-expiration and with the patient supine which, while allowing for reproducibility, is likely not an entirely accurate physiologic assessment of the patient's hemodynamics during their day to day activities^[12].

The Swan-Ganz catheter, in part due to its perceived safety, was widely adopted in a number of clinical scenarios and as a result, a number of significant associated adverse events were reported^[8]. Therefore, the ESCAPE trial was undertaken to assess the value of PA catheterization in HF patients. Published in 2005, ESCAPE showed that the routine use of PA catheterization for patients admitted with HF was not associated with a significantly decreased length of stay, due in part to an increased infection risk; however, its applicability to disease states such as overt cardiogenic shock has not been shown^[9] and such patients were, in large part, excluded from ESCAPE.

IHM AND HF MANAGEMENT

Although the indwelling PA catheter has fallen out of favor with clinicians for uncomplicated HF, the overall

goal of accurate and reproducible hemodynamic monitoring to assess volume status, filling pressures and cardiac output remains very valuable in preventing adverse events in this group, including hospital readmissions. As a part of the Affordable Care Act in the United States, the Centers for Medicare and Medicaid Services has identified HF as a disease state warranting readmission measures and the assessment of penalties are to begin in 2016 for readmission rates deemed to be in excess of the national average^[13].

With a view to managing volume in the ambulatory setting, a number of different IHMs have been developed. Perhaps the most frequently used at present are those devices that measure thoracic impedance *via* the RV lead on an implanted cardiac defibrillation or cardiac resynchronization device. Specifically, these devices attempt to gauge the degree of pulmonary congestion by measuring the resistance to flow of a current passed across the lung. Since tissue will conduct current more readily with increasing amounts of fluid, impedance will drop as a patient's volume status expands. In clinical practice, this is usually reported by the device using an algorithm that indexes these values and can signal the clinician of an abnormal trend upon device interrogation. The FAST study showed that decrements in thoracic impedance were more closely correlated with negative HF endpoints than standard home weight monitoring^[14] but these readings have proved difficult for clinicians to incorporate in clinical practice^[15].

Other IHM devices have targeted intravascular pressures directly with a view to increasing sensitivity and applicability to clinical practice. The first of these devices used a diaphragm-tipped pressure catheter that would be passively placed in a vascular structure. In the case of the Medtronic Chronicle device, a generator was implanted subcutaneously and attached to a lead with its electrode tip placed subcutaneously in the RV by passive fixation. This allowed for remote measurement of RV systolic and diastolic pressure, imputed PA diastolic pressure, heart rate, activity, RV dp/dt, and core body temperature^[16]. The HeartPOD was a device from St Jude Medical deployed *via* a femoral venous approach and then crossing the intra-atrial septum to sit directly in the left atrium. An antenna coil could then be subcutaneously implanted in the femoral region or reflected back into a superior venous position^[17].

COMPASS-HF^[18] was a single-blinded prospective study designed to use the Chronicle device in patients with HFpEF and HFrEF and tailor medical therapy based either on standard assessments alone (control arm) or with the use of the device data. They randomized a total of 274 patients and although the primary endpoint of HF-related events, including hospitalizations and urgent clinic visits, decreased by 21% it failed to reach statistical significance. The device was not granted FDA approval and so did not reach market.

The initial study HeartPOD study^[19] showed promise but the follow-up study LAPTOP-HF was terminated

early for safety reasons due to procedural complications related to the required trans-septal puncture.

More recently, the CardioMEMS device from St Jude was studied in the CHAMPION trial^[20]. This was a multicenter, single-blind, prospective trial which enrolled 550 patients total to both arms and, similarly to previous studies, both HFpEF and HFrEF were included. As with previous studies, medical therapies in the treatment arm were guided by the use of the PA pressures provided by the device. Patients were followed for a mean of 15 mo. The primary endpoint was HF related hospitalizations and this was significantly reduced by 37% with minimal device-related adverse events (1.4%) and 100% device reliability. Follow-up data showing open-access to the PA pressures reported by the device led to a 48% readmission rate reduction in the former control group and, in patients who had repeat RHC, the mean difference in the mean PA pressure between the device and direct invasive measurement was 1 mmHg^[21].

Unlike the aforementioned devices, the CardioMEMS device is a percutaneously delivered pressure sensor that is placed in a PA branch and interrogated *via* a wireless detection system which can then be remotely reviewed by clinicians in close to real-time *via* upload to a website (Figures 1 and 2). This has the benefit to the clinician of understanding a patient's ambulatory right-sided pressures and, by extension, volume status in a format similar to RHC. In addition, this device did not have a percutaneous lead or generator that was prone to failure or infection and could last for the life of the patient. These factors and the success of the CHAMPION trial led to the approval of the device by the FDA in order to reduce hospitalizations in HF patients.

There may also be a role for IHMs in the risk stratification of patients requiring advanced HF therapies including left ventricular assist device (LVAD) implantation and transplantation. Data from the CHAMPION trial showed that treatment group progressed faster to LVAD therapy (167 d vs 266 d), had faster declines in PA pressures and ultimately, a quicker bridge to cardiac transplantation (177 d vs 370 d)^[22]. Furthermore, the CardioMEMS device may provide a way to monitor exercise responsiveness in patients with LVAD implants^[23] and could provide a novel way to measure PA pressure in those with total artificial hearts (TAH) whose PA pressures were not previously measureable due to the inherent limitations of the TAH implant.

IHMS AND PULMONARY HYPERTENSION MANAGEMENT

Although neither the Medtronic Chronicle nor the CardioMEMS device were expressly designed for the management of PAH, ongoing knowledge of a patient's PA pressures in this disease might be extremely valuable, especially if an estimate of cardiac output could be derived from the sensor to calculate total pulmonary



Figure 1 The CardioMEMS Heart Failure System Comprised of Implanted Wireless Sensor, Hospital Remote Unit and Home Remote Unit with Cushion.

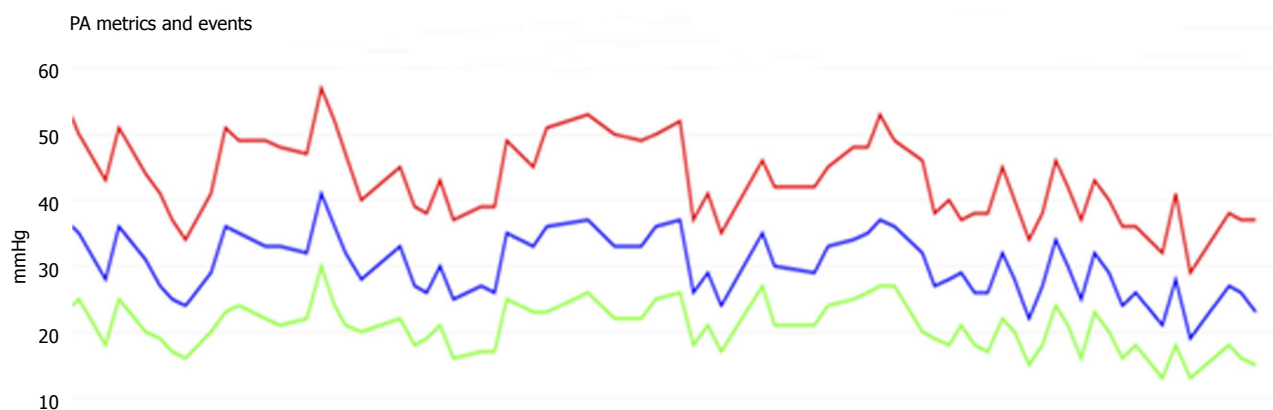


Figure 2 Sample Screenshot of CardioMEMS Website showing PA pressure trends over 90 d, systolic (red), mean (blue), and diastolic (green).

resistance. With regard to therapeutic interventions in PAH, the device could allow for guided up and down titration of therapy and thus prevent the sequelae of RV failure or high cardiac output states *via* direct measurement of these parameters. It could also give some insight into those patients with medication compliance issues. There have been several case series that have been conducted to investigate the role of these monitors in guiding PH therapies.

The Chronicle device was studied in 5 patients with PAH who were prescribed iloprost - a prostacyclin analog - *via* an inhaled, aerosolized delivery. The device clearly demonstrated a drop in RV systolic pressures in the immediate post-inhalation period and importantly showed that the duration of drug effect was much shorter than was expected^[24]. The authors postulated that patients who were at rest during the delivery of the drug may even have a more pronounced pressure lowering and indeed, further study with iloprost and IHMs showed that in fact, with exercise, there was a significantly blunted pressure lowering effect^[25]. The Chronicle device also identified 13 out of 15 PAH

patients who had a greater than 30 mm decrease in 6 min walk distance on the basis of improvement in pressure measurements^[26].

The CardioMEMS device is currently being studied in PAH as part of an NHLBI funded pilot study in PAH. This is a single center study investigating long-term pressure measurements and titration of therapy based on device interpretations. Early data has shown that instead of titrating to a pre-specified, protocolled dose of parenteral prostacyclin, IHM-guided therapy has allowed for early recognition of optimal dosing. As compared with standard therapy, this has allowed for enhanced cost savings due to lower drug dosing (in one case, approximately United States \$ 29000 was saved), minimization of prostacyclin-related side effects, and decreased risk associated with repeat RHC izations^[27].

CONCLUSION

Ambulatory hemodynamic monitoring in HF and PAH is clearly still developing but the use of these devices is being gradually expanded outside the traditional role of

fluid management in HFrEF. As we gain more experience with the current generation of devices such as the CardioMEMS IHM in clinical practice, device design will continue to evolve and already a variety of even more sophisticated sensors are under development.

As the CardioMEMs sensor continues to be evaluated in the management of PAH, IHMs hold the promise of a more precise and accurate titration of medical therapies and may also allow for determining which patients are at higher risk of adverse events, thus allowing for earlier and more aggressive interventions.

Clinicians should eagerly await and critically scrutinize data from forthcoming studies looking at expanding roles for these devices.

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Tregs and kidney: From diabetic nephropathy to renal transplantation

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Abstract

Kidney transplantation is recognised as the most effective treatment for patients with end-stage renal disease (ESRD). Kidney transplantation continues to face

several challenges including long-term graft and patient survival, and the side effects of immunosuppressive therapy. The tendency in kidney transplantation is to avoid the side effects of immunosuppressants and induce immune tolerance. Regulatory T-cells (Tregs) contribute to self-tolerance, tolerance to alloantigen and transplant tolerance, mainly by suppressing the activation and function of reactive effector T-cells. Additionally, Tregs are implicated in the pathogenesis of diabetes, which is the leading cause of ESRD, suggesting that these cells play a role both in the pathogenesis of chronic kidney disease and the induction of transplant tolerance. Several strategies to achieve immunological tolerance to grafts have been tested experimentally, and include combinations of co-stimulatory blockade pathways, T-cell depletion, *in vivo* Treg-induction and/or infusion of *ex-vivo* expanded Tregs. However, a successful regimen that induces transplant tolerance is not yet available for clinical application. This review brings together certain key studies on the role of Tregs in ESRD, diabetes and kidney transplantation, only to emphasize that many more studies are needed to elucidate the clinical significance and the therapeutic applications of Tregs.

Key words: Diabetes; Foxp3; Kidney transplantation; Regulatory T-cells

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Core tip: This review brings together certain key studies on the role of regulatory T-cells (Tregs) in end-stage renal disease, diabetes and kidney transplantation, only to emphasize that many more studies are needed to elucidate the clinical significance and the therapeutic applications of Tregs.

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INTRODUCTION

Immunological self-tolerance in the periphery is achieved by the negative regulation exerted on the immune response by a variety of cells of which the best characterized populations are the regulatory T cells (Tregs)^[1]. Tregs mediate self-tolerance and tolerance to alloantigens by suppressing the activation of effector T-cells (Teffs), and exerting anti-inflammatory activity^[2]. Of Tregs the best characterized and studied cells are the CD4⁺CD25⁺Foxp3⁺ Tregs, especially in the context of autoimmune diseases and organ transplantation^[2,3].

Kidney transplantation is considered the most effective therapy for end-stage renal disease (ESRD); however, a major unresolved challenge is to avoid the side effects of immunosuppression by inducing immune tolerance^[4]. Transplant tolerance has been defined as graft acceptance without long-term use of immunosuppressive drugs^[5]. Transplant tolerance is characterized by decreased alloreactive Teffs and increased Treg count in grafts and associated lymphoid tissues in the periphery^[4].

Diabetic nephropathy is the leading cause of ESRD^[6]. Diabetes type I is a chronic autoimmune disease^[7] and Tregs have been implicated in the pathogenesis of insulin resistance^[8]. On the other hand, in a model of murine diabetes, adoptive transfer of Tregs improved insulin resistance and diabetic nephropathy^[8], suggesting a complicated relationship between Tregs, diabetes and kidney transplantation^[8,9].

Several strategies to achieve immunological tolerance to grafts have been tested experimentally, and include combinations of co-stimulatory blockade pathways, T-cell depletion, *in vivo* Treg-induction and/or infusion of ex-vivo expanded Tregs^[5,10]. However, a successful regimen that induces transplant tolerance is not yet available for clinical application.

TREGS

Several subsets of regulatory or tolerogenic cells have been characterized or partially characterized so far.

In the 1970s, Gershon *et al.*^[11] reported that a subset of T-cells called "suppressor cells" might exhibit suppressive activity. In recent years, the term "suppressor T-cells" was replaced by the term "Tregs". In 1995, Sakaguchi *et al.*^[12] reported that a subset of CD4⁺CD25⁺ T-cells exhibit regulatory functions *in vitro* and *in vivo*. In addition, Piccirillo *et al.*^[13] observed that murine CD4⁺CD25⁺ T-cells suppress the proliferation of CD4⁺ or CD8⁺ Teffs *in vitro*^[13]. Subsequently, Dieckmann *et al.*^[14] identified a similar population of T-cells in humans. These cells play an important role in autoimmunity, allergy, inflammation, maintenance of

maternal tolerance to the foetus, infections and cancer. In 2002, Graca *et al.*^[15] reported that the presence of Tregs mediated transplant tolerance. In addition, the authors observed that Tregs in tolerant skin grafts transfer transplant tolerance to fresh skin allografts if re-transplanted into naive recipients^[15]. In 2007, Lair *et al.*^[16] reported that in a rat heart transplant model, long-term survival is achieved in rat recipients by pre-graft donor-specific blood transfusion that resulted in splenic Tregs that were not only able and sufficient to mediate graft tolerance, but were also able to transfer long-term survival to naive recipients.

Tregs include natural (n)Tregs that are generated in the thymus and inducible (iTregs) that are generated in the periphery. nTregs arise in the thymus and express the forkhead/winged helix transcription factor Foxp3 that, in turn, controls nTreg differentiation^[4]. iTregs arise in the periphery from memory and naive CD4⁺ Teffs following stimulation by self- or allo-antigens in the presence of IL-4, IL-10, TGF- β and IL-2. iTregs may or may not express the transcription factor Foxp3, and exert their suppressive activity mainly *via* the secretion of anti-inflammatory cytokines, mainly TGF- β and IL-10^[17,18]. TGF- β induces the expression of Foxp3, converting CD4⁺CD25⁺ naive Teffs to Tregs in the periphery. nTregs are antigen non-specific, while iTregs are usually antigen-specific^[17,18].

iTregs are further subdivided into Tr1 cells that mainly secrete IL-10 and Th3 cells that mainly secrete TGF- β . Both iTreg types inhibit the maturation of dendritic cells (DCs) and the activation and proliferation of both memory and naive Teffs^[18].

Regulation of Tregs

A well-studied regulator of Tregs at the molecular level is the transcription factor Foxp3, the expression of which is critical for their development and function^[19-21]. Data from animal studies have provided evidence that Foxp3 deficiency causes loss of Treg suppressive activity leading to the development of a lethal autoimmune syndrome^[5]. In accordance, adoptive transfer of CD4⁺CD25⁺Foxp3⁺ T-cells from wild-type mice can prevent the development of severe autoimmune diseases observed in Foxp3-deficient mice^[5]. In humans, Foxp3 deficiency has been associated with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome^[22-24].

Both DNA and histone protein modifications are implicated in the epigenetic regulation of Foxp3^[25]. Regarding DNA modifications, the methylation status of cytosine at cytosine-phosphate diester-guanine sites in the locus of Foxp3 influences its expression^[25].

Histone modifications entail the acetylation of lysine residues at the amino terminus of the histone tail, inducing *Foxp3* gene expression. Interestingly, these epigenetic regulators can be used to enhance the function and number of Tregs, for potential therapeutic applications^[26].

Suppressive mechanisms of Tregs

Tregs express the T-cell receptor and may suppress innate and adaptive immune responses^[4]. Tregs exert a cell-cell contact-dependent suppression, and they also exert suppressive activity mediated by cytokines, mainly IL-10 and TGF- β ^[27,28]. Tregs can block Teffs at any stage of their activation, proliferation, differentiation and effector functions^[5,28,29].

Tregs suppress the activation of antigen presenting cells (APCs) through the expression of membrane-associated inhibitory molecules such as the cytotoxic T lymphocyte antigen 4 (CTLA4) and lymphocyte activation gene-3, a CD4-related trans-membrane protein that binds HLA II on APCs (DCs in particular) and inhibits their activation and the ensuing antigen presentation^[30].

In addition, Tregs induce the apoptosis of target cells by producing several cytolytic molecules such as granzymes A and B, perforin and galectin 1^[5]. Tregs also exert suppressive activity by causing metabolic disruption of Teffs through IL-2 consumption (IL-2 is an essential growth factor for naive Teffs), suppression of cyclic adenosine monophosphate synthesis, and inhibition of the CD39-CD73 pathway^[28,31]. Specifically, CD39 hydrolyzes ATP or ADP to AMP. CD39 is a dominant ectoenzyme expressed by Tregs. Catalytic inactivation of extracellular ATP by CD39 can be considered as an additional anti-inflammatory mechanism mediated by Tregs. Co-expression of CD39 and CD73 generates pericellular adenosine. Adenosine is an inhibitor of T-cell responses and exerts its effect *via* binding to the A2A receptor^[28,31].

Wu *et al.*^[32] reported that the suppressive function of Tregs is mediated through a complex formed by the transcription factors NFAT and Foxp3, whereas in Teffs, NFAT forms a complex with the activator protein-1 (AP-1). The authors suggested that a strategy to induce tolerance is to inhibit the NFAT:AP-1 interaction by small molecules, without interfering with the NFAT:FoxP3 interaction.

The recent finding that NFAT is a common regulator for both Teffs and Tregs^[32,33], indicate that NFAT is an essential transcription factor for the functional integrity of both populations^[32,33]. Therefore, immunosuppressive drugs targeting NFAT activity in stimulated T-cells, such as calcineurin inhibitors, may also suppress the activity of Tregs.

Both nTregs and iTregs also suppress B cell activation and the ensuing antibody production^[34]. It has been reported that nTregs kill B cells directly by secreting perforin and granzyme B, whereas iTregs inhibit B-cell activation through the secretion of IL-10 and TGF- β ^[35].

Site of action of Tregs

In the setting of autoimmune diseases, Tregs are activated in the draining lymph nodes to prevent priming and clonal expansion of autoreactive Teffs; they then migrate to the inflamed tissues, exerting their suppressive activity in the periphery^[36].

In the setting of transplantation, Treg migration to the graft is required to prevent graft rejection. Early trafficking of Tregs to the graft prevents the exit of donor-derived DCs to the drained lymph nodes, decreasing thus the extent of alloimmune priming^[10].

TREGS AND DIABETIC NEPHROPATHY

Diabetes is one of the major causes of ESRD^[6]. Type 1 diabetes (T1D) has been described as a chronic autoimmune disease due to T-cell mediated destruction of pancreatic β -islets leading to insulin deficiency^[7]. Data from experimental studies indicate that Treg cells are involved in the pathogenesis of T1D^[37-39].

It is not clear whether the peripheral blood count of CD4⁺CD25⁺ Foxp3 Tregs is altered in T1D patients^[40]. Jailwala *et al.*^[41] reported that the frequency of Tregs in T1D patients is not altered but that these cells have an increased sensitivity to apoptosis. Studies in non-obese diabetic (NOD) mice showed that depletion of CD4⁺CD25⁺ T-cells, leads to T1D development^[42]; in addition, abolishment of the CD28 and ICOS co-stimulatory pathways, that are critical for Treg homeostasis and function, exacerbate T1D^[43]. Also in NOD mice, T1D progression is linked with a reduction in Treg number and suppressive activity in the inflamed pancreatic islets, together with a diminished IL-2 production by Teffs. In addition, Tregs may lose Foxp3 expression with concomitant loss of their suppressive activity during T1D progression^[37].

Although type 2 diabetes is considered to be a metabolic disorder with no autoimmune etiology, recently an adiposity-associated chronic inflammation process mediated by immune mediators has been proposed as an underlying mechanism of this disease^[44-46]. Interactions between metabolic disorders, hemodynamic changes, oxidative stress, inflammation and genetic predisposition, seem to contribute to the pathogenesis of diabetes and diabetic nephropathy. Interestingly, an increased expression of CD4⁺CD25⁺Foxp3 cells has been revealed in type 2 diabetic patients with micro and macroalbuminuria^[47,48] suggesting a potential link between Tregs and disease progression. However, the relationship between CD4⁺CD25⁺Foxp3 Tregs and type-2 diabetic nephropathy is not well studied. In the db/db mouse with type 2 diabetes, CD4⁺CD25⁺Foxp3 Treg depletion with anti-CD25 monoclonal antibody, enhanced insulin resistance, albuminuria and glomerular hyperfiltration^[8]. Adoptive transfer of CD4⁺CD25⁺Foxp3 Tregs increased FoxP3 mRNA synthesis in the recipients and improved insulin sensitivity and type 2 diabetic nephropathy^[8].

TREGS AND KIDNEY TRANSPLANTATION

Tregs in transplantation tolerance and acute rejection

A large body of evidence supports the notion that CD4⁺CD25⁺Foxp3⁺ Tregs play a fundamental role in the establishment and maintenance of operational tolerance

Table 1 Regulatory cells in humans

Cell	Phenotype	Properties	Ref.
T-cells (Treg)	CD4 ⁺ CD25 ⁺	Secrete mainly IL-10	[1-4,17,77-79]
	CD4 ⁺ CD25 ⁺ FoxP3 ⁺	and TGF-β; some	
	CD4 ⁺ CD25 ⁺ CD127 ^{low}	secrete IL-35 or	
	CD4 ⁺ CD45RO ⁺	IFN-γ	
	CD8 ⁺	Secrete mainly	[80]
	CD28 ⁺	IL-10 but also	
	CD8 ⁺ CD28 ⁺ (FoxP3 ⁺)	TGF-β, IFN-γ, CCL4; downregulate APC or DC maturation; direct killing of CD4 ⁺ Tregs and APCs	
	CTLA-4	Mainly inhibition of Tregs	[81]
	CD4 ⁺ CD8 ⁺ TCRαβ ⁺	Suppress antigen- specific T-cells; secrete mainly IFN-γ but also IL-4	[82]
	TCRγδ ⁺	Secrete IL-10, TGF-β, IL-4	[83]
T-cells or monocytes	HLA-G	Secrete IL-10, IL-35, TGF-β, soluble HLA-G	[84,85]
iNKT	CD3 ⁺ CD16 ⁺ CD56 ⁺	Can secrete IFN-γ ± IL-4 ± IL-10 ± TGF-γ, direct killing of target cells	[86]
B-cells (Breg)	CD19/20 ⁺ , CD80/86 ⁺ , CD40 ⁺ , TLR4 ⁺ , mainly IgG and IgA BCR	Secrete IL-10 and IL-35, induce Tregs, downregulate DC maturation	[87]
tDC	PD-L1/L2 ⁺ , FasL ⁺	Secrete IL-10 and TGF-β; downregulate Teff activation	[88]

APC: Antigen presenting cell; DC: Dendritic cell; BCR: B-cell receptor; tDC: Tolerogenic dendritic cells; iNKT: Natural killer T regulatory cells; TGF: Transforming growth factor; IL: Interleukin; IFN: Interferon; HLA-G: Human leukocyte antigen-G; CTLA-4: Cytotoxic T lymphocyte-associated antigen 4.

to renal allografts^[15,49].

In animal models of transplantation, Tregs were present in tolerant allografts and were shown to migrate to the allograft tissue^[15,50]. It was also shown that Tregs, induced *in vitro*, *in vivo* or expanded *ex vivo* after alloantigen stimulation, promoted transplant tolerance to the allograft^[16,51-54].

Salama *et al.*^[55] were the first to demonstrate the existence of antigen-specific Tregs capable of suppressing alloresponses to donor HLA peptides in human kidney transplant recipients. In accordance, data from renal liver and lung transplantation in humans showed a high number of circulating and intra-graft Tregs in tolerant stable recipients^[56-59]. On the other hand, recruitment of Tregs into the graft, as part of an allogeneic inflammatory response, suggests a role for Tregs in immune-mediated graft injury^[60].

Reports on the clinical and prognostic significance of Foxp3⁺ cell infiltrates in renal allograft recipients with acute rejection are contradictory^[61]. Muthukumar

et al.^[62] reported that renal transplant patients with an acute rejection episode expressed high levels of Foxp3 mRNA in the urine, and that the lower levels of Foxp3 were associated with a poorer response to anti-rejection therapy, postulating that this could be a future non-invasive marker for the level of renal graft function. Bunnag *et al.*^[63] reported that Foxp3 expression in human kidney biopsies was linked to rejection and did not correlate with a favourable outcome. In accordance, data from studies that used Foxp3 analysis from graft biopsy cores, have demonstrated a higher Foxp3 expression in the allografts with acute rejection in comparison with stable renal allografts or with those displaying antibody-mediated rejection^[64,65]. It should be emphasized that these studies did not report any potential benefit of Foxp3-enriched infiltrate on renal allografts outcome, or even associated the level of *in situ* Foxp3 expression with tubulitis, higher scarring scores and worse prognosis of renal allografts survival^[61]. Contradictory, in the context of lower graft inflammation such as borderline changes and subclinical episodes of acute rejection, it seems that Treg-enriched graft infiltrate has a protective role in interstitial inflammation and graft function^[66-68]. Data from protocol biopsies in recipients with episodes of subclinical cellular rejection, reported a correlation of low Foxp3/CD3 ratio with a poor graft function up to five years post-transplantation^[67,68].

Tregs in chronic allograft nephropathy

The number of CD4⁺CD25⁺Foxp3⁺ Tregs usually decreases after transplantation. Renal transplant recipients with chronic rejection have a lower number of peripheral CD4⁺CD25⁺Foxp3⁺ Tregs compared to those with stable renal graft function^[69,70]. In accordance, Al-Wedaie *et al.*^[71] reported a decreased count of CD4⁺CD25⁺ Tregs in the blood of renal allograft recipients with chronic rejection.

A decreased synthesis of Foxp3 mRNA in renal recipients with chronic rejection has been reported in comparison to stable or operationally tolerant renal allograft recipients or healthy controls^[69,70]. On the other hand, an increased frequency of infiltrating Foxp3⁺ T-cells in renal grafts with chronic rejection and poor graft function has been reported^[57,72]. It can be hypothesized that higher numbers of Tregs reflect an effort to suppress the immune response at the site of inflammation.

Interestingly, Ashton-Chess *et al.*^[73] reported that the expression of Foxp3 both in blood and renal graft did not distinguish rejecting from non-rejecting renal recipients. The authors suggested that Foxp3 expression does not correlate with rejection but it depends on the time post-transplantation and the age of the patients.

An important issue that needs to be addressed is whether Tregs in renal allograft recipients have a normal suppressive capacity. Data from several studies on the development of chronic rejection have shown a quantitative defect of Tregs whereas data from other studies a functional deficit of Tregs^[61,74]. Given that

immunosuppressive drugs can have detrimental effects on the number^[74], induction, function and survival of Tregs, the answer to this question is difficult because all the renal allograft recipients enrolled in these studies were on double or triple immunosuppressive regimens. Thus it could be assumed that the decreased number of Tregs or their functional deficit reported in recipients with chronic rejection was partially due to the effect of immunosuppression.

In addition, Tregs may contribute to chronic allograft nephropathy through new onset post-transplant diabetes, hypertension^[75] and hyperlipidemia^[76], but these hypotheses need to be explored in experimental models and in the clinic.

CONCLUSION

Regarding the entire spectrum of studies on chronic kidney disease and renal transplantation, Tregs are clearly implicated both in the pathogenesis of diabetic nephropathy and in the induction of transplant tolerance. Nevertheless, up to date, a relatively small number of clinical and experimental studies have explored the mechanism of Treg involvement in diabetic nephropathy. In addition, although a large body of evidence implicates Tregs in the immune mechanisms of acute and chronic rejection, their exact role remains unclear. The therapeutic potential of Tregs in kidney transplantation is promising but challenging for human patients. More studies are needed to elucidate the clinical significance and the therapeutic applications of Tregs and, also, of all the emerging types of regulatory and tolerogenic cells (Table 1) in kidney diseases and transplantation.

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Current techniques for ABO-incompatible living donor liver transplantation

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Abstract

For a long time, it was considered medical malpractice to neglect the blood group system during transplantation. Because there are far more patients waiting for organs than organs available, a variety of attempts have been made to transplant ABO-incompatible (ABOi) grafts. Improvements in ABOi graft survival rates have been achieved with immunosuppression regimens and plasma treatment procedures. Nevertheless, some grafts are rejected early after ABOi living donor liver transplantation (LDLT) due to antibody mediated rejection or later biliary complications that affect the quality of life. Therefore, the ABOi LDLT is an option only for emergency situations, and it requires careful planning. This review compares the treatment possibilities and their effect on the patients' graft outcome from 2010 to the present. We compared 11 transplant center regimens and their outcomes. The best improvement, next to plasma treatment procedures, has been reached with the prophylactic use of rituximab more than one week before ABOi LDLT. Unfortunately, no standardized treatment protocols are available. Each center treats its patients with its own scheme. Nevertheless, the transplant results are homogeneous. Due to refined treatment strategies, ABOi LDLT is a feasible option today and almost free of severe complications.

Key words: Living-donor liver transplantation; ABO-incompatible; Rituximab; Desensitization; Iso-titer; Biliary complications

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Core tip: Due to refined treatment strategies, ABO-incompatible living donor liver transplantation (ABOi LDLT) is a feasible option today and almost free from severe complications, but biliary complications still affect the quality of life after ABOi LDLT. Until now, the best improvement could be reached with the prophylactic

use of rituximab more than one week before AB0i LDLT.

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INTRODUCTION

Blood group antigens are expressed in almost every cell in the body, and an individual develops antibodies against blood group antigens (anti-A/B antibodies) absent in his or her own tissue. Grafts expressing foreign A/B antigens are usually hyperacutely rejected^[1]. For a long time, it was considered medical malpractice to neglect the blood group system during cadaveric transplantation. Because there are far more patients waiting for organs than organs available, a variety of attempts have been made to transplant AB0-incompatible (AB0i) grafts. Most AB0i liver transplantations (AB0i LTs) have had a lower graft survival rate due to hepatic arterial thrombosis, various biliary complications or acute rejection episodes^[2-4]. In those rejection episodes, the graft was damaged by necrosis or disseminated intravascular coagulopathy^[4,5]. This susceptibility to rejection can be explained sufficiently by blood group antigens that are expressed on the vascular endothelium and in large bile ducts for up to 150 d after transplantation^[6-10].

Young children with an incompletely developed immune system seem to be an exception. In 1979, Starzl's group reported eleven human AB0i LTs without evidence of acute rejection after transplantation^[11].

Because AB0i LTs need a certain amount of prearrangement, we focus in this review on AB0i living donor liver transplantation (LDLT), which is conducted electively, and neglect cadaveric AB0i LT.

In Western Europe and the United States, few case reports of AB0i LDLT exist, even though new techniques are available to overcome the blood group barrier^[6,12-17]. In Asia, Japan and South Korea, elective AB0i LDLT is performed with excellent results. Due to religious beliefs, fewer organs of deceased individuals are donated, and AB0i LDLT has become well established^[18,19]. Patients demonstrate survival with an AB0i graft for nearly as long as patients with an AB0-compatible (AB0c) graft^[18-21]. Improvements in AB0i graft survival rates have been achieved with immunosuppression and plasma treatment procedures (PTPs). The antibody titer (iso-titer) level cannot explain all clinical findings. However, hyperacute or acute antibody-mediated rejection (AMR) is closely related to hepatic necrosis or intrahepatic biliary complications^[22]. Additionally, patients with a history of immunizations are at higher risk for AMR. Blood group incompatibility, recipient age, etiology of liver disease and transplant era were found

to be significant predictors of overall survival, too^[23].

Various treatment protocols have been used for iso-titer elimination in AB0i LDLT patients. They originate from AB0i kidney transplantation protocols and do not follow a common standard. The iso-titer itself has also not been standardized. The results as well as its interpretation depend on the examining laboratory. Therefore, this review compares several treatment possibilities and their effect on graft outcome from 2010 to the present.

INDICATIONS FOR AB0i LDLT WITH SPECIAL REFLEXIONS

Pediatrics

The younger the child, the fewer iso-titers have been developed. In the first month of life, children are able to tolerate an AB0i graft very well. Preformed antibodies are absent, and the immune system is highly tolerant^[24].

Gurevich *et al.*^[25] examined 58 pediatric patients undergoing AB0i LDLT with a preoperative iso-titer of < 1:16. No graft rejection or death occurred and 93% survived beyond the first 10 years. Patients with biliary atresia had fewer rejection episodes in situations where the graft was donated by the mother (mother:father vs 40%:55%)^[25-27]. Most data in children have been collected in Asia^[25,28]. Okada *et al.*^[29] described rituximab to be successful in pediatric AB0i LDLT. Kasahara *et al.*^[23] analyzed 2224 pediatric transplantations, the largest cohort worldwide. They found 1-, 5-, 10- and 20-year patient survival rates of 88.3%, 85.4%, 82.8% and 79.6% in the 294 patients undergoing AB0i LDLT.

Acute liver failure

In Europe and the United States, emergency AB0i LDLT is conducted only if no compatible donor can be acquired in time^[8,30]. In Asia, this concept is more common. Shen *et al.*^[31] for example, reported 3-year patient survival rates in AB0c vs AB0i LDLT of 83.1% vs 86%. The graft survival was 80% vs 86%. Two AB0i patients developed AMR, but no other patients had cellular rejection, biliary complications or infections. A model of end stage liver diseases (MELD) score > 30 put patients at high risk for mortality. For this reason, in the Asian Medical Center, the largest LDLT center in the world, Lee *et al.*^[18] excluded high-urgency patients from AB0i LDLT. Shinoda *et al.*^[32] in contrast, found no difference between AB0c and AB0i LDLT.

Hepatocellular carcinoma

Living donation provides an alternative curative treatment option for patients with hepatocellular carcinoma (HCC) in cirrhosis if no offers for deceased donor organs exist. This can be due to low laboratory MELD scores or if the tumor burden is beyond the Milan criteria. There are only a few reports of successful AB0i LDLT in patients with HCC outside Milan^[33]. After Lee *et*

Table 1 Research regarding ABO-incompatible living donor liver transplantation published since 2010

Ref.	Pat No.	Splenectomy local graft infusion	Rituximab	IVIG	PTP Target iso-titer	IS	AMR
Lee <i>et al</i> ^[59]	15	-/-	-14 d 300 mg/m ²	+1, +4 d 0.8 g/kg bw	First -7 d 1:8	Triple	No
Shen <i>et al</i> ^[31]	35	n.s.	Z 375 mg/m ²	Z 0.4 g/kg bw	Rescue	Quadruple	2
Lee <i>et al</i> ^[59]	15	-/-	-14 d, 300 mg/m ² Z, +4 d, 200 mg/m ²	No	TPE < 1:8	Triple	No
Kim <i>et al</i> ^[36]	14	-/-	-7 d 375 mg/m ²	+1, +3, +5 d 0.6 g/kg bw	TPE 1:32	-3 d MMF 1.5 g triple	No
Song <i>et al</i> ^[52]	10	-/+	-14 d, 375 mg/m ²	No	TPE 1:32	Triple with Cyc	No
Kim <i>et al</i> ^[20]	22	-/-	-14 d, 375 mg/m ²	No	PP 1:32	PGE1 Triple	No
Lee <i>et al</i> ^[34]	20	-/-	-15 d 300 mg/m ²	+1, +4 d 0.8 g/kg bw	TPE < 1.16	Quadruple	No
Song <i>et al</i> ^[66]	20	-/+	-21, -14 d 300, 375 mg/m ²	No	TPE 1:8	Triple with Cyc	No
Song <i>et al</i> ^[66]	21-127	-/-	-21, -14 d 300, 375 mg/m ²	No	TPE 1:8	Triple	No
Song <i>et al</i> ^[66]	128-235	+/-	-21, -14 d 300, 375 mg/m ²	No	TPE 1:8	Triple	17
Yasuda <i>et al</i> ^[67]	5	+/-	-15, -3 d 500 mg/m ²	No	TPE n.s.	Triple	4
Lee <i>et al</i> ^[35]	19	-/-	-10 d 300-375 mg/m ²	No	TPE 1:32	-7 d Tac 0.1 mg/kg, quadruple	No
Lee <i>et al</i> ^[68] (Initial iso-titer < 1:64)	20	-/-	+1 d, 375 mg/m ²	No	< 1:64	Quadruple	No
Lee <i>et al</i> ^[68] (Initial iso-titer > 1:64)	26	-/-	-21 d, 375 mg/m ² +1 d, 187 mg/m ²	No	TPE/PP < 1:64	Quadruple	No

Quadruple: Tacrolimus, mycophenolate mofetil, basiliximab, steroids; Triple: Tacrolimus, mycophenolate mofetil, steroids; TPE: Therapeutic plasma exchange; IS: Immunosuppression: 5 d before transplantation - 5 d, 5 d after TX - +5 d, day of TX - Z; Cyc: Cyclophosphamide; PP: Plasmapheresis, not otherwise specified; PGE1: Prostaglandin E1, gabexate mesilate; Tac: Tacrolimus.

a^[34] experienced a recurrence of 57% in the first year after ABOi LDLT, they recommended refraining from transplanting HCC patients^[34].

Peter and Werny investigated a distinctly higher anti-A/B titer in patients with severe emaciating diseases compared to healthy blood donors^[30]. HCC patients seem to have very high anti-A/B titers and a strong rebound. This increase could relate from altered expression of blood group antigens on the biliary tree in pathological conditions^[23]. Neexpression or aberrant expression of A or B substances in malignant cells possibly boost the production of antibodies^[24]. In this situation, the tumor bulk might define the antibody titer and rebound.

Hepatitis B/C

Lee *et al*^[34] described ABOi LDLT in 20 patients. The etiology of liver diseases consisted mostly of HBV infections ($n = 15$) and one hepatitis C virus (HCV) infection. To prevent hepatitis C virus (HBV) recurrence, Lee *et al*^[34] used entecavir or tenofovir with a high dose of intravenous (IV) HB-hyperimmune globulin. If HCV was confirmed by a liver biopsy or an abnormal liver function test with elevated HCV RNA loads, PEGylated-interferon and ribavirin were administered. Other authors describe ABOi LDLT in patients with HBV or HCV cirrhosis and in patients with HCC, as well. Unfortunately, they provide

no information about their hepatitis therapy or antibiosis (Table 1)^[20,35,36]. No data are available on ABOi LDLT in HCV patients with the new antivirals.

TREATMENT STRATEGIES TO OVERCOME BLOOD GROUP BARRIER

ABOi LDLT requires careful planning and logistical preparation prior to surgery. As treatment regimens vary distinctly, we would like to present them in the following way. All regimens have the focus on antibody reduction in common. To reach this goal and to prevent antibody rebound as well, therapeutic apheresis is combined with immunosuppressive therapy. A good overview is given in a South Korean treatment schedule: Prior to transplantation rituximab and plasma exchange is started. When the anti-A/B titer has decreased to at least a titer of 1:8, transplantation takes place without local infusion or splenectomy. Afterward, immunoglobulins and quadruple immunosuppression are administered.

Anti-A/B iso-titer

As Warner *et al*^[37] summarized, "The durable survival of ABOi solid organ allografts seems to be primarily dependent on 3 conditions: (1) the low expression of antigen on the graft, as in case of A2 positive organs; (2)

a low titer of anti-donor ABO antibodies in the recipient before transplantation; and (3) the ability to maintain low titers of antidonor ABO antibodies in the recipients after transplantation, at least for the first 3 to 6 week^[37]. In the setting of ABOi LDLT, iso-titers naturally rise during the first two days after transplantation^[38]. In addition to the natural rebound, de novo alloantibodies have the potential to develop. This alloimmune reaction induces a higher rebound and can lead to AMR, putting the graft at risk. This makes the first two weeks, or even four to six weeks, after ABOi LDLT critical for AMR^[39].

After this period, the graft has been mostly adapted to its new environment. This state is called accommodation.

Furthermore, the target titer for IgG and IgM in ABOi LDLT varies from center to center. Some centers estimate 1:8 to be appropriate, others 1:16^[39]. However, a titer of 1:64 or above should be avoided due to an increased risk of complications during transplantation and AMR^[30,40]. In the studies we compared in Table 1, titers of 1:64 or above were not accepted and lead to further PTPs (Table 1).

Therapeutic apheresis

Therapeutic apheresis is the most effective way to control the humoral antibody response to prevent rejection^[41]. There are a variety of PTPs, which differ mainly in their selectivity toward immunoglobulin elimination.

Therapeutic plasma exchange: Therapeutic plasma exchange (TPE) is a widely accepted nonselective PTP to eliminate antibodies in patients with solid-organ transplants which are sensitive to HLA antigens or undergo ABOi transplantation. Still, no controlled studies of TPE in ABOi LDLT or therapy standards have been published. With TPE, usually 1.2 times (1.0-1.5) the patient's plasma volume is treated. The amount of treated plasma volume correlates with the removal of 63% to 72% of the original plasma constituents. At the end of a TPE procedure, IgM is very low. High levels of IgM are usually reduced with one or two TPE^[42]. The American Society of Apheresis guidelines designate the perioperative use of TPE in ABOi LDLT as a category I with 1C recommendation^[43]. Moreover, the use of double-volume TPE pre-transplant eliminated more than 90% of the antibodies, lead to an iso-titer of < 1:16 and decreased the episodes of rejection^[44]. In the studies we reviewed, PTP was conducted before and after ABOi LDLT. Almost all centers used TPE to eliminate anti-A/B iso-titers (Table 1).

Immunoadsorption: Immunoadsorption (IA) is mainly performed in Western Europe. Controlled studies of IA are still lacking in the setting of ABOi LDLT. With IA, it is possible to deplete a large amount of circulating antibodies without considerable loss of essential plasma constituents. Two IA-methods are available to selectively reduce antibodies. The first is the blood group

antigen-specific apheresis (Glycosorb® ABO, Glycorex Transplantation, Lund, Sweden). This technique is preferred to reduce the iso-titer. Because the IA-column is highly selective for anti-A/B antibodies, other antibodies are not affected and no replacement fluid is required. With each plasma volume treated with Glycosorb®, the iso-titer of IgG and IgM is reduced by one titer. Compared to the baseline, a reduction to 59% for IgG iso-titer and to 30% for IgM iso-titer is considered average^[45].

The second is the semiselective antibody removal (Immunosorba®, Globaffin®, Fresenius Medical Care, Bad Homburg, Germany, Therasorb®, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). These columns mainly bind IgG and, to a lesser degree, IgM, regardless of their specificity. This unspecific removal is beneficial for transplant candidates with an additional sensitization. In ABOi kidney transplant patients, a single session of IA decreased anti-A/B IgG iso-titers more effectively than antigen-specific apheresis. IgG was reduced to 28% of the baseline value and IgM to 74%^[45]. In the studies we compared, the use of IA was not reported, as IA is only common in Europe. Asian centers use TPE or double-filtration plasmapheresis instead (Table 1).

Double-filtration plasmapheresis: Outside of Japan, the use of double-filtration plasmapheresis for ABOi LDLT is very limited. The Evaflux™ 2A (Kawasumi laboratories, Japan) eliminates IgG as well as IgM. After processing 1000 mL plasma, the ratio of solute returned to the patient, or the sieving coefficient, is 0.00 for IgM and 0.19 for IgG. As the value of 0.00 for IgM indicates, these pore-based filter columns are most effective for IgM depletion. The target iso-titer < 1:16 was reached with only 4 treatments, even in cases with very high initial iso-titers (> 1:2048)^[46].

Intravenous immunoglobulin G

Intravenous immunoglobulin G (IVIG) are suggested to be beneficial in immunoregulation because they block Fc receptors on mononuclear phagocytes and directly neutralize alloantibodies. They also inhibit the expression not only of CD19 on activated B cells and the complement system but also of alloreactive T cells^[13]. In the field of transplantation, IVIG was used with PTPs in pre-sensitized recipients or to treat AMR^[47,48]. IVIG can be used as a rescue therapy, in the case of severe AMR, if there is not enough time (three days) for rituximab to exert an effect^[39]. When IVIG is part of the therapeutic protocol, graft survival is estimated to be greater than 87%^[47,49,50]. Hanto *et al.*^[44] compared ABOi recipients receiving TPE and IVIG with patients receiving only TPE during the post-transplant period. In this study, the patient group with IVIG did not develop AMR, but 27.3% of the patients in the other group did develop AMR post-transplant. Unfortunately, a transient increase of anti-A/B titers is observed after IVIG administration due to the passive transfer of anti-A/B. Thus, IVIG should not be administered prior to ABOi LDLT. All

centers that we have compared report using IVIG after ABOi LDLT (Table 1).

Immunosuppression

Immunosuppression consists of steroids, calcineurin inhibitors and antimetabolites. In our center, we use quadruple immunosuppression: Monoclonal antibodies, calcineurin inhibitors, antimetabolites and steroids.

In 1998, Tanabe *et al*^[51] described a new protocol in which they, in addition to perioperative TPE and splenectomy, supplemented systemic immunosuppression with portal vein infusion therapy (PVIT). Methylprednisolone, prostaglandin E1 and gabexate mesilate were used in the PVIT. If PVIT causes portal vein thrombosis, Kozaki *et al*^[41]'s hepatic arterial infusion therapy (HAIT) could be conducted. The two most feared complications after PVIT or HAIT were thrombosis and bleeding.

In 2013, local graft infusion, in the form of hepatic arterial infusion (HAI) or portal vein infusion (PVI), with PGE1 was only performed by Kim *et al*^[20] and Song *et al*^[52]. Since 2010, only Song *et al*^[52] have also administered cyclophosphamide as immunosuppression. The therapeutic regimen after LDLT includes antifungal, antimicrobial and cytomegalovirus prophylaxis. However, dosage, medication and duration of the medication have not yet been standardized.

MONOCLONAL ANTIBODIES

Rituximab is a monoclonal chimeric human-murine anti-CD20 antibody that depletes B cells. It acts by complement- and antibody-dependent cell-mediated cytotoxicity. The CD20 antigen is expressed on pre- and mature B cells, but not on long living plasma cells persisting in the bone marrow. Hence, rituximab does not directly affect antibody-producing plasma cells. A single dose of rituximab in ABOi LDLT suppresses B cells for more than six months after transplantation in the peripheral blood^[4,50]. However, because B cells in the lymph node are unaffected, they are activated by the ABOi graft, and the anti-A/B titers rise for the first four to six weeks after transplantation^[4,50,53]. But even if antibody production is possible at low levels, de novo production of antibodies is sufficiently delayed due to rituximab^[28]. Monteiro *et al*^[54] reported the first case of ABOi LTX using rituximab in 2003. Usuda *et al*^[55] reported the first case of rituximab prophylaxis in ABOi LDLT in 2005. Egawa *et al*^[4] reported in 2014 that rituximab prophylaxis significantly decreased the incidence of AMR, especially severe AMR leading to hepatic necrosis ($P < 0.001$)^[4]. However, other B cell desensitization therapies have shown no additional effects in the rituximab group. Multiple or large rituximab doses significantly increased the incidence of infection and early administration held no advantage^[4]. All the transplantation centers we compared treated their ABOi LDLT patients with rituximab, with most of them administering it before transplantation. Two weeks before surgery tends to be an opportune time

(Table 1). Regarding the safety of rituximab in ABOi LDLT, pharmacodynamic studies have to be conducted to determine the safest dose. Currently, therapeutic regimens are adopted from the kidney transplantation protocols.

Basiliximab is a chimeric mouse-human monoclonal antibody to CD25 of the interleukin (IL)-2 receptor, located on the surface of activated T lymphocytes. It inhibits T cell proliferation and prevents cell-mediated rejection in liver transplantation^[56,57]. It prevents T-helper cells from replicating, blocks the activation of B cells and restricts the production of antibodies, including anti-donor isoagglutinin antibody. Recently, the regimen that combines rituximab with basiliximab in ABOi LDLT has been questioned^[4].

Splenectomy

The spleen is a major antibody reservoir, containing large amounts of B cells and plasma cells. Splenectomy before ABOi LDLT to prevent antibody rebound is becoming more controversial. Most Asian centers use protocols with splenectomy in addition to other immunosuppressive measures^[18]. However, several reports have shown that splenectomy does not offer any immunological advantage in ABOi LDLT. For example, Raut *et al.* observed no statistically significant differences in anti-A/B IgM and anti-A/B IgG titers between "splenectomy" and "non-splenectomy" groups^[58]. Several reports have also shown that splenectomy may not offer any immunological advantage in ABOi LDLT. The clinical outcomes, including AMR, biliary complications, infections and survival, were also similar in the two groups^[52,59,60]. An exception to this general rule are patients with imminent "small for size" syndrome, who have better outcomes after splenectomy^[4,61]. Only two centers of the ones compared carried out splenectomy. In these centers, 21 of 23 patients had AMR occurrence (Table 1).

Complications after ABOi LDLT

Biliary complications, which are still a major issue in ABOi LDLT, are likely related to immunological mechanisms. Donor blood group antigens are expressed for up to 150 d on the bile duct's epithelium after transplantation^[59,62-64]. Song *et al*^[7] reported a higher incidence of biliary strictures, especially diffuse intrahepatic biliary strictures (DIHBS), in ABOi LDLT than in ABOc grafts. These strictures significantly affected the overall survival^[15]. In Lee *et al*^[18]'s study, 5.6% of the patients developed complications, such as DIHBS, 2.1-5.2 mo post-transplant. In 2005, Kozaki *et al*^[41] showed that high preoperative anti-IgM iso-titer led to bile duct complications. High preoperative anti-IgG iso-titer led to hepatic necrosis and high postoperative anti-IgM and anti-IgG iso-titers lead to hepatic necrosis as well. Once hepatic necrosis occurred, no patient survived.

Biliary complications developed in 54%-82% of the ABOi allograft recipients, compared to 6% in

ABO matched allografts. Hepatic artery thrombosis also occurred in 24% of ABOi allografts^[3,28]. In 2011, the meta-analysis of Wu *et al.*^[64] showed increased complications and AMR in ABOi LDLT, as well.

Another complication, such as the “small for size” syndrome in ABOi LDLT, can be avoided *via* a new dual split technique from Asia^[65]. Dual LDLT with ABOi and ABOc grafts is a feasible solution for simultaneously overcoming both the ABO blood group barrier and small-for-size graft.

CONCLUSION

Since 2010, no new techniques in ABOi LDLT have been reported in medical journals, but the treatment options have been refined. The outcomes of ABOi LDLT are still inferior to those of ABO-compatible and identical LDLTs, and anti-A/B antibodies reappear after the transplant. However, due to refined treatment strategies, ABOi LDLT is a feasible option today and is almost free from severe complications. We compared the regimens of 11 transplant centers, as well as their outcomes from 2010 to the present. The best improvement in outcomes next to PTPs has been observed with the prophylactic use of rituximab more than one week before ABOi LDLT. Although each center treats its patients with its own scheme, the transplant results are homogeneous. In our center, we have had positive experiences starting quadruple immunosuppression with basiliximab before transplantation. We also use TPE or IA and reduce the iso-titer at least down to 1:8 prior to transplantation. If the iso-titer rises again afterward, we mainly perform TPE.

A new approach for overcoming both the ABO blood group barrier and small-for-size grafts seems to be the dual split LDLT with ABOi and ABOc grafts that has been conducted in Asia.

Still, ABOi graft survival in adults is poorly understood. Neither is the emergence of de novo anti-A/B, nor their impact. Graft accommodation gives a possible explanation for ABOi graft survival in the presence of donor specific antibody titers.

In the long term, iso-titer rebound prevention might be necessary to lower the risk of iso-titer mediated rejection even further. However, no specific medication is available yet to meet this need.

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Updates on antibody-mediated rejection in intestinal transplantation

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Abstract

Antibody-mediated rejection (ABMR) has increasingly emerged as an important cause of allograft loss after intestinal transplantation (ITx). Compelling evidence

indicates that donor-specific antibodies can mediate and promote acute and chronic rejection after ITx. However, diagnostic criteria for ABMR after ITx have not been established yet and the mechanisms of antibody-mediated graft injury are not well-known. Effective approaches to prevent and treat ABMR are required to improve long-term outcomes of intestine recipients. Clearly, ABMR after ITx has become an important area for research and clinical investigation.

Key words: Intestinal transplantation; Antibody-mediated rejection; Hyperacute rejection; Chronic rejection; Donor-specific antibodies; C4d deposition; Outcomes

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Core tip: Antibody-mediated rejection (ABMR) has increasingly surfaced as an important cause of allograft loss after intestinal transplantation. The presence of donor-specific antibodies (DSAs) should alert the clinician of the increased risk of ABMR. The avoidance of a known donor-specific antibody target at the time of transplant remains a primary preventive strategy. The development of newly-formed DSAs usually portends a poor prognosis with an increased risk of refractory acute rejection, chronic rejection, and allograft loss. The better understanding of mechanisms of antibody-mediated graft injury, establishment of the diagnostic criteria, and optimal management of these antibodies may improve clinical outcomes of intestine transplants.

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INTRODUCTION

The intestine is often deemed one of the most difficult

organs to be transplanted because of its unique structure and enhanced immune response^[1-3]. Over the past several decades, intestinal transplantation (ITx) has achieved remarkable advancement not only in volume of transplants but also in outcomes, owing to progress in various aspects of organ preservation, surgical technique, immunosuppression, and postoperative management^[4-7]. Despite improvements in short-term outcome, long-term survival of both patient and graft after ITx has been well behind other solid-organ transplants, with 10-year survival rates under 50%^[5,8]. Allograft dysfunction and/or loss due to acute and chronic rejection continue to be major barriers to the success of intestinal allografts^[6]. Therefore, it is essential to further delineate mechanisms for graft failure and to develop treatment strategies that will provide long-term intestinal graft function.

Traditionally, intestinal allograft rejection has mainly been regarded as a T-cell-mediated process, whereas the humoral immunity has received less attention in the evaluation of intestinal rejection. A potential role for antibodies in graft rejection has long been suspected because antibodies to human leukocyte antigens (HLA) are often detected in patients with rejection^[9-11]. To date, HLA antibodies are well recognized as causes for hyperacute rejection, acute antibody-mediated rejection (ABMR) and chronic ABMR following kidney or heart transplantation^[12-14]. Isolated reports suggest that HLA antibodies also affect lung, liver, or pancreas transplants^[15-17]. Much of the evidence indicates that an early diagnosis and aggressive treatment of acute ABMR are critical for improving graft and patient outcomes in kidney or heart transplantation^[18,19]. In recent years, several groups demonstrate that, as with other solid-organ transplantation, HLA antibodies appear to be a significant risk factor for the development of acute and chronic rejection after ITx and worsen the overall prognosis for both patient and graft^[20-22]. ABMR has increasingly emerged as a potential form of graft dysfunction after ITx. The strategies to decrease or eliminate preformed HLA antibodies, early recognition and appropriate management of newly-formed (*de novo*) antibodies may further improve outcomes in intestinal allograft recipients.

This review summarizes what is currently known regarding antibody-mediated injury to the intestine and potential solutions to this problem and to emphasize the areas that require further study.

DONOR-SPECIFIC ANTIBODIES AND PRETRANSPLANT SENSITIZATION

Alloantibodies directed against donor HLA, called donor-specific antibodies (DSAs), may be present at the time of transplantation (preformed DSA) or develop *de novo* following organ grafting. These donor HLA antigens are commonly expressed on endothelial cells, epithelial cells, or other organ specific targets. Over the past several

decades, analyzing transplant recipients for DSAs has become an important part of immune monitoring before and after transplantation^[23]. The earliest method developed in the 1960s was complement-dependent cytotoxicity (CDC) cross-matching of the recipient's serum with the donor's lymphocytes in the presence of complement. This simple test substantially reduces the occurrence of hyperacute rejection, but its sensitivity and specificity (due to non-HLA antibodies) are very low. Flow cytometry cross-matching developed in the 1970s is based on the detection of serum antibodies binding to donor lymphocytes, and it is more sensitive than CDC cross-matching. Current solid-phase immunoassays such as Luminex single-antigen beads provide important advantages in sensitivity and specificity over cell-based assays and are widely used in most transplant centers around the world^[24].

Compared with other solid-organ transplants, sensitization is relatively higher in intestinal allograft recipients, most likely due to previous multiple operations, blood transfusions, recurrent line infections, or pregnancies. High panel reactive antibody (PRA) levels are observed in 18%-30% of intestinal transplant candidates on the waiting list, compared to the sensitization rate of 10%-15% in kidney and heart transplant candidates^[22,25,26]. Indeed, in our experience the incidence of sensitization was as high as 30%, implying that intestine recipients are an immunologically high-risk population^[21].

HYPERACUTE REJECTION

As with other solid-organ transplants, an intestinal allograft placed into a highly sensitized recipient may be subject to very rapid loss because of hyperacute rejection. This severe form of acute rejection was originally described for clinical kidney allografts transplanted into recipients with circulating antibody against the donor^[27]. The kidney graft rapidly develops a beefy red or blue appearance and immediately fails^[28]. The pathogenesis involves the binding of preformed DSA to HLA on endothelial cells and the subsequent activation of the classical complement cascade leading to the formation of the membrane attack complex and endothelial damage. Because of its strong clinical relevance, cross-matching of the recipient's serum and the donor's lymphocytes prior to transplantation became a standard protocol of kidney transplant programs throughout the world.

The kidney and heart are most susceptible to hyperacute rejection, and the liver is relatively resistant^[29,30]. To date, hyperacute rejection has not been sufficiently studied in ITx^[31]. Hyperacute rejection, although rare, can occur in intestinal allograft recipients who are highly sensitized with the presence of DSAs. This aggressive form of rejection occurs almost exclusively in the pre-sensitized patient with a very high titer of preformed HLA antibodies and is the result of a severe antibody-mediated response to the vasculature

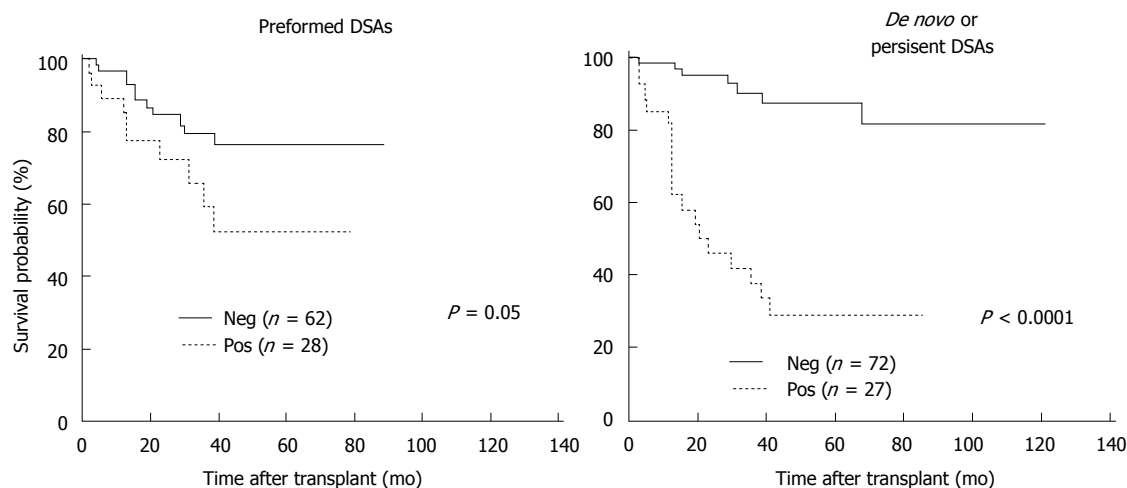


Figure 1 The Kaplan-Meier graft survival for the presence of performed donor-specific antibodies before transplant and newly formed (*de novo*) donor-specific antibodies after transplant. Patients with preformed donor-specific antibodies (DSA) had significantly lower graft survival than those without preformed DSA. The graft survival was markedly worse in patients with *de novo* DSA or persistent DSA.

endothelium, characterized histologically by vascular injury, thrombosis, and ischemia. In a case report of hyperacute rejection, Ruiz *et al*^[32] described an isolated intestinal allograft recipient with the presence of a positive cross-match and multiple preformed DSAs. The intestinal allograft became dusky immediately following graft reperfusion and the recipient showed hypoxia, hypotension, and acidosis. Subsequent mucosal biopsy specimens exhibited severe vascular congestion with thrombi, hemorrhage, and leukocyte infiltration. Immunofluorescence revealed the deposits of IgG, IgM, C4d, and C3 on the endothelium, suggesting that antibodies can directly injury the intestinal allograft. In this isolated case, the intestinal graft was successfully saved after a combination of intensified tacrolimus, alemtuzumab, rituximab, and plasmapheresis.

ACUTE ABMR

In the earlier series, Bond *et al*^[9] reported outcomes of 23 cross-matching positive grafts in 124 recipients (18%) and illustrated that a positive cross-match was associated with increased frequency of acute rejection after ITx, especially with an isolated intestine. They showed 43.5% (10 out of 23 positive cross-matching) allografts failed at a follow-up of two years. The simultaneous liver allograft as part of a composite visceral transplant appeared to improve the negative effect of the preformed antibodies and positive cross-matching. Later, Ruiz *et al*^[33] in Miami and Wu *et al*^[10] in Pittsburgh respectively described the vascular changes of intestinal allograft recipients in the setting of a positive cross-match. In the recipients with a higher PRA and a positive cross-match, the pathology showed significant vascular congestion and submucosal hemorrhage with deposition of C4d, IgG, and IgM. They found a lower graft survival in the recipients with the early significant vascular lesions^[33]. Based on these early results and lessons learned from the other solid-organ

transplantation, a positive CDC cross-match has been considered relatively prohibitive for an isolated intestine transplant in most intestinal transplant programs.

A decade later, Wu *et al*^[34] evaluated an adverse impact of HLA antibodies on intestinal allograft outcome. This study initially retrospectively analyzed a total of 117 recipients who received a primary liver-exclusive intestine allograft during the period between 2000 and 2009. The results further confirmed that a positive cross-match with preformed DSA significantly increased rate and severity of acute rejection after transplant and the formation of *de novo* DSA after ITx was associated with the worst clinical outcome (Figure 1). Tsai *et al*^[20] prospectively examined the impact of pre- and post-transplant DSA on intestinal allograft rejection. Thirteen recipients were subsequently followed up for DSA levels by a sensitive Luminex assay pre- and posttransplant. They found that the presence of DSA was closely related to an increasing number of rejection episodes and severe acute rejection grading. A combination of rituximab, plasmapheresis, IVIg, or bortezomib therapies to eliminate DSA was associated with clinical improvement of acute rejection. The authors suggest that frequent intestinal graft biopsies combined with serial measurement of DSAs are valuable for evaluation of cellular and humoral immunity of acute rejection.

Our group further analyzed 194 primary intestinal/multivisceral allograft recipients in which one-third had a positive CDC cross-match prior to surgery^[21]. In 156 recipients, 49 (31%) had preformed DSA before ITx; 19 (39%) had persistent DSA after ITx; and 19 (18%) developed *de novo* DSA. The authors again showed preformed DSA significantly increased frequency and severity of acute rejection. Overall cumulative risk of acute rejection was significantly higher in a positive cross-match compared to a negative cross-match. The recipients with higher levels of DSAs, as measured by a single antigen Luminex assay, developed an increased incidence of steroid-resistant rejection which responded

poorly to OKT3 treatment, and 1-year graft survival in DSA-positive recipients was significantly inferior to that of DSA-negative recipients. Twenty-one (11%) of recipients were diagnosed with acute ABMR, and most ABMR cases occurred in the first three months after transplant. The incidence of acute ABMR was substantially elevated in recipients with performed, persistent DSA and *de novo* DSA and 11 (52%) of acute ABMR cases led to allograft failure.

It is important to note that intestinal transplant recipients can mount humoral immune response after transplantation even in the setting of a negative cross-math. Gerlach *et al*^[35] reported thirteen patients undergoing intestinal/multivisceral transplants with non-donor-specific HLA antibodies before ITx and found that the development of *de novo* DSAs after ITx was associated with severe graft dysfunction. They observed that only three recipients had non-donor-specific HLA antibodies before transplantation; 15 (50%) cases developed *de novo* DSA during the first 6 mo; and only two recipients developed DSA 10 years after transplantation. In their small series, all patients with *de novo* DSAs showed simultaneous acute cellular rejection at the time of DSA occurrence. Luckily, nine of the 10 patients diagnosed with acute ABMR were successfully treated with a combination of plasmapheresis and intravenous immunoglobulin (IVIg). In case of persistence of DSA and/or treatment-refractory rejection, additional rituximab and/or bortezomib were beneficial.

DIAGNOSIS OF ACUTE ABMR

Up to date, diagnostic criteria for acute ABMR after ITx have not been established and there is no consensus on the characteristic clinicopathologic features. However, several reports addressing a unique form of allograft rejection that is consistent with the definition of acute ABMR which was defined by The National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplantation in kidney and heart transplantation^[36,37].

Wu *et al*^[10] initially described a characteristic clinical and pathologic syndrome during the early postoperative course in intestine recipients with a positive cross-math. They observed that the strongly positive cross-match recipients exhibited serious mucosal damage instantly after graft reperfusion, including mucosal congestion, bluish discoloration, and focal hemorrhage in the allograft. Pathology showed severe capillary congestion, neutrophilic infiltration, hemorrhage, epithelial injury, and thrombi within the lamina propria microvasculature, but without evidence of histologic neutrophilic or necrotizing arteritis, and the immunofluorescent findings were unremarkable. In contrast, the recipients with a weakly positive crossmatch, as well as the cross-match negative recipients, had none of these characteristic clinical, endoscopic, or microscopic findings.

C4d is a footprint of antibody-triggered classical complement activation and its deposition has become

pivotal to the diagnosis of acute ABMR in kidney and heart transplants^[37,38]. However, there is no generally acceptable consensus on the use of C4d staining in diagnosing acute ABMR after ITx. Earlier studies showed that C4d deposition had no difference in biopsies between acute rejection and no rejection and claimed that C4d had no clinical relevance as diagnosing humoral rejection in intestinal allografts^[39,40]. Unfortunately these earlier studies neither correlated C4d with the levels of HLA antibodies nor examined these antibodies by a relatively sensitive methodology. Ruiz *et al*^[33] demonstrated that post-transplant vascular lesions in intestinal allografts at earlier time periods were associated with higher levels of pre-transplant PRA or a positive CDC cross-match. In intestinal recipients with the vascular changes, C4d staining can be seen in the small vasculatures. Of the patients with no significant vascular alterations, C4d deposition was negative or trace. Our team evaluated the utility of C4d in intestinal biopsies at the time of suspected acute ABMR and showed a diffuse C4d staining was mainly observed in recipients with a positive DSA, while focal or minimal C4d staining was observed in intestinal biopsies with no evidence of rejection^[21]. Similar to other solid-organ transplants, our results emphasize clinical significance of a diffuse C4d deposition in intestinal allografts, suggesting that C4d together with higher titers of DSA, is a very useful marker to detect acute ABMR after ITx.

Based on the established diagnostic criteria for kidney transplant, including the presence of circulating DSAs, acute tissue injury, C4d deposition and clinical allograft dysfunction, we performed a retrospective single-center analysis to investigate the incidence, risk factors and clinical outcomes of acute ABMR after ITx (unpublished data). Acute ABMR was diagnosed in 18 (10.3%) out of 175 primary intestinal/multivisceral transplants with a median occurrence of 10 d (range, 4-162) after ITx. All eighteen patients were sensitized to HLA class I and/or II antigens with the presence of performed DSAs. A cross-match was positive in 14 (77.8%) recipients. Twelve of 18 patients (66.7%) developed *de novo* DSA after ITx. Pathological features of acute ABMR include C4d deposition, prominent hemorrhage and congestion with scattered fibrin thrombin in the lamina propria (Figure 2). Despite initial improvement after treatment, eleven (61.1%) lost graft due to rejection. Of those, nine (50%) received enterectomies and four (22.2%) underwent retransplantation after acute ABMR. At a median follow-up of 32.3 mo (range, 13.3-76.4 mo), eight (44.4%) recipients died. We conclude that acute ABMR can be a fulminant form of intestinal rejection, especially in a liver-free transplant and survivors are at an increased risk of developing refractory rejection. Our studies suggest that no morphologic findings are specific for acute ABMR in intestinal allografts, and the diagnosis is best made using serologic, clinical, and histologic data

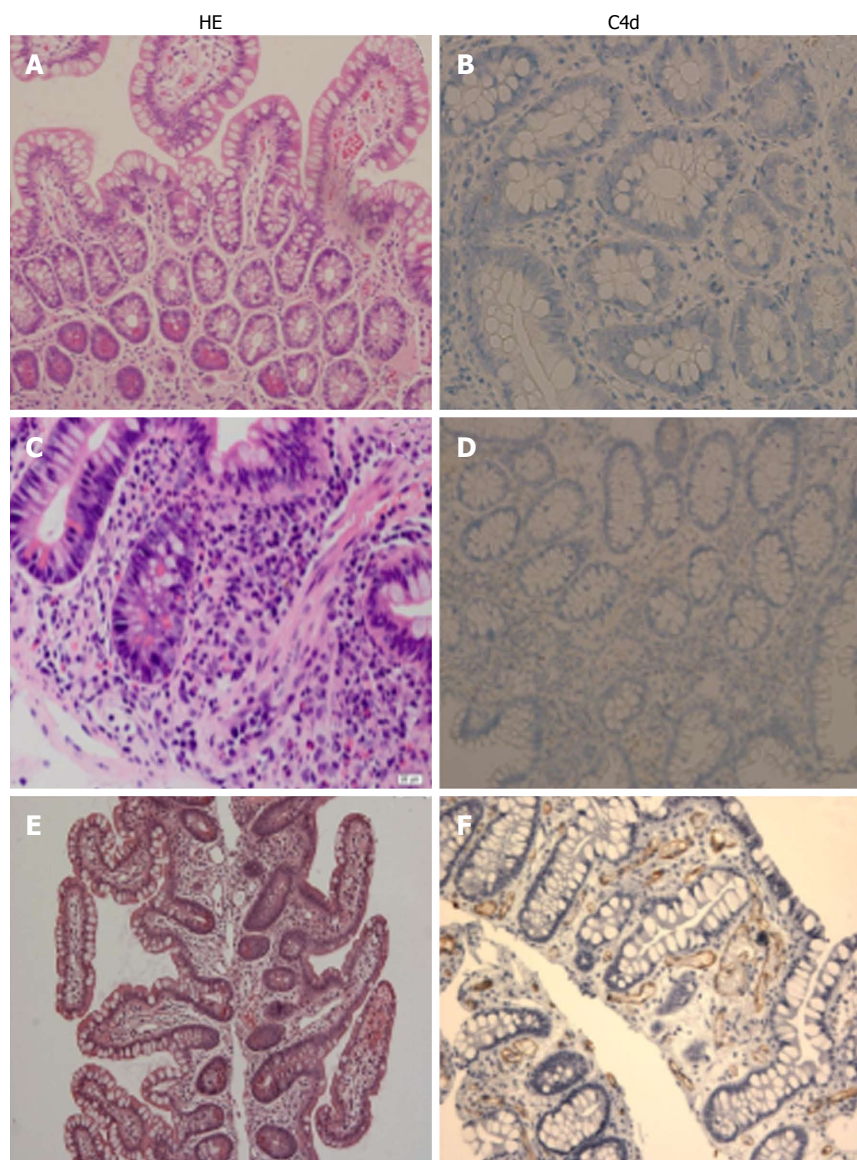


Figure 2 Histopathology of the intestinal allografts. A and B: No rejection: Normal mucosal architecture of small bowel biopsy after transplant. No staining for C4d is seen in the capillaries of the lamina propria; C and D: Acute cellular rejection (ACR): There is mononuclear infiltration, crypt epithelial injury, and apoptotic bodies in the lamina propria. A weak staining for C4d is sometimes present in a patient with ACR; E and F: Acute antibody-mediated rejection: There is prominent hemorrhage and congestion with scattered fibrin thrombin in the lamina propria. Widespread and bright staining for C4d is present in the capillaries of the lamina propria.

together.

PREVENTION AND TREATMENT OF ACUTE ABMR

Due to rarity of ITx, no standard protocols are currently available for prevention and treatment of acute ABMR. Therapeutic strategies are predominantly based on case reports, small series, and renal transplant data.

The avoidance of a known HLA DSA target at the time of transplant remains a primary preventive strategy. With the development of solid-phase assays, the ability to detect and minimize DSA prior to transplantation is possible. Luminex single-antigen assay of DSA has led to the application of the virtual cross-match, in which known recipient HLA antibodies are compared to donor HLA prior to transplantation. At the time of a donor organ offer, the

titer, MFI, and total number of DSA can be evaluated for the virtual cross-match. Hawksworth *et al*^[25] evaluated the virtual cross-matching for organ allocation and immunological risk reduction in sensitized isolated intestinal transplants. In their study, higher DSA titers (more than 1:16) were considered a contraindication for an isolated intestinal transplant. They observed that clinical outcomes were comparable between sensitized (PRA > 20%) and control (PRA < 20%) recipients in terms of 1-year freedom from rejection, 1-year patient survival, and 1-year graft survival. The authors conclude that a virtual cross-matching strategy to optimize organ allocation is valuable in sensitized patients to successfully undergo isolated ITx with good short-term outcomes. However, this strategy may affect the sensitized potential recipient's access to ITx.

The use of preoperative desensitization strategies

to decrease DSA titers with plasmapheresis, ATG, IVIg, and mycophenolate has been described with good tolerability and reduction of early rejection episodes and equivalent posttransplant outcomes to unsensitized patients^[41]. The Indiana group reported their experience with combined rabbit ATG and rituximab as induction therapy, a positive cross-match was not related to an increased risk of acute rejection and graft failure. They suggested that combined use of anti-IL2 receptor antibody may be beneficial in the liver-free intestinal transplant. The authors conclude that with anti-thymocyte globulin plus rituximab induction, a positive cross-match had reasonable outcomes after intestinal/multivisceral transplantation. Garcia-Roca *et al*^[42] recently presented two living donor intestinal candidates with a positive cross-match that was successfully converted to a negative cross-match using desensitization protocol prior to transplantation. The first case had 67% for PRA HLA class I and 100% for class II and had DSA with a positive flow cytometry cross-match with a potential donor. The second case was sensitized with 80% for PRA class I and 26% for class II; no DSAs were identified. In this case, the standard cytotoxic cross-match was negative, but the flow cytometry cross-match was positive for B cell. Both cases were successfully desensitized with steroids, thymoglobulin, multiple plasmapheresis, followed by IVIg, achieving a complete negative cross-match at the time of transplant. ITx was successfully performed in both cases after desensitization protocol. Humoral rejection did not occur during the initial 6 and 9 mo follow-up.

It has been well-known that combined liver and ITx can be performed against a positive cross-match, suggesting that the liver graft protects the subsequent intestinal transplant from the harmful antibodies. Testa *et al*^[43] described a highly sensitized case in which a cross-match remained positive after multiple plasmapheresis. With a liver transplant, the cross-match quickly became negative allowing subsequent bowel grafting in one week. We described our single-center experience in retransplanted recipients and compared cases who underwent liver-free retransplants with those who underwent liver-inclusive retransplants^[44]. The graft survival rates at 1, 3 and 5 years in liver-free retransplants were markedly worse than those in liver-inclusive retransplants. The majority of liver-free retransplants underwent enterectomy due to either severe acute cellular rejection or chronic rejection. Six recipients died due to rejection-related complications. Compared to liver-free retransplants, the frequency and grading of acute rejection were markedly decreased in liver-inclusive retransplants. We did not see cases with chronic rejection during the study period and two patients died due to graft-vs host disease and infection in this group, respectively. We conclude that a liver-inclusive retransplant offers a better long-term clinical outcome, suggesting that the liver-intestine combined transplantation should be considered when

retransplantation is unavoidable.

The treatment of confirmed acute ABMR has routinely included a combination of corticosteroids, IVIg, plasmapheresis, ATG, and rituximab. Bortezomib, a proteasome inhibitor, has been reported to reduce or eliminate DSA after transplantation^[45]. Gerlach *et al*^[46] described ten intestinal recipients with a diagnosis of acute ABMR. After combined therapies including bortezomib, 9 cases were successfully treated with a good graft function. DSAs were completely cleared in 8 patients, and detectable in only one. Eculizumab, a humanized monoclonal antibody against complement C5, has successfully been used to treat acute ABMR in renal transplant. Recently, Fan *et al*^[47] described a case in which eculizumab was administered to reverse acute ABMR in a desensitization-resistant intestinal retransplant patient. His primary intestinal allograft failed due to ABMR eight years after ITx. Two donors were used in his initial allograft (one for the intestinal graft and another for the abdominal wall graft). He underwent a second intestinal graft which had to be resected a month later due to uncontrolled severe acute ABMR. The patient became highly immunized due to three HLA unmatched different organs, as reflected by 100% PRA and serum high titers of DSAs. He received the third liver-inclusive multivisceral transplant and developed severe acute ABMR on day 3 post-transplantation. Acute ABMR was successfully salvaged with antibody-targeted desensitization regimens. Although PRA levels remained higher, the titers of DSAs significantly decreased below the cut-off level of 3000 MFI (mean fluorescent intensity) within a month after the third transplant. The favorable outcomes in this extremely difficult case may be attributed to the use of Eculizumab and the immunoprotective effect of the liver graft.

CHRONIC REJECTION

Chronic rejection or enteropathy is a significant barrier to long-term graft and patient survival of intestinal allograft. The incidence of chronic rejection ranges between 15%-20% after ITx^[6,48]. Pathologically, it is characterized by concentric vasculopathy, luminal occlusion, leukocyte infiltration, and a marked fibrotic change^[49]. These histologic findings are the end results of a complex, multi-stage process of repeated immune- and non-immune-mediated cellular injury and inflammation. Repetitive insults exhaust the recipient's natural repair mechanisms leading to fibrotic replacement and intestinal failure^[50]. An isolated small bowel transplant appears to render the graft more susceptible to chronic rejection compared to a liver-inclusive transplant^[6,44,51] (Figure 3).

The causes of chronic rejection resulting from graft tissue injury are multifactorial and both immune- and non-immune-mediated factors can contribute to graft injury. Emerging evidence suggests that immune-mediated injuries to the graft are the fundamental cause

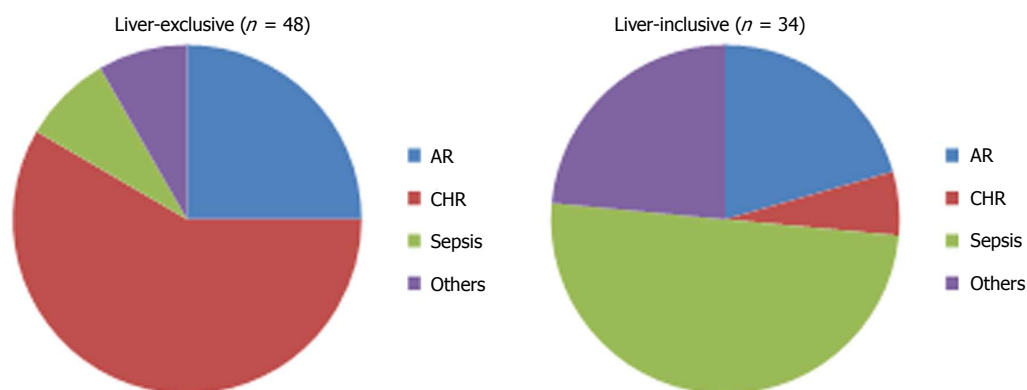


Figure 3 The causes of graft loss in the liver-exclusive and liver-inclusive intestinal transplants. In liver-exclusive transplants, chronic rejection was the leading cause of graft loss. In liver-inclusive transplants, however, infection was the major cause of graft loss. AR: Acute rejection; CHR: Chronic rejection.

of chronic rejection^[3,52]. Several studies have identified severe acute rejection, recurrent episodes of rejection, the cumulative burden of acute rejection, and late-onset acute rejection as risk factors for chronic rejection^[6,21]. Recently, the role of humoral alloimmunity has also appeared to be closely related to chronic rejection^[21,53]. The major target of humoral immunity appears to be the graft endothelium, which can be activated and injured by HLA antibodies. However, the mechanism by which humoral alloimmunity leading to chronic rejection is not well understood, and whether the presence of antibody is an initiating event or merely a response to tissue damage remains to be defined.

A large observational study investigating the potential effect of HLA antibodies on the intestinal chronic rejection came from our group^[21]. We retrospectively analyzed 194 consecutive intestine transplants which showed the incidence of chronic rejection at 36 cases (19%) with an average of 21 ± 10 mo (range 2-88 mo) follow-up. Cumulative risk of chronic rejection was slightly higher in recipients with a positive cross-match vs a negative cross-match. Cumulative probability of chronic rejection was markedly elevated in recipients in the setting of the presence of preformed DSAs before ITx together with persistent DSAs after ITx. The formation of *de novo* DSAs was closely related severe chronic rejection and subsequent graft loss. The graft survival was markedly decreased in the DSA-positive patients and the graft loss due to chronic rejection was irreversible in one-third patients. The liver-inclusive transplant was associated with better clearance of preformed DSAs, lower rates of *de novo* DSA formation, and therefore reduced rates of chronic rejection. The results illustrate a strong relationship between DSAs and an increased risk of chronic rejection and allograft failure.

CONCLUSION

Increasing and compelling evidence indicates that antibody-mediated graft injury is closely related to poor outcomes in ITx. The presence of preformed

DSAs should alert the clinician of the increased risk of ABMR. The avoidance of a known DSA target at the time of transplant remains a major preventive strategy and may improve unsatisfactory outcomes in intestine recipients. The development of *de novo* DSA after ITx usually portends a poor prognosis with an increased risk of uncontrolled acute rejection, chronic rejection, and allograft loss. The better understanding of mechanisms of antibody-mediated graft injury, establishment of the diagnostic criteria, and optimal management of DSAs are needed to improve clinical outcomes of ITx.

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Basic Study

Pharmacological Tie2 activation in kidney transplantation

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Conflict-of-interest statement: Vasculotide has been patented and is being commercialized by Vasomune Therapeutics. Van Slyke P and Dumont DJ are co-inventors of Vasculotide and Van Slyke P is the Chief Scientific Officer, while Dumont DJ sits on the Scientific Advisory Board. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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Abstract

AIM

To investigate the therapeutic potential of vasculotide (VT) - a Tie2 activating therapeutic - in kidney transplantation.

METHODS

We performed a murine MHC-mismatched renal transplant model (C57Bl/6 male into Balb/c female) with 60 min cold and 30 min warm ischemia time. 500 ng VT was administered *i.p.* to donor mice 1 h before organ removal. In addition, recipients received 500 ng VT *i.p.* directly and 3 d after surgery. Survival was monitored and remaining animals were sacrificed 28 d after transplantation. In this model, we analyzed: (1) organ function; (2) Kaplan-Meier survival; (3) organ damage (periodic acid Schiff staining) *via* semi-quantitative scoring [0-4 (0 = no injury/inflammation to 4 = very severe injury/inflammation)]; (4) expression of renal endothelial adhesion molecules (ICAM-1) *via*

immunofluorescence (IF) staining, immunoblotting and qPCR; (5) infiltration of inflammatory cells (IF Gr-1, F4/80); and (6) fibrosis *via* staining of α -smooth muscle actin (α SMA), Sirius red staining and immunoblotting of SMAD3 activation.

RESULTS

Exogenous activation of Tie2 with VT resulted in diminished expression of peritubular and glomerular endothelial adhesion molecules. Consequently, infiltration of inflammatory cells (analyzed as ICAM-1, Gr-1 and F4/80 positive cells) was reduced in VT-treated mice compared to controls. Additionally, VT was protective against fibrogenesis after kidney transplantation. Trends towards lower serum creatinine (vehicle: $142 \pm 17 \mu\text{mol/L}$ *vs* VT: $94 \pm 23 \mu\text{mol/L}$), urea (vehicle: $76 \pm 5 \text{ mmol/L}$ *vs* VT: $60 \pm 8 \text{ mmol/L}$) and lactate dehydrogenase (vehicle: $1288 \pm 383 \text{ iU}$ *vs* VT: $870 \pm 275 \text{ iU}$) were observed on day 6 after transplantation. Kaplan-Meier survival analysis showed improved survival rates in the VT-treated mice that did not reach statistical significance (27% *vs* 54%, $P = 0.24$, $n = 11$ per group). Exogenous activation of Tie2 *via* VT might reduce infiltration of inflammatory cells into renal tissue thereby protecting the transplant from early graft dysfunction potentially affecting long-term function.

CONCLUSION

Protection of the endothelial microvasculature *via* the Tie2 axis in the early transplant setting might hold promise as a therapeutic target.

Key words: Vasculotide; Tie2; Kidney transplantation; Endothelium; Angiopoietin

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Core tip: Activation of the Tie2 receptor has been shown to be beneficial in different models of disease. Here, we demonstrate that agonistic stimulation of Tie2 *via* the drug-like putative therapeutic termed "vasculotide" (VT) ameliorates outcome in a murine MHC-mismatched kidney transplant model. VT treatment (*i.e.*, activation of endothelial Tie2) prevented inflammation and fibrosis thereby preserving graft function. Moreover, single administration at the time of transplantation was also sufficient to prolong survival compared to control group.

Thamm K, Njau F, Van Slyke P, Dumont DJ, Park JK, Haller H, David S. Pharmacological Tie2 activation in kidney transplantation. *World J Transplant* 2016; 6(3): 573-582 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i3/573.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i3.573>

INTRODUCTION

Graft failure and ultimately graft loss are still major

problems in solid organ transplantation. The endothelium hereby plays a pivotal role in mediating inflammation and subsequent organ dysfunction. In general, a healthy endothelium is essential for vascular homeostasis, and preservation of endothelial cell (EC) function is critical for maintaining transplant allograft function. Damage to the microvascular ECs is a characteristic feature of acute vascular rejection, an important predictor of later graft function and loss^[1]. Innovative therapeutic strategies preventing IRI and maintaining stable renal function are highly desirable.

The angiopoietin (Angpt)/Tie2 system consists of the transmembrane endothelial tyrosine kinase Tie2 and its four circulating ligands (Angpt1-4)^[2-5]. This system regulates baseline endothelial barrier function and its response to injury^[6,7]. Previous work has shown that the balance between the Tie2 agonist (Angpt-1) and the antagonist (Angpt-2) controls Tie2 phosphorylation^[6]. Angpt-1 which is mainly secreted by pericytes binds Tie2 as a natural agonist thereby promoting vascular quiescence^[8]. Canonical downstream effects of Tie2 signaling are activation of PI3K/Akt^[9,10], inhibition of the inflammatory transcription factor NF κ B^[11] and consecutive control of adhesion molecule expression^[12] as well as cytoskeletal regulation *via* the scaffolding protein IQGAP1^[13]. All together Tie2 activation promotes an anti-inflammatory, pro-survival, and anti-permeability phenotype of the vasculature. In contrast, Angpt-2 which is released from ECs upon pro-inflammatory stimuli inhibits Tie2 phosphorylation and consequently disrupts protective Tie2 signaling^[14].

Few data indicate a beneficial role of Tie2 activation in solid organ transplantation. In kidney transplant recipients, it has been shown that increased Angpt-2 levels (the natural Tie2 antagonist) correlate with mortality indicating that a dysbalanced Angpt/Tie2 system might be unfavorable in renal transplantation^[15]. Interestingly, it has very recently been demonstrated that a chimeric Angpt-1 mimetic, termed COMP-Ang1, is able to reduce endothelial permeability and inflammation in a murine heart transplantation model^[16].

Vasculotide (VT) - a PEGylated synthetic Tie2 agonistic peptide (CHHHRHSF) - has proven its potency to activate Tie2 *in vivo* even stronger and longer than its natural ligand Angpt-1. The therapeutic use of VT was first described in a murine diabetes model where it improved wound healing^[17]. Additionally, we and others have shown that VT can reduce vascular leakage and endothelial inflammation in different murine models of acute systemic inflammation^[18-21].

Given the beneficial properties of Tie2 activation on multiple levels of intracellular signaling with clinically relevant functional effects, we hypothesized that exogenous manipulation of the Angpt/Tie2 system might be protective in transplantation. To test this, we exogenously activated the Tie2 receptor with VT. The aim of our study was to investigate the potential beneficial effects of VT treatment in a murine kidney transplant model on graft

function. We analyzed inflammation, fibrous tissue deposition, renal function and overall survival to better understand if Tie2 activation might improve outcome after transplantation.

MATERIALS AND METHODS

Mouse studies and experimental design

All experiments were approved by the local authorities and conducted in accordance with institutional and governmental guidelines. Mice were housed in a room with 12 h day/night cycle, constant temperature and humidity as well as water and food ad libitum. All appropriate measures were taken to minimize pain or discomfort. Eight-week-old male C57Bl/6 or Balb/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Briefly, kidneys from C57Bl/6 male (donor) were transplanted into Balb/c female (recipient) ($n = 23$). Donor mice received 500 ng VT ($n = 11$) or vehicle (PBS) ($n = 11$) intraperitoneally (*i.p.*) 1h prior to surgery. Recipients were injected with 500 ng VT or vehicle directly and on day 3 after kidney transplantation *i.p.*. Dosage of VT was carefully adjusted before^[18]. Mice were anesthetized with isoflurane and the donor kidney, ureter, and bladder were harvested en block, including the renal artery with a small aortic cuff and the renal vein. Cold ischemia time is 60, and warm ischemia time 30 min. After explantation, kidneys are stored in vehicle solution at 4 °C for 60 min. These ischemia times induce a moderate degree of ischemia-reperfusion injury (IRI) in this model. After left nephrectomy of the recipient, vascular cuff and vein are anastomosed to the recipient abdominal aorta and vena cava, respectively, below the level of the native renal vessels. The ureter is directly anastomosed into the bladder. A second dose of VT or vehicle was administered systemically (*i.v.*) 30 min post-transplantation. The right native kidney was removed on post-transplantation day 4 so that survival becomes graft dependent. Within a given experiments/analysis, we only used samples from single mice. We did not pool samples to increase protein amounts. Blood was taken on days 0, 6, 14, 21 and 28. Survivors were sacrificed 28 d after transplantation for further analysis. Renal function was estimated by serum lactate dehydrogenase (LDH), creatinine and urea measurements (Olympus).

Antibodies and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. GR-1 (AbD Serotec, MCA7716), F4/80 (Biolegend, 122602), Alexa Fluor 555 (Life Technologies), intercellular adhesion molecule (ICAM-1) (M-19) (Santa Cruz, sc-1511), α -smooth muscle actin (α SMA) (Abcam, ab7817) and pSMAD3 (Cell Signaling, C25A9) were utilized for immunoblot or immunohistochemistry. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (FL-335) (Santa Cruz, 25778) served as loading control

for immunoblots.

Immunoblotting

Protein was extracted by using RIPA buffer [including 1 mmol/L Na₃VO₄, 50 mmol/L NaF, protease inhibitors (Roche Diagnostics, Mannheim, Germany)] and resolved with a 10% polyacrylamide gel, followed by blotting on a polyvinylidene fluoride membrane (Merck Millipore, Darmstadt, Germany). Membranes were blocked with 3% bovine serum albumin and incubated with a primary antibody overnight (4 °C). Incubation with the second antibody was performed for 1 h at room temperature. All washing steps were carried out in TBST [20 mmol/L Tris, 150 mmol/L NaCl, 0.1% Tween20 (Merck)]. Bands were visualized with SuperSignal™ West Pico Chemiluminescent Substrate (Life Technologies) and Versa Doc Imaging System Modell 3000 (BioRad).

Immunohistochemistry

Ice-cold acetone-fixed cryosections (6 μ m) were blocked with 10% donkey serum (Dianova) and stained with primary antibodies against ICAM-1. Paraformaldehyde-fixed (Merck, Darmstadt, Germany) and paraffin-embedded tissue sections (1.5 μ m) were dehydrated and rehydrated with ascending and descending ethanol series including deparaffinising with Histoclear (Bioszym, Hessisch Oldendorf, Germany). After blocking with 10% donkey serum, paraffin sections were stained with primary and a secondary antibody. Mounting was accomplished with VectaShield DAPI (Vector Laboratories Inc., Burlingame, CA).

Periodic acid Schiff and sirius red staining

Paraffin-embedded sections were prepared as described above. Periodic acid Schiff staining was performed with periodic acid (0.5%) (Merck), Schiff's reagent (Merck) and hematoxylin (Fluka). For Sirius red staining, sections were treated with 0.2% phosphomolybdic acid, 0.1% Sirius red in 3% picric acid, 0.01 mol/L HCl, 70% and 100% ethanol in the order specified.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from murine kidneys using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Equal amounts of total RNA were reverse transcribed with the Transcriptor First Strand cDNA Synthesis kit from Roche Diagnostics. Real-time-quantitative polymerase chain reaction (RT-qPCR) was performed by a LightCycler 480 II (Roche). Triplicate RT-qPCR analyses were executed for each sample, and the obtained threshold cycle values (CT) were averaged. Gene expression was normalized to the expression of the housekeeping gene, yielding the Δ CT value.

Statistical analysis

Statistical significance was assessed by independent samples and unpaired *t* test as well as Mann-Whitney

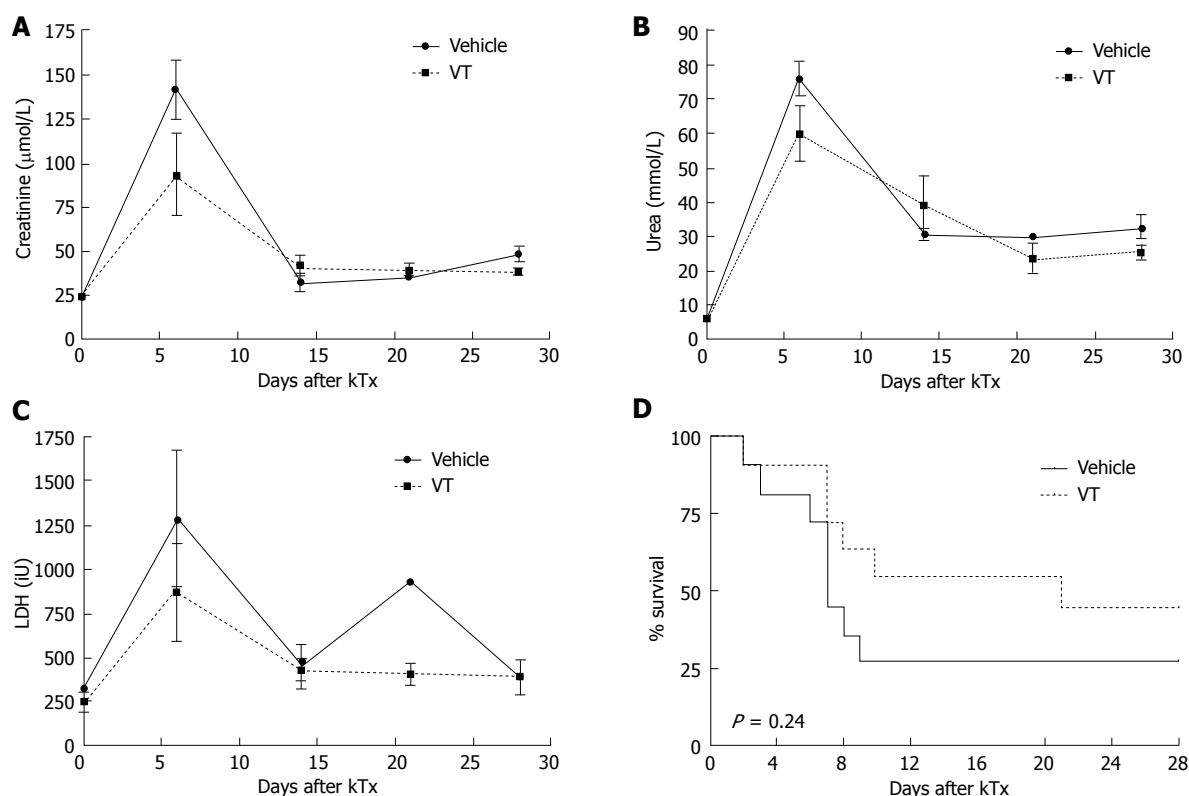


Figure 1 Vasculotide shows trends to improved kidney function and survival in an MHC-mismatched renal transplant model. C57Bl/6 donor mice received 500 ng VT or vehicle prior to surgery (-1 h). Balb/c recipients were injected with 500 ng VT *i.p.* or vehicle directly and on day 3 after kidney transplantation. A-C: Kidney function [serum creatinine, urea and lactate dehydrogenase (LDH) levels] was monitored on day 6, 14, 21 and 28 after transplantation in murine serum and analyzed with unpaired *t* test (day 6, *n* = 7-10); D: Kaplan-Meier survival after MHC-mismatch kidney transplantation (*n* = 11 per group). VT: Vasculotide.

test as indicated. Survival data were analyzed by Log-Rank test. All experimental results are presented as mean \pm SEM or median and a two-tailed *P* value of less than 0.05 was considered to be statistical significant. Analysis and graph generation were performed in GraphPad Prism 6.0 (La Jolla, CA).

RESULTS

VT improves renal transplant function and survival

Given the beneficial properties of Tie2 activation on the endothelial function, we hypothesized that early exogenous activation of Tie2 might also be beneficial in long-term transplant function. Therefore, we established an MHC-incompatible murine kidney transplant model^[22] and treated the mice with 2 doses of VT or vehicle control. Serial blood measurements after transplantation (day 3, 6, 14, 21, 28) showed that renal function was indeed slightly improved upon VT treatment at early time points (serum creatinine vehicle-treated (*n* = 8): 142 \pm 17 $\mu\text{mol/L}$ vs VT-treated (*n* = 9): 94 \pm 23 $\mu\text{mol/L}$, *P* = 0.12; urea level vehicle-treated (*n* = 8): 76 \pm 5 mmol/L vs VT-treated (*n* = 10): 60 \pm 8 mmol/L, *P* = 0.13 using unpaired *t* test) was observed on day 6 (Figure 1A and B). LDH as a broad surrogate marker for cell death showed a similar trend in vehicle-treated animals compared to the VT group potentially indicating that VT might reduce apoptosis/necrosis [vehicle-treated (*n* =

7): 1288 \pm 383 iU vs VT-treated (*n* = 8): 870 \pm 275 iU, *P* = 0.38 using unpaired *t* test] (Figure 1C). Additionally, we analyzed survival after transplantation and observed a trend towards improved survival in VT- compared to vehicle-treated mice (27% vs 54%, *P* = 0.24, *n* = 11) (Figure 1D). Together, these data indicate that early VT treatment might improve kidney function after renal transplantation.

Infiltration of inflammatory cells is diminished upon VT treatment

We next studied histological changes to investigate graft rejection and inflammation in transplanted kidneys. One can easily appreciate the glomerular as well as the interstitial inflammatory infiltrates in the vehicle-treated mice on day 28 after transplantation (Figure 2A and C, left side). In the lower left panel (Figure 2C, left side) almost no intact tubular structures are detectable anymore. VT treated mice exhibited a much weaker inflammatory burden both in the glomerulus as well as the interstitium (Figure 2, right side). These results were confirmed by a histological semi-quantification (Table 1) regarding interstitial inflammation [vehicle (*n* = 3): Median = 4.0 (25% quartile: 4.0%-75% quartile: 4.0) vs VT-treated (*n* = 5): 2.0 (2.0-2.0), *P* = 0.02 using Mann-Whitney test] and glomerular injury [vehicle (*n* = 3): 4.0 (3.0-4.0) vs VT-treated (*n* = 5): 2.0 (1.0-2.0), *P* = 0.04 using Mann-Whitney test] (Figure

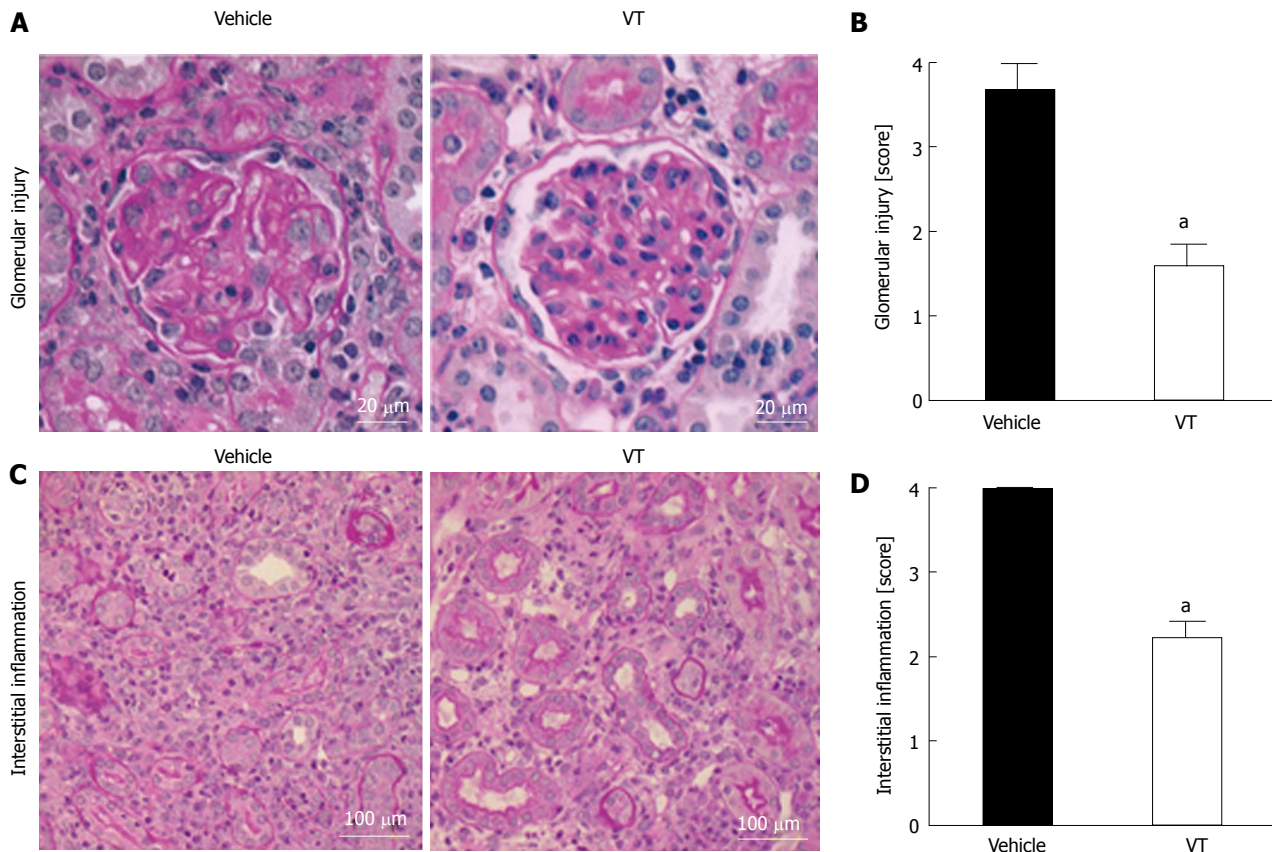


Figure 2 Vasculotide-treated mice show less infiltration of inflammatory cells in the kidney. A: Exemplary PAS staining of kidneys from mice treated with vehicle or VT. Glomerular injury was evaluated on day 28 after transplantation; B: Semi-quantification of glomerular injury by surveying kidney cross-sections. Scoring was done as described above (see Table 1) (vehicle $n = 3$; VT $n = 5$) ($^aP < 0.05$); C: PAS staining for peritubular injury in transplanted kidneys from mice treated with vehicle or VT; D: Semi-quantification of interstitial inflammation by surveying kidney cross-sections from transplanted mice ($^aP < 0.05$). Scoring was done as described above (Table 1) (vehicle $n = 3$; VT $n = 5$). Semi-quantification was assessed using Mann-Whitney test. VT: Vasculotide; PAS: Periodic acid Schiff.

Table 1 Scoring system for semiquantification of periodic acid Schiff staining

Interstitial inflammation	0 = no interstitial inflammation, < 5% of interstitium affected
	1 = mild interstitial inflammation, 5%-25% of interstitium affected
	2 = moderate interstitial inflammation, 25%-50% of interstitium affected
	3 = severe interstitial inflammation, 50%-75% of interstitium affected
	4 = very severe interstitial inflammation, > 75% of interstitium affected
Glomerular injury	0 = no glomerular injury
	1 = mild glomerular injury, < 10% of glomeruli damaged
	2 = moderate glomerular injury, 10%-50% of glomeruli damaged
	3 = severe glomerular injury, 50%-75% of glomeruli damaged
	4 = very severe glomerular injury, > 75% of glomeruli damaged

2B and D). These results suggest that two early doses of VT are sufficient to reduce infiltration of inflammatory cells into the graft thereby potentially preventing graft dysfunction and rejection.

VT reduces vascular inflammation and tissue infiltration

Keeping in mind the profound histological changes indicating that VT prevents infiltration of immune cells, we wanted to further analyze vascular inflammation and the infiltrative cell population. Therefore, we performed fluorescent immunohistochemistry for ICAM-1, for Gr-1 (a marker of granulocytes), as well as F4/80 (macrophages). Kidney cross-sections from VT-treated mice exhibit much less ICAM-1 expression than vehicle-treated mice (Figure 3A). Additionally, whole kidney homogenates also depicted less ICAM-1, as shown by immunoblotting (Figure 3D). Presumably as a consequence of less adhesion molecule expression we also noted a reduction of Gr-1 and F4/80 in the peritubular interstitium of VT-treated mice (Figure 3B and C). Together these data indicate that early VT regulates vascular adhesion molecule expression thereby reducing overwhelming tissue infiltration of inflammatory cells in the later post-transplant course.

VT ameliorates fibrosis progression

Fibrosis as a consequence of acute or chronic inflammation is a key contributor to organ dysfunction. After kidney transplantation we observed an increased expression of α SMA, a broad marker of fibrosis (Figure

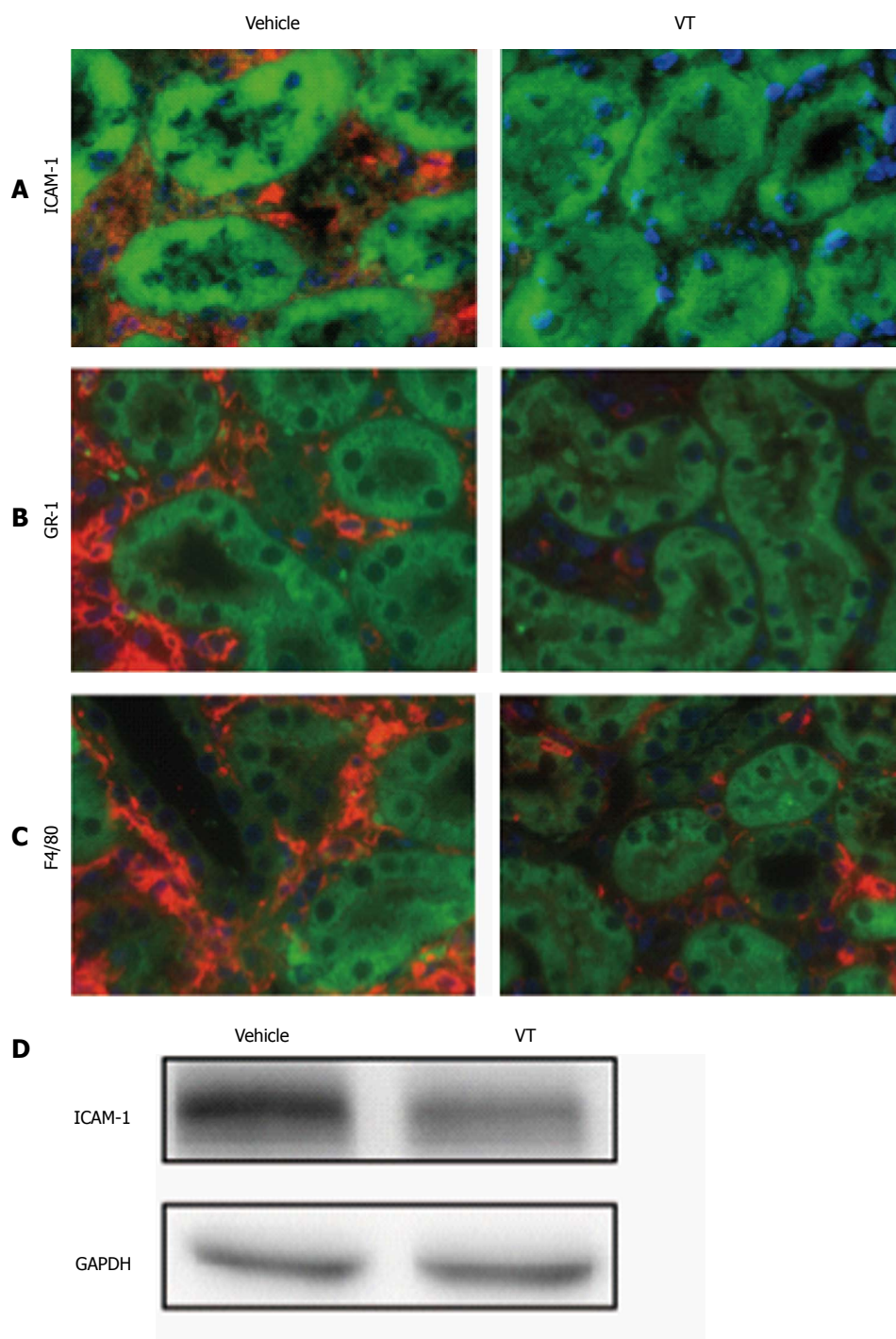


Figure 3 Vasculotide treatment reduces vascular inflammation and tissue infiltration in kidney transplantation. Fluorescent immunohistochemistry for (A) ICAM-1 (red), (B) Gr-1 (red) and (C) F4/80 (red) in kidney cross-sections of transplanted mice (vehicle or VT-treated) on day 28 after transplantation. Autofluorescence is shown in green. Images are exemplary for $n = 5$ /condition; D: Immunoblot of murine kidney homogenate for ICAM-1 and GAPDH for the same conditions. VT: Vasculotide; ICAM-1: Intercellular adhesion molecule; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

4A and B, left side). However, upon VT treatment tubular as well as glomerular damage were reduced with regard to α SMA expression (Figure 4A and B, right side). To further substantiate our finding, we visualized collagen fibers by Sirius red (Figure 4C) and observed profound differences between vehicle and VT-treated mice. VT appears to prevent inflammation-driven collagen formation. Pathological phosphorylation of SMAD3 a canonical downstream target of TGF β signaling after transplantation was also reduced in mice treated with

VT (Figure 4D). Taken together, VT might prevent the induction of TGF β signaling and collagen formation leading to reduced fibrosis and organ dysfunction.

VT does not prevent induction of inflammation on the transcriptional level

To further investigate the anti-inflammatory properties of VT in murine kidney transplantation, we analyzed different markers of inflammation (ICAM-1, VCAM-1, TGF β , collagen-1, collagen-3 and fibronectin) on the

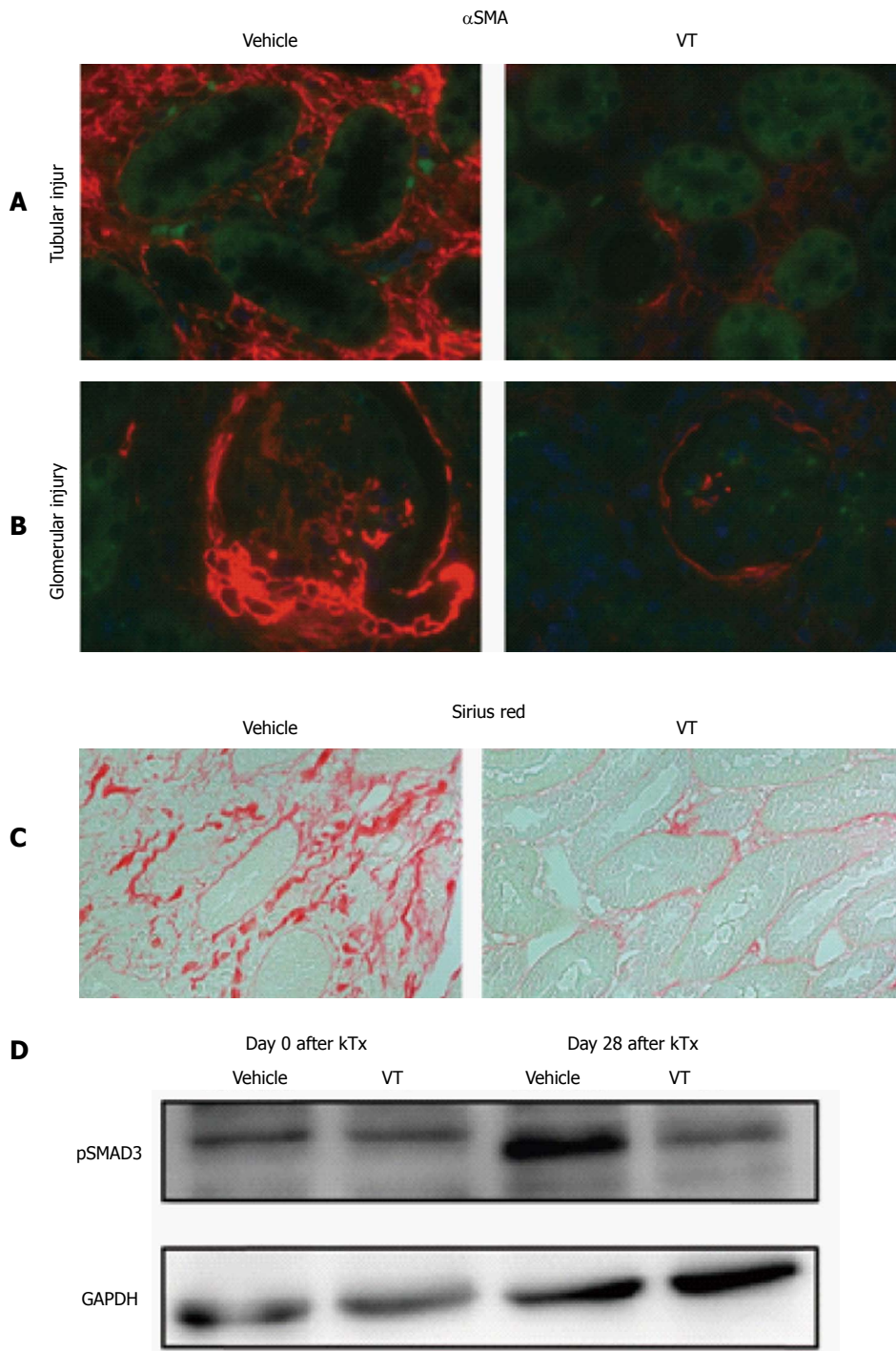


Figure 4 Vasculotide ameliorates fibrogenesis. Fluorescent immunohistochemistry of α -smooth-muscle-actin (red) regarding (A) tubular and (B) glomerular injury in kidney cross-sections of transplanted mice (vehicle or VT-treated) on day 28 after transplantation. Autofluorescence is shown in green. Images are exemplary for $n = 5$ /condition; C: Sirius red staining for the same conditions (D) Immunoblot of murine kidney homogenates for pSMAD3 in healthy (day 0) and kidney transplanted mice (day 28) upon vehicle or VT treatment. VT: Vasculotide; α SMA: α -smooth-muscle-actin; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

transcriptional level. Notably, we detected a dramatic upregulation of these markers in vehicle- and VT-treated animals on day 28 after transplantation compared to explanted donor kidneys on day 0. Despite the differences on protein level demonstrating that VT-treatment indeed reduces inflammation in a murine renal transplant model, differences on the transcriptional are not present on day 28 after transplantation (Figure 5 analyzed using Mann-Whitney test, $n = 3-5$).

DISCUSSION

The endothelium plays an important role in maintaining organ function and homeostasis in health and disease. As part of the rejection process of solid organ transplants, the endothelium is characterized by a highly activated proinflammatory phenotype. In routine kidney transplant pathology this has nowadays been implicated in the grading of rejection by using a so-called C4d

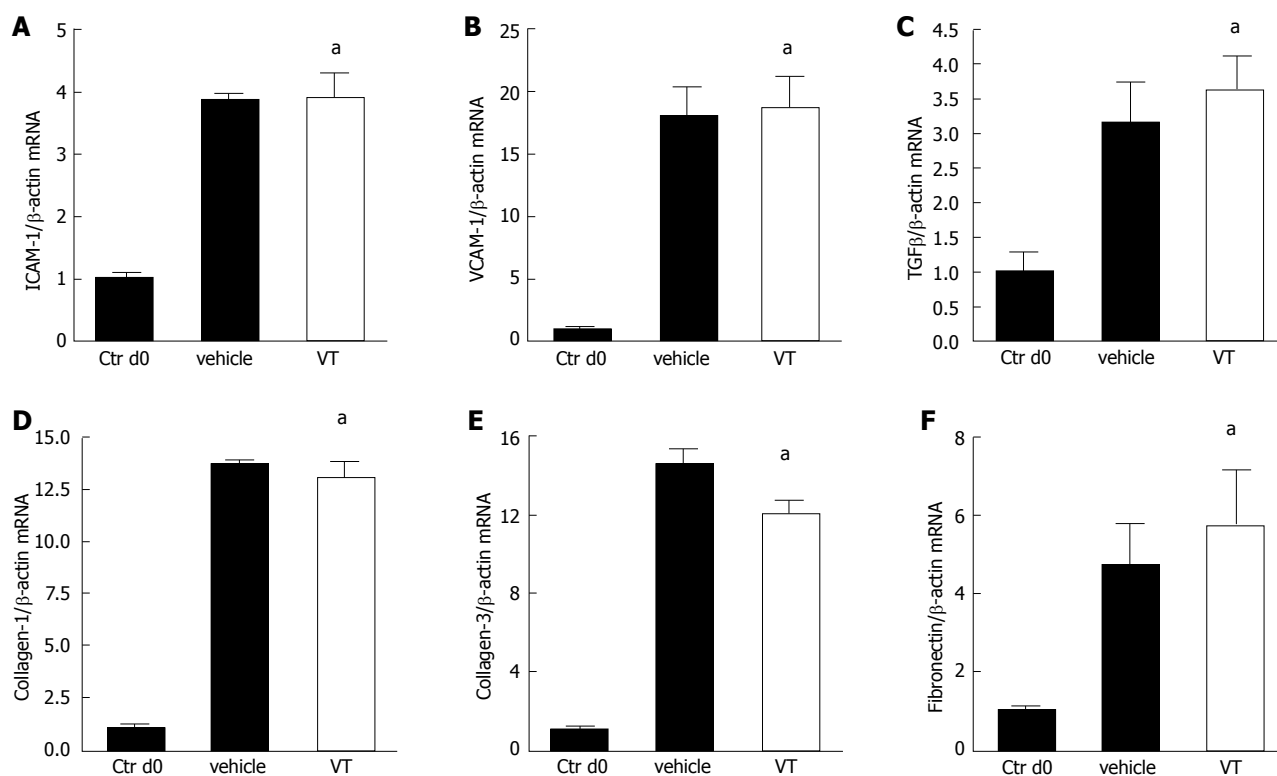


Figure 5 Anti-inflammatory properties of vasculotide are not regulated on the transcriptional level. A-F: Mice were treated with vehicle ($n = 3$) or VT ($n = 5$) and underwent kidney transplantation. Transplanted kidneys were harvested on day 28 after transplantation. Explanted donor kidneys from day 0 served as control ($n = 3$). Expression levels of Inter cellular adhesion molecule (ICAM-1), Vascular cell adhesion protein 1 (VCAM-1), Transforming growth factor β (TGF β), collagen-1, collagen-3 and fibronectin in kidney homogenates were determined *via* RT-qPCR and analyzed using Mann-Whitney test ($^aP < 0.05$). VT: Vasculotide.

staining that does reflect complement activation in the endothelium^[23]. We therefore hypothesized that pharmacological stabilization of the vasculature might be beneficial.

Our approach demonstrated that exogenous activation of the endothelium-stabilizing Tie2 receptor with the drug-like compound, termed VT, might prevent graft dysfunction and inflammation. Administration of VT showed trends toward improved organ function and survival in a renal transplant model. One beneficial effect of VT in kidney transplantation could be attributed to phosphorylation of the Tie2 receptor thereby activating the PI3K/Akt pathway and suppressing NF κ B signaling. This assumed canonical mechanisms of action of VT resulted in reduced tissue infiltration of immune cells and expression of endothelial adhesion molecules. Furthermore, early VT administration was sufficient to ameliorate classical fibrogenic signaling (e.g., SMAD3/TGF β) thereby reducing collagen formation and the development of fibrosis. Interestingly, we could not detect any transcriptional regulation of neither adhesion molecules nor inflammatory mediators. How VT regulates endothelial inflammation has to be thoroughly investigated in future projects.

Additionally, organ function in VT treated mice was slightly better at early time points exclusively. Due to the fact, that animals were treated at day 0 and 3 after transplantation, the beneficial effect of VT would be expected to decrease over time. Re-dosing could

further ameliorate outcome after kidney transplantation. To investigate and improve the beneficial effects of VT in kidney transplantation, pharmacokinetics of this Tie2 agonist need to be further investigated. Most experimental data on VT that showed improved outcome are derived from acute short-term injury models, such as sepsis and influenza^[19,21]. These data confirm however that the endothelium indeed plays an important role in the pathogenesis of various medical conditions and that maintaining endothelial homeostasis early in the pathogenesis might provide protection. Some work on slow-progressing disease models, such as diabetes and tumor growth used extensive re-dosing of VT to maintain beneficial effects at the highest possible level^[17,24].

Due to the small number of animals that survived until day 28, we were not able to include more animals into our studies. Nevertheless, our VT-treated mice show a clear trend towards improvement after kidney transplantation indicating a potential type II error in our statistical analysis.

Another aspect that we did not investigate but that is - at least theoretically - of high relevance is the putative long-term effect of an early short-term VT treatment. It might very well be that an improved early graft function has relevant implications for long term graft performance, as our histological data at day 28 suggest and as it has been demonstrated for delayed graft function^[25].

In summary, our study demonstrated that early VT treatment slightly improves graft function in an MHC-mismatched kidney transplant model potentially *via* regulation of endothelial activation and transmigration of harmful inflammatory cells into the transplant's interstitium. The Tie2 agonistic strategy might hold promise as a potential therapeutic in transplant medicine and future examination of long-term results are highly desirable.

ACKNOWLEDGMENTS

We thank Yvonne Nicolai for technical assistance.

COMMENTS

Background

Early graft dysfunction as well as acute rejection after solid organ transplantation are characterized by a proinflammatory microvascular endothelium. The Angiopoietin/Tie2 system plays an important role in maintenance of baseline endothelial barrier function and its response to injury. Activation (*i.e.*, phosphorylation) of the Tie2 receptor promotes endothelial homeostasis as well as anti-inflammatory properties. The authors therefore analyzed the potential of the Tie2 activating drug-like compound "vasculotide" (VT), as a novel therapeutic strategy in an MHC-mismatched renal transplant model.

Research frontiers

As long as the mystery of tolerance remains unsolved pharmacotherapy for transplanted patients is obligatory based on immunosuppressive regimens that come with a high burden of adverse events. Novel approaches aim to find therapeutic strategies that do not weaken the host or graft function. Here, the authors present a putative approach that promotes vascular stability/quiescence thereby preventing graft dysfunction and loss.

Innovations and breakthroughs

Targeting the endothelium as a direct interface between self and non-self offers the opportunity to interfere with graft specifically at the site of rejection.

Applications

The synthetic Tie2 agonist VT promotes vascular quiescence and improves graft function after allogeneic solid organ transplantation. The potency of VT has recently been demonstrated in different models of vascular diseases underlining its potential therapeutic relevance. In the future, toxicity studies and first clinical trials are planned, specifically for the treatment of acute kidney injury.

Terminology

Angpt: Angiopoietin; α SMA: α -smooth muscle actin; EC: Endothelial cell; ICAM-1: Intercellular adhesion molecule; LDH: Lactate dehydrogenase; VT: Vasculotide.

Peer-review

This is an interesting study in which authors show that VT - a synthetic Tie2 agonist- may improved renal transplant outcome.

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Retrospective Cohort Study

Thromboelastographic reference ranges for a cirrhotic patient population undergoing liver transplantation

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Abstract

AIM

To describe the thromboelastography (TEG) "reference" values within a population of liver transplant (LT) candidates that underline the differences from healthy patients.

METHODS

Between 2000 and 2013, 261 liver transplant patients with a model for end-stage liver disease (MELD) score between 15 and 40 were studied. In particular the adult patients (aged 18-70 years) underwent to a first LT with a MELD score between 15 and 40 were included, while

all patients with acute liver failure, congenital bleeding disorders, and anticoagulant and/or antiplatelet drug use were excluded. In this population of cirrhotic patients, preoperative haematological and coagulation laboratory tests were collected, and the pretransplant thromboelastographic parameters were studied and compared with the parameters measured in a previously studied population of 40 healthy subjects. The basal TEG parameters analysed in the cirrhotic population of liver candidates were as follows: Reaction time (r), coagulation time (k), Angle-Rate of polymerization of clot (α Angle), Maximum strength of clot (MA), Amplitudes of the TEG tracing at 30 min and 60 min after MA is measured ($A30$ and $A60$), and Fibrinolysis at 30 and 60 min after MA ($Ly30$ and $Ly60$). The possible correlation between the distribution of the reference range and the gender, age, MELD score (higher or lower than 20) and indications for transplantation (liver pathology) were also investigated. In particular, a MELD cut-off value of 20 was chosen to verify the possible correlation between the thromboelastographic reference range and MELD score.

RESULTS

Most of the TEG reference values from patients with end-stage liver disease were significantly different from those measured in the healthy population and were outside the suggested normal ranges in up to 79.3% of subjects. Wide differences were found among all TEG variables, including r (41.5% of the values), k (48.6%), α (43.7%), MA (79.3%), $A30$ (74.4%) and $A60$ (80.9%), indicating a prevailing trend to hypocoagulability. The differences between the mean TEG values obtained from healthy subjects and the cirrhotic population were statistically significant for r ($P = 0.039$), k ($P < 0.001$), MA ($P < 0.001$), $A30$ ($P < 0.001$), $A60$ ($P < 0.001$) and $Ly60$ ($P = 0.038$), indicating slower and less stable clot formation in the cirrhotic patients. In the cirrhotic population, 9.5% of patients had an r value shorter than normal, indicating a tendency for faster clot formation. Within the cirrhotic patient population, gender, age and the presence of hepatocellular carcinoma or alcoholic cirrhosis were not significantly associated with greater clot firmness or enhanced whole blood clot formation, whereas greater clot strength was associated with a MELD score < 20 , hepatitis C virus and cholestatic-related cirrhosis ($P < 0.001$; $P = 0.013$; $P < 0.001$).

CONCLUSION

The range and distribution of TEG values in cirrhotic patients differ from those of healthy subjects, suggesting that a specific thromboelastographic reference range is required for liver transplant candidates.

Key words: Thromboelastography; Liver cirrhosis; Blood coagulation disorder; Liver transplantation; Reference values

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Core tip: Thromboelastography provides a more comprehensive coagulation assessment than routine tests in cirrhotic patients. We evaluated the baseline thromboelastography (TEG) tracing and preoperative laboratory tests of cirrhotic patients undergoing liver transplant (LT) to generate a reliable picture of their coagulation profile. We also studied how TEG value distribution in cirrhotic patients could be modified by gender, age, model for end-stage liver disease score and liver disease characteristics. End-stage liver disease is associated with considerable changes in TEG variables, which should be allowed for when interpreting TEG traces in cirrhotic patients. TEG reference values derived from a healthy population could be misleading in the management of cirrhotic patients during LT.

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INTRODUCTION

Laboratory evaluations of bleeding disorders have been conducted with standard clotting assays such as prothrombin time (PT) and activated partial thromboplastin time (PTT) for a long time. However, standard laboratory tests fail to give comprehensive information about the bleeding tendency of cirrhotic patients. Tripodi *et al*^[1] showed that patients suffering from chronic liver disease as well as healthy subjects have the ability to generate the same amount of thrombin in stable liver disease conditions.

PT International Normalized Ratio (INR) tests performed in the absence of thrombomodulin are of little use in representing the real state of coagulation in cirrhotic patients. Furthermore, such tests are not standardized across centres when they are used for patients with liver disease^[2,3].

Because of these limits, the interest in assays performed with thromboelastography (TEG), which offers a more targeted approach to assess the overall outcome of the interactions of clotting factors beyond the initiation of clot formation, has progressively increased. However, even though thromboelastography is a useful tool for measuring global haemostasis during hepatic surgery and liver transplant, allowing the optimization of blood product selection and usage, its methodology is not standardized. Normal TEG values, as reported by manufacturers and in the literature, are determined from the average clotting time of healthy volunteers^[4]. Although investigators have tested the correlation between TEG values and the risk of bleeding in various surgical populations^[5,6], it is possible that standard TEG

cut-off values derived from a healthy population have a different and misleading meaning in the management of cirrhotic patients during liver transplantation (LT). Addressing the issue of the reference values, the TEG analyzer manufacturer suggests that each new user should test 20 healthy volunteers to generate normal values to be used locally as reference values at each institution, prior to clinical use^[7]. The consequence is that TEG suffers from a lack of proven reliability^[8,9], also motivated by the large range of normal values. However, this wide normal range defined for healthy people, is unreliable when applied to patients with liver disease, making it necessary to define thromboelastographic "reference ranges" for cirrhotic patients.

Under physiological conditions, the haemostatic system of these patients reaches a new equilibrium determined by a parallel decline of the pro- and anticoagulant drivers, which is represented by specific thromboelastographic values^[10]. The main aim of the present study was to describe the thromboelastographic preoperative coagulation condition of cirrhotic patients undergoing liver transplant to generate a more reliable picture of their common coagulation profile. A further aim of the study was to compare the TEG range distribution of cirrhotic patients with a population of healthy subjects, verifying that the range corrected for cirrhotic patients could be modified by gender, age and model for end-stage liver disease (MELD) score as well as liver disease characteristics.

MATERIALS AND METHODS

Between 2000 and 2013, 473 patients underwent LT in Liver Transplant Center of Policlinico di Modena (Italy). After the approval of the local Ethical Authority and the receipt of written informed consent, the thromboelastographic parameter distribution of a selected population of cirrhotic patients was studied according to the following inclusion and exclusion criteria: adult patients (aged 18-70 years), first LT, and MELD score between 15 and 40. The exclusion criteria were as follows: acute liver failure, congenital bleeding disorders (*i.e.*, haemophilia A and B), and anticoagulant and/or antiplatelet drug use. Therefore, the analysis was performed in 261 (55%) patients who underwent LT. A MELD score between 15 and 40 was chosen because it is the most frequently used in the literature, and the AISF (Italian Association for Liver Study) also recommends it for listing a patient for LT^[11]. In this population of cirrhotic patients, preoperative haematological and coagulation laboratory tests were collected, and thromboelastographic traces were studied and compared with those obtained from a previously studied population of 40 healthy subjects. The study protocol approved by the Institutional Review Board of Azienda Ospedaliera-Universitaria, Modena (N°:139/14 TRIGGER) was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Blood samples were collected with the double-syringe technique from a clean venipuncture. The first 6 mL of each sample was discarded. All the healthy subjects (20 males and 20 females), selected from among residents, students and nurses, had not taken drugs known to affect coagulation parameters or platelet aggregation for at least 1 wk before the collection of blood samples.

Distribution ranges of the basal TEG parameters (r , k , α , MA , $A30$, $A60$, $Ly30$ and $Ly60$) in the cirrhotic population of patients were analysed. The possible correlation of the distribution of reference ranges with gender, age, MELD score (higher and lower than 20) and indications for transplantation (liver pathology) were also investigated. In particular, a MELD cut-off value of 20 was chosen to verify the possible correlation between thromboelastographic reference range and MELD score. This cut-off is the most frequently used parameter in the literature for predicting mortality risk after LT^[12,13]. Two TEG® 5000 Hemostasis Analyzers (Haemoscope Inc., Skokie, Illinois, United States) were used. The strength of clot formation is graphically represented over time as the tracing shown in Figure 1.

Maintenance and quality controls were performed daily in accordance with manufacturer recommendations. Native arterial blood samples were collected from a radial artery cannulated before induction of anaesthesia and were analysed without adding anticoagulant or activator. We routinely use heparinase TEG, only after reperfusion in all cases and from the baseline only in patients with fulminant liver failure.

Blood samples were always handled by the same three anaesthesiologists. TEG tracings were started within 4 min after sampling. Clot formation was triggered by contact activation. TEG tracings were displayed before the surgical procedure in the operating room. Parameters normally used to assess the process of coagulation are as follows^[8,14]: r (*coagulation time*) is the time from the start of the TEG tracing until the TEG trace amplitude reaches 2 mm. This represents the rate of initial fibrin formation and is functionally related to plasma clotting factors and circulating inhibitor activity. Prolongation of the r time may be a result of coagulation factor deficiencies or severe hypofibrinogenemia; k (*Clot Formation time*) is measured from r to the point where the amplitude of the tracing reaches 20 mm. This is the time taken to reach a standard clot firmness and is affected by the activity of the intrinsic clotting factors, fibrinogen and platelet; α *Angle* (Angle-Rate of polymerization of clot) is the angle formed by the slope of the TEG tracing from the r to the k value. This represents the rate of clot growth and describes the polymerization of the structural elements involved in clotting^[15]; MA (Maximum Clot Firmness) is the maximum amplitude of the TEG tracing. This reflects the strength of the clot and is a direct result of the function of platelets and plasma factors and their interaction; the $A30$ and $A60$ parameters are the amplitudes of the TEG tracing at 30 min and 60 min after MA is measured; the

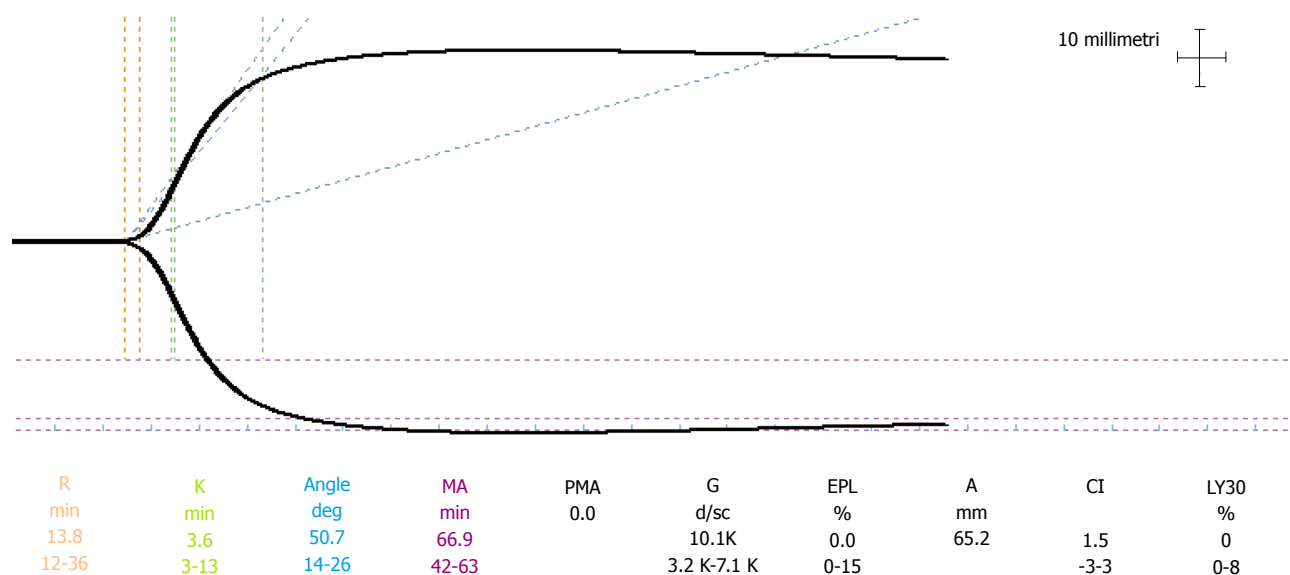


Figure 1 Normal trace. The reference ranges are those defined by manufacturer thromboelastography® 5000 Hemostasis Analyzers (Haemoscope Inc., United States). PMA: Projected MA.

Table 1 Demographic and laboratory data of the Patient Population and their indication for liver transplantation

Study group (n = 261)	
Males/females (n/n), %	(193/68) 73.9%/26.1%
Age (yr)	53.5 ± 9.4
Body mass index (kg/m ²)	26.18 ± 6.40
MELD score	24 ± 6.5
Indication for liver transplantation (n, %)	
Alcoholism	40 (15.3 %)
Viral	189 (72.4%)
Colestatic	15 (5.7%)
Other	17 (6.5 %)
HCC	107 (41 %)
Laboratory data	
Hb (g/dL)	11.3 ± 2.2 (nv:12-16)
Hct (%)	3.4 ± 6.2 (nv: 36-46)
PLT (10 ³ /μL)	83.2 ± 66.7 (nv: 150-450)
PT (%)	53.6 ± 22.4 (nv: 70-100)
INR	1.7 ± 0.7 (nv: 0.84-1.24)
aPTT ratio	2.0 ± 9.3 (nv: 0.82-1.24)
Fibrinogen (mg/dL)	190 ± 120 (nv: 200-400)
ATIII (%)	50 ± 27(nv: 80-120)

Data are expressed as the median ± SD. MELD: Model for end stage liver disease; HCC: Hepatocellular carcinoma; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; nv: Normal values.

Ly30 and Ly60 (Fibrinolysis at 30 and 60 min after MA) parameters measure percent lysis at 30 and 60 min after MA is reached. The Ly30 and Ly60 measurements are based on the reduction of the area under the TEG tracing from the time MA is measured until 30 (or 60) min after the MA.

Statistical analysis

Continuous data are reported as the mean ± SD (range) and/or median (reference ranges) and were compared using the two-sided Student's *t* test for normally distributed parameters. Continuous non-

normally distributed data were compared using the Wilcoxon-Mann-Whitney test. Comparisons between groups for categorical variables were performed using the χ^2 test with Yates' correction or Fisher's exact test when appropriate. Descriptive methods were used to calculate the 2.5% and 97.5% percentiles according to the NCCLS guidelines to establish reference ranges^[16]. Reference ranges were not calculable for groups of less than 40 cases. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 19.0., IBM Corp., Armonk, NY. The statistical review of the study was performed by a biomedical statistician.

RESULTS

The demographic profiles and laboratory data of the patient population and their indication for LT are shown in Table 1.

Reference value distribution in the whole population

Median, minimum and maximum value and reference ranges, for the whole population of cirrhotic patients undergoing LT and comparison with healthy subjects, are presented for *r*, *k*, α , MA, A30, A60, Ly30 and Ly60 in Table 2.

Most TEG reference values from patients with end-stage liver disease (ESLD) were found to be outside the suggested normal ranges and were abnormal in up to 79.3% of subjects. Wide differences were found for all TEG variables, including *r* (41.5% of the values), *k* (48.6%), α (43.7%), MA (79.3%), A30 (74.4%) and A60 (80.9%), indicating a prevailing trend to hypocoagulability. The differences between mean TEG values obtained from healthy subjects and the cirrhotic population were statistically significant for *r* ($P = 0.039$), *k* ($P < 0.001$), MA ($P < 0.001$), A30 ($P < 0.001$), A60

Table 2 Medians, means, ranges and reference ranges (2.5%-97.5% percentiles) for thromboelastographic variables obtained from the study population (261 cirrhotic patients) and from the 40 healthy patients

	<i>r</i> (min)	<i>k</i> (min)	α (degree)	MA (mm)	A(30) mm	A(60) mm	Ly30 (%)	Ly60 (%)
Cirrhotic patient population (<i>n</i> = 261)								
Reference values	6.2-58.5	4.2-39.2	3.4-42.8	10.4-63.5	9.8-62	92-62	0-4	0-10
Mean \pm SD	23.7 \pm 12.5	14.9 \pm 9.6	18.2 \pm 10	35.3 \pm 12.8	33.8 \pm 12.8	32.3 \pm 12.6	0.38 \pm 1	2.28 \pm 4.3
Median (range)	21.8 (2.2/75.4)	12.3 (1.6/68.1)	16.1 (1.7/67)	33.6 (2.2/71.9)	33 (2/86)	31 (2.2/85.5)	0.0 (0/11)	0.40 (0/44)
Healthy population (<i>n</i> = 40)								
Reference values	11-26	3-14	15-46	43-64	41-64	42-63	0-4	0-5
Mean \pm SD	19.6 \pm 1.3	9.8 \pm 0.9	20.6 \pm 1.2	43.7 \pm 2.9	43.2 \pm 3.1	42.9 \pm 0.8	0.8 \pm 2.5	0.9 \pm 2.1
Median (range)	17.8 (8-27)	7.2 (2-15)	18.1 (13-48)	41.5 (41-66)	42 (39-67)	41.7 (41-65)	0.7 (0-5)	0.76 (0-7)
¹ <i>P</i>	0.039	< 0.001	0.131	< 0.001	< 0.001	< 0.001	0.06	0.038
Number of tests below normal	25 (9.5%)	2 (0.76%)	112 (42.9%)	200 (77%)	192 (74%)	207 (79%)	0	0
Number of tests above normal	84 (32%)	125 (47.9%)	2 (0.8%)	6 (2.3%)	5 (1.9%)	5 (1.9%)	2 (0.76%)	28 (10.7%)
Total number of tests outside the healthy population range	109 (41.5%)	127 (48.6%)	114 (43.7%)	206 (79.3%)	197 (74.4%)	212 (80.9%)	2 (0.76%)	28 (10.7%)

Number of test results outside the normal reference range proposed by the manufacturer. *r*: Time to initial fibrin formation; *k*: Time to clot formation; α : Alpha angle, rate of clot formation; MA: Maximum amplitude, absolute clot strength; A30: Maximum amplitude at 30 min after MA; Ly30: Fibrinolysis at 30 min after MA; Ly60: Fibrinolysis at 60 min after MA; ¹*P* value expresses the significant differences between the mean values obtained from the study population and from the healthy population.

(*P* < 0.001) and Ly60 (*P* = 0.038), indicating slower and less stable clot formation in cirrhotic patients (Table 2). In the cirrhotic population 25 (9.5%), patients had *r* values shorter than normal, indicating a tendency to faster clot formation.

Reference values distribution according to patient gender, age and liver disease characteristics

A comparison of the average values of TEG parameters in the cirrhotic patient population did not show any statistically significant difference for gender and age (Table 3). Gender and age were not significantly associated with greater clot firmness or with enhanced whole blood clot formation (Table 3).

Patients with a MELD score less than 20 showed greater clot firmness (higher MA, A30 and A60) compared with patients with a MELD score above 20, with MA (*P* < 0.001), A30 (*P* < 0.001) and A60 (Table 3, *P* < 0.001).

As shown in Table 3, the presence of hepatocellular carcinoma (HCC) or alcoholic cirrhosis did not result in faster coagulation activation (shorter *r* and *k*) or greater clot firmness (higher MA, A30, or A60). Patients with a MELD score under 20 showed no thromboelastographic difference based on the presence of HCC. Patients with HCV-related cirrhosis did not show faster activation of the coagulation process but showed significantly greater clot firmness compared with the other patients enrolled in the study because of end stage liver disease, according to MA (*P* = 0.013), A30 (*P* = 0.021) and A60 values (*P* = 0.023). Instead, hepatitis B virus-related cirrhosis did not appear to have any significant influence on clot activation or strength.

The clot strength of patients transplanted for cholestatic disease was enhanced (higher MA, A30, and A60; all with *P* < 0.001) compared with patients without cholestatic liver disease, and activation of the coagulation process did not result in faster activation

(Table 3).

DISCUSSION

Several authors have found a relatively poor correlation between bleeding and laboratory indices of coagulation in patients with chronic liver disease^[17,18]. INR and PTT explore only the first 5% of whole thrombin formation^[19,20] and are performed without adding thrombomodulin, making these techniques less optimal for exploring the physiological mechanisms regulating thrombin formation. The inadequacy of laboratory methods and the production of technologies applied to blood coagulation analysis have increased interest in thromboelastography for the management of acute peri-operative bleeding^[21-24]. TEG offers a rapid and global view of the coagulation processes^[15,25-27], but in spite of these advantages, users should keep in mind the poor reproducibility, the wide boundaries of normality, the lack of standardization^[8,28] and the need to define local normal ranges^[28].

Although TEG is a useful viscoelastic test for haemostatic monitoring, interpretation of its results requires care. In particular, the normal ranges of TEG variables may not apply under different operating and patient conditions such as in the cirrhotic patient population.

In the present study, we determined the range of distribution for TEG variables in a population of patients receiving a first liver graft for ESLD or HCC, with a MELD score between 15 and 40. We also underlined the differences in TEG values obtained from cirrhotic patients from those recorded in the normal, healthy population. In the cirrhotic population the *r* and *k* values were above the upper limit of normality in 32% and 47.9% of the population, respectively, indicating significant reduced activation of clot formation. In our population, the mean plasma fibrinogen concentration, PT, INR, aPTT and platelet number were outside

Table 3 Median and reference ranges for thromboelastography assay in the study population according to gender, age, model for end-stage liver disease, liver disease and presence of hepatocellular carcinoma

	<i>r</i> (min)	<i>k</i> (min)	α (degree)	<i>MA</i> (mm)	<i>A</i> (30) mm	<i>A</i> (60) mm	<i>Ly30</i> (%)	<i>Ly60</i> (%)
Females (<i>n</i> = 68)	22.7 (7.6-58.6)	12.5 (3-38.5)	16.5 (4.1-52.2)	38.1 (10.3-70)	37.7 (8.5-71.1)	35.5 (6.7-71.1)	0.0 (0-4)	0.25 (0-26.5)
Males (<i>n</i> = 193)	22.8 (5.8-61.5)	13.5 (3.2-44.9)	15.8 (3.9-49.8)	34 (8.1-71.2)	33.4 (8.1-75)	3.3 (6.7-75)	0.0 (0-4)	0.4 (0-10)
<i>P</i>	0.9	0.97	0.74	0.57	0.37	0.29	0.64	0.9
< 60 yr (<i>n</i> = 181)	21 (5.1-57.6)	12.2 (4.1-40.9)	16.7 (3.7-42.9)	32.5 (10.4-62.6)	32 (9.8-59.4)	30.2 (9.2-57.7)	0.0 (0.0-4.1)	0.2 (0.0-9.8)
≥ 60 yr (<i>n</i> = 80)	22.7 (10.2-65.1)	13 (5.3-40)	15.6 (2.4-35.4)	37.8 (6.7-70.7)	37.2 (6.7-70.7)	35.2 (6.7-70.7)	0.0 (0-3.5)	0.2 (0-9.8)
<i>P</i>	0.08	0.8	0.1	0.2	0.2	0.12	0.76	0.54
MELD < 20 (<i>n</i> = 90)	19.4 (8-59.8)	11.6 (2.6-40.5)	18.3 (4.1-56.8)	38.9 (19.9)	38.4 (17-69.6)	35.9 (8.2-71.6)	0.0 (0-4.9)	0.8 (0-25.3)
MELD ≥ 20 (<i>n</i> = 171)	22.3 (5.7-58.6)	13 (4.4-40.3)	15.4 (3.2-42.2)	31.3 (9-62.2)	31 (9.1-61.5)	30 (9.1-60)	0.0 (0-4)	0.10 (0-9.6)
<i>P</i>	0.9	0.66	0.07	< 0.001	< 0.001	< 0.001	0.19	0.76
Not alcoholic (<i>n</i> = 216)	21.2 (5.6-58.7)	13.1 (4.1-41)	15.4 (1.8-32.4)	30.2 (2.8-70.4)	30 (2.8-70.4)	29 (2.8-70.4)	0.0 (0-1.4)	0.3 (0-6.9)
Alcoholic (<i>n</i> = 45)	22.5 (10.4-63.3)	12.8 (3.6-30.8)	15 (2.2-45.3)	33.9 (5.7-64.2)	33.8 (5.7-64.2)	33.1 (5.7-63.9)	0 (0-10.4)	0.4 (0-42.5)
<i>P</i>	0.68	0.81	0.2	0.16	0.18	0.19	0.95	0.97
HCV absence (<i>n</i> = 111)	21.8 (7.4-68.5)	11.5 (4.5-54.4)	16.7 (3-40.6)	37.6 (8.9-70.1)	36.6 (8.9-70.1)	33.9 (8.9-68.6)	0.0 (0.0-3.6)	0.4 (0-9.4)
HCV presence (<i>n</i> = 150)	21.5 (5.2-53.7)	13.1 (4.1-36)	15.7 (4-43.3)	31.4 (10.5-59.3)	30.9 (9.9-59.1)	30 (8.5-57.7)	0 (0-4.2)	0.15 (0-13.9)
<i>P</i>	0.31	0.62	0.43	0.013	0.021	0.023	0.65	0.43
HBV absence (<i>n</i> = 206)	22.0 (6.4-57.9)	13 (4.2-38.1)	16 (3.8-42.8)	33.9 (10.2-65.8)	33.6 (9.1-67.3)	31.2 (7.4-67)	0 (0-4)	0.3 (0-10.4)
HBV presence (<i>n</i> = 55)	21 (3.8-65)	11.2 (2-67.3)	16.7 (3.2-56.1)	33.3 (10.7-55.7)	32.5 (10.7-55.6)	30.1 (10.7-53.4)	0 (0-4.1)	0.4 (0-14.4)
<i>P</i>	0.34	0.4	0.36	0.25	0.2	0.16	0.74	0.99
Not cholestatic (<i>n</i> = 246)	21.7 (6.2-57.9)	12.3 (4.1-40)	15.5 (3.4-42.4)	33 (10.2-58.6)	32.1 (9.6-58.5)	30.2 (9-57.1)	0.0 (0-3.9)	0.4 (0-10.4)
Cholestatic (<i>n</i> = 15)	22.2 (NA)	11.5 (NA)	18.1 (NA)	53.4 (NA)	53.4 (NA)	53.3 (NA)	0.0 (NA)	0.3 (NA)
<i>P</i>	0.15	0.53	0.38	0.001	0.001	0.001	0.26	0.5
HCC absence (<i>n</i> = 154)	2.7 (7.6-65.6)	12.2 (4.2-42.2)	16.5 (3.1-42.9)	33.9 (10.7-65.1)	33 (10.7-62.8)	30.8 (9.7-62.8)	0 (0-4)	0.4 (0-9.1)
HCC presence (<i>n</i> = 107)	23.2 (4.8-55.8)	13 (4.1-39.6)	15.8 (3.4-42.8)	33.3 (9.3-64.9)	33.3 (8-64.9)	31.5 (8.3-64.6)	0.0 (0-3.4)	0.1 (0-12.5)
<i>P</i>	0.56	0.6	0.3	0.76	0.84	0.82	0.6	0.87

MELD: Model for End-stage Liver Disease; HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; *r*: Time to initial fibrin formation; *k*: Time to clot formation; α : Alpha angle, rate of clot formation; *MA*: Maximum amplitude, absolute clot strength; *A30*: Maximum amplitude at 30 min after *MA*; *Ly30*: Fibrinolysis at 30 min after *MA*; *Ly60*: Fibrinolysis at 60 min after *MA*.

the normal laboratory reference range, indicating a reduction in clotting factors and platelet number, which are typical features of ESLD and could be a possible explanation for prolonged *r* and *k* values. The heparin-like effect (HLE) may also be another possible explanation for the longer *r* time recorded in the baseline tracings. This effect is not often represented in the first basal tracing (before the beginning of the surgical operation) and is usually less pronounced than that observed after reperfusion or in patients with acute liver failure^[29]. Because only 6% of patients undergoing LT have a severe HLE at baseline, which does not seem to correlate with an increase in blood requirements^[30], we do not usually perform this test at baseline, and we can only argue that a basal prolongation of the *r* time may more often be related to coagulation factor deficiencies or hypofibrinogenemia than to HLE, as shown in the laboratory data.

If a large percentage of *r* and *k* values were abnormally prolonged, then in 58.5% and 51.4% of cases, the same parameters were within the range of normality, expressing normal clot activation and firmness. This observation is in line with Stravitz' study^[31] that showed that the mean and median TEG parameters were within normal limits in a cohort of 273 patients with stable cirrhosis. Nevertheless, we studied a population of patients with decompensated cirrhosis, and we observed normal coagulation parameters in half of the cases and a shorter than normal *r* value in 9.5% of cases, indicating a tendency to faster clot activation.

These observations are in line with the new concept of rebalanced haemostasis, which better describes the coagulation condition of cirrhotic patients and is usually not represented in conventional laboratory tests^[10]. However, the haemostatic balance in a patient with liver disease is relatively unstable as evidenced by the occurrence of both bleeding and thrombotic complications^[27]. The shorter *r* values observed in 9.5% of patients could indicate cirrhotic patients' tendency to develop thromboembolic complications at appreciable rates (between 0.5% and 1.9%)^[32,33]. Another observation derived from the comparison of the two studied groups was the reduced clot firmness observed in the cirrhotic patient group. *MA*, *A30* and *A60* values were below the lower limit of normality for healthy people in up to 77%, 74% and 79% of patients, respectively. Thrombocytopenia, a typical feature of chronic liver disease^[34,35], may justify the high number of patients with lower values of *MA*, *A30* and *A60* compared with the normal population. Thrombocytopenia, *i.e.*, platelet counts between 30 and 100 × 10⁹/L^[36], is usually a sign of advanced liver atrophy^[37] and is frequently observed in cirrhotic patients arriving in the operating room for LT. Because of increased levels of von Willebrand factor and low levels of ADAMTS 13 metalloproteinase, cirrhotic patients can compensate for platelet abnormalities^[38]. Another possible explanation for these deteriorating TEG parameters may be the hypo- and dysfibrinogenemia associated with liver disease^[39,40]. In our patient population, the mean pre-

operative platelet number was $83.2 \pm 66.7 \times 10^9/L$, which has been shown in experimental observations to be sufficient to secure *in vitro* thrombin generation^[41], whereas the mean plasma fibrinogen concentration was 190 ± 122 mg/dL, a value that can require correction in cases of severe bleeding^[24]. So, a possible explanation for the reduced MA amplitude observed in the study could be a reduction in plasma fibrinogen concentration or fibrinogen function. Specific thrombelastographic tests^[42,43] may be helpful for determining the combined effects of thrombocytopenia and hypofibrinogenaemia. Unfortunately, we have only been using TEG functional fibrinogen assays to detect signs of functional fibrinogen deficit in our intraoperative management since 2013, and we did not have enough data to identify the role of platelets and fibrinogen in determining MA amplitude.

Ly30 and *Ly60*, unlike the other parameters studied, have been shown to differentiate between the values recorded in healthy patients in a smaller number of subjects. *Ly30* and *Ly60* reference ranges were different from the healthy population in 0.76% and 10.7% of samples that were above the upper limit of normality. Cirrhosis has been variably associated with an increased tendency to fibrinolysis; however, hypofibrinolysis can also be the result of reduced levels of plasminogen and increased levels of plasminogen activator inhibitor^[34].

Therefore, although contrasting results have been reported, the balance of fibrinolytic processes is most likely restored in patients with liver disease by the parallel changes in the circulating levels of pro-fibrinolytic and anti-fibrinolytic agents^[18]. This phenomenon could explain the low number of patients who showed abnormal *Ly30* and *Ly60* values. During liver transplant, primary hyperfibrinolysis may occur in up to 60% of cases but is usually confined to the phase of hepatectomy and reperfusion^[44,45].

Because of the unique haemostatic behaviour of cirrhotic patients, specific thrombelastographic ranges have to be considered when managing liver transplant patients. Even if it was not the point of the study to demonstrate the clinical advantage of interpreting the TEG traces, taking into account the "reference ranges" for cirrhotic patients in term of blood products saving, we think that when managing bleeding during surgery, it would most likely be useful to correct TEG values while keeping in mind the reference ranges for this category of patients and not for healthy patients.

Realizing the wide variation in patient characteristics and in the causes of ESLD, we divided our cirrhotic population into subgroups of patients based on gender, age, MELD score and liver disease characteristics. For the potential effect of gender on TEG values, our analysis did not find any difference in coagulation activation and in clot firmness between females and males. Our results do not support the findings of Gorton *et al*^[46] who showed enhanced coagulation activity in females with non-activated thromboelastography. Chronic liver disease induces a severe dysfunction of sex hormone metabolism, causing feminization in men

and infertility and amenorrhoea in women^[47]. This may explain the absence of difference in coagulation activation between males and females observed in our study. Lang *et al*^[48] showed small differences in ROTEM variables between males and females that were not always statistically significant and argued that a sex-related definition of reference ranges in thromboelastometry is not necessary.

For age, we were not able to find any thromboelastographic signs of increased coagulability related to advanced age as otherwise described by Ng *et al*^[49] who showed that hypercoagulability increases progressively beyond age sixty. In our study, *r*, *k*, α and *MA* were not dependent on age. The variables are functionally related to levels of plasma clotting factors, fibrinogen, platelets and activity of circulating inhibitors. It is possible that hypercoagulability, which is usually associated with advancing age due to increased plasma concentrations of fibrinogen, factor VII and factor IX, has not been observed in aged patients because of ESLD and coagulation factor synthesis impairment^[49,50].

In accordance with another study^[15], we found significantly higher clot firmness in cholestatic patients compared with cirrhotic patients undergoing liver transplant for other causes. Usually, patients with cholestatic cirrhosis show higher fibrinogen levels as well as stable or even increased platelet function^[51], which can justify the significantly higher clot firmness observed in the group of patients transplanted for cholestatic disease.

Patients with HCV-related cirrhosis showed a significant tendency towards higher clot firmness (higher *MA*, *A30* and *A60*), which was not observed in patients without HCV infection. In HCV liver diseases, Panasiuk *et al*^[52] showed evidence of *in vivo* platelet activation, as suggested by the increased concentrations of b-thromboglobulin and platelet factor 4 in serum. Furthermore, plasma-soluble P-selectin levels have been shown to be markedly elevated in chronic hepatitis C^[53], and this infection might be directly responsible for *in vivo* platelet activation and for the higher *MA* values observed in patients suffering from this disease.

The presence of HCC nodules has been associated by Samonakis *et al*^[54] and by Krzanicki *et al*^[55], even if with a very low prevalence of hypercoagulability, with a thrombophilic tendency and with thrombotic complications. For this reason, we would have expected to see faster coagulation activation (shorter *r*) and/or greater clot firmness (higher *MA*), but we did not observe any signs of hypercoagulation. HCC did not appear to be responsible for a higher thrombophilic tendency in the study population, even in subgroups of patients with a low MELD score (15-20) and a minor coagulation impairment.

Patients affected by alcoholic or hepatitis B cirrhosis did not show any significant difference in clot formation or strength.

Cirrhotic patients with a MELD score under 20 had significantly better *MA*, *A30*, and *A60* values than

patients with a score above 20 ($P < 0.001$), which could be an expression of greater stability of the clot related to less severe liver disease and better coagulation function^[56,57]. In particular, r , k , and α were within normal limits, although the maximum amplitude was decreased. As previously showed by Stravitz *et al.*^[31] in patients with stable cirrhosis, global haemostasis is maintained, while the mean maximum amplitude of clot formation can be below normal limits. Our cohort of patients with a MELD score less than 20 represents a lower grade of liver disease severity and, for this reason, is more similar to the results described by Stravitz.

Our study showed how TEG value distribution in patients with ESLD is very different from that obtained from a healthy population. The coagulation system in healthy patients is characterized by a greater functional reserve of both procoagulants and anticoagulants, and it is unlikely that the thromboelastographic reference ranges of a healthy population are also representative of patients with ESLD. In healthy people, "normal" range also means normal coagulation balance. Patients with liver disease may show a satisfactory coagulation balance without spontaneous bleeding, even if their TEG values are outside the normal ranges observed in healthy people. However, this was a descriptive and not an outcome study, and we think that this study's findings should always be kept in mind when TEG data are interpreted in patients with ESLD. It was not possible to directly demonstrate the clinical effect of interpreting the TEG in cirrhotic patients with or without taking these "normal" variations into account. Thromboelastographic ranges in liver transplant candidates are so different from normal subjects that specific ranges for cirrhotic patients have to be defined. Because of the unique coagulation condition of cirrhotic, TEG ranges representative of this category of patients, have probably to be considered in all bleeding conditions avoiding to correct these parameters to normal TEG ranges for healthy patients. In the last few years, several transfusion algorithms have been proposed, aiming at developing a better treatment for haemostasis in patients with coagulopathy and bleeding, but none of these algorithms have been built using values typically obtained from cirrhotic patient candidates. For this reason, our group has already shown how specific thromboelastographic cut off values, adapted for cirrhotic patients, can be used to guide blood product infusions before invasive procedures, ensuring patient safety and avoiding bleeding episodes^[58]. Similarly, Wang *et al.*^[21] showed that TEG values higher than normal in transplant recipients may not have a reliable predictive value of increased blood loss during surgery. In their study, the authors adopted a TEG-guided transfusion protocol using higher threshold values to initiate transfusions, without observing any negative consequences. Therefore, standard TEG values obtained from healthy volunteers may be misleading for patients with liver disease.

This study presents the following possible limitations: TEG suffers from a lack of proven standardization^[8,9],

and pre-analytical factors such as sampling and sample handling could play a significant role in coagulation testing. Due to the manual steps, such as placement of pin and cup or pipetting a sample, operator-to-operator variability had to be considered. Another possible limitation is that the range of distribution described in this population could most likely only be applied to our reality and is not necessarily representative of other liver transplant centres.

In conclusion, the comparison between thromboelastographic parameters of cirrhotic patients and those of healthy subjects have shown many differences that are the ultimate expression of the different coagulation balance typical of cirrhotic subjects. The analysis of the cirrhotic population has also demonstrated how a MELD score greater than 20 and HCV infection-related cirrhosis may be related to the formation of a less stable clot, and patient candidates for LT due to cholestatic liver diseases are capable of forming more stable and durable clots. The TEG values described in this population of candidates for liver transplantation, although very different from those of a healthy population, are however an expression of a new haemostatic balance that cirrhotic patients reach and, in conditions of stability, does not result in spontaneous bleeding. The observation of a shorter than normal r value in 10% of cirrhotic patients should make the reader remember that such a population of patients can face thrombotic as well as haemorrhagic problems during surgery because of their unstable haemostatic balance. Determining a range of distribution for TEG values in a very specific population of cirrhotic patients could be important for the implementation of a transfusion protocol based on a point-of-care device that could help in properly guiding coagulation therapy. If the imperative is the correction of the thromboelastographic parameters only in the presence of active bleeding, aiming to restore TEG values to those suggested as "normal" could lead to an over-correction of the coagulation abnormalities typical of cirrhotic patients. This hypothesis needs to be confirmed by detailed clinical trials on the medical utility of new TEG reference ranges for the management of perioperative haemostasis in cirrhotic patient clinical settings.

COMMENTS

Background

Standard laboratory tests (international normalized ratio, activated partial thromboplastin time) fail to give comprehensive information about the bleeding tendency and coagulation status of cirrhotic patients because they are not standardized across centres when used for patients with liver disease and are performed in the absence of thrombomodulin. All of these limits have progressively increased the interest in thromboelastography (TEG), which assesses the overall coagulation process beyond the initiation of clot formation. However, this methodology is not standardized, and when defining reference values, the TEG analyzer manufacturer suggests that each new user should test 20 healthy volunteers to generate "his own" normal values to be used locally as reference values. The normal TEG values reported by manufacturers and the literature are determined from the average clotting time of healthy volunteers, making them unreliable and potentially misleading in the

management of patients with liver disease. It is very important to try to generate a more reliable picture of a common cirrhotic patient coagulation profile to properly manage these patients during liver transplant (LT).

Research frontiers

Many publications have shown that TEG-based transfusion algorithms are useful in the management of blood products during LT, but the proposed cut-off value for transfusion is subject to great variability. The values proposed as indices of transfusion are often detected in patients with cirrhosis without being associated with bleeding. In this study, similar to reference values obtained from healthy people, the authors tried to study TEG value distribution in a group of patient candidates for LT. Stravitz, in a cohort of 273 patients with stable cirrhosis, found that the mean and median TEG parameters were within normal limits, although the maximum amplitude was decreased in proportion to the severity of thrombocytopenia due to hypersplenism. In contrast with this author, the authors studied patients with decompensated cirrhosis who arrived in the operating theatre with rebalanced haemostasis, which differs considerably from healthy people but can be "normal" for cirrhotic patients.

Innovations and breakthroughs

Stable cirrhotic patients do not have inherent bleeding diathesis but rather a reduced reserve that can be readily tipped towards a bleeding or thrombotic tendency. In the last few years, several transfusion algorithms have been proposed, aiming to develop a better treatment for haemostasis in patients with coagulopathy and bleeding, but none of these algorithms have been built using values typically obtained from cirrhotic patient candidates. In contrast with Stravitz, the authors studied a population of patients with decompensated cirrhosis, with candidates for liver transplant having normal coagulation parameters in almost half of cases and more rapid clot formation in a small percentage of patients. The authors could show which reference range distributions in a population of patient candidates for LT should be taken into account when administering blood products during LT. However, this is a descriptive and not an outcome study, and the authors think that these findings should always be kept in mind when TEG data are interpreted in patients with end-stage liver disease.

Application

Stable cirrhotic patients do not have an inherent bleeding diathesis but rather a reduced reserve that can be readily tipped towards a bleeding or thrombotic tendency. The liver disease patient has a new balanced haemostatic profile that corresponds with TEG values that are very different from those observed in healthy people but that are within the range of normality in almost half of the liver transplant candidates studied. Even if it was not possible to directly demonstrate the clinical effect of interpreting the TEG traces, taking into account the "reference ranges" for cirrhotic patients, the authors think that in cases of bleeding episodes or intraoperative haemorrhage, it would most likely be useful to correct TEG values while keeping in mind the reference ranges for this category of patients to avoid unnecessary blood product transfusions.

Terminology

TEG offers a more targeted approach for assessing the overall outcome of the interactions of clotting factors beyond the initiation of clot formation. Although TEG is a useful viscoelastic test for haemostatic monitoring, interpretation of its results requires care, especially in cirrhotic patients in whom they have already shown that specific cut off values are necessary to guide blood products infusion. Liver transplantation is the only therapeutic approach for end-stage liver disease. It is a surgical procedure characterized by deep haemodynamic, coagulation and biochemical repercussions that are different depending on the surgical stage (laparotomy, pre-anhepatic, anhepatic, or reperfusion phase) observed.

Peer-review

This is a very interesting observational study and the manuscript has been well written.

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Retrospective Study

Underutilization of palliative care services in the liver transplant population

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Abstract

AIM

To evaluate use of palliative care services in patients with end-stage liver disease who do not have access to liver transplant.

METHODS

Evaluated were end-stage liver disease patients who were removed from the liver transplant wait-list or died prior to transplant at a single transplant center over a 2-year period. Those who were removed due to noncompliance or ultimately transplanted elsewhere were excluded from this study. Patient characteristics associated with palliative care consultation were assessed using logistic regression analysis.

RESULTS

Six hundred and eighty-three patients were listed for liver transplant in 2013-2014 with 107 (16%) dying ($n = 62$) or removed for clinical decompensation prior to liver transplant ($n = 45$): Median age was 58 years, and the majority were male (66%), Caucasian (53%), had Child C cirrhosis (61%) or hepatocellular carcinoma (52%). The palliative care team was consulted in only 18 of the 107 patients (17%) who died or were removed, 89% of which occurred as inpatients. Half of these consultations occurred within 72 h of death. In univariable analysis, patients of younger age, white race, and higher end-stage liver disease scores at time of listing and delisting were more likely to receive palliative care services. Only younger age [Odds ratio (OR) = 0.92; $P = 0.02$] and Caucasian race (OR = 4.90; $P = 0.02$) were still associated with integration of palliative care services through multivariable analysis.

CONCLUSION

Palliative care services are grossly underutilized in older, non-white patients with cirrhosis on the liver transplant wait-list. We encourage early integration of these ser-

vices into clinical decision-making in the transplant population, with further studies aimed at understanding barriers to consultation.

Key words: Cirrhosis; Hospice; End of life; Symptom management; Palliative care

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Core tip: Without liver transplant, patients with cirrhosis have 50% mortality at 5 years; these patients represent a population that would benefit from palliative care services. Palliative care services are grossly underutilized in older, non-white patients with cirrhosis on the liver transplant wait-list. We encourage early integration of these services into clinical decision-making in the transplant population, with further studies aimed at understanding barriers to consultation.

Kathpalia P, Smith A, Lai JC. Underutilization of palliative care services in the liver transplant population. *World J Transplant* 2016; 6(3): 594-598 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i3/594.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i3.594>

INTRODUCTION

Decompensated cirrhosis is characterized by ascites, hepatic encephalopathy, and variceal bleeding. Mortality is high with 50% death rate due to complications of cirrhosis within five years^[1,2]. In addition to these medical complications, patients with decompensated cirrhosis experience a large symptomatic burden including debilitating fatigue, muscle wasting, anorexia, and intractable pruritus. Self-reported quality of life is poor in cirrhotics; in one study, 38% of elderly patients with cirrhosis had difficulty in independently completing at least one daily living activity including dressing, walking few steps, or bathing while 10% had impaired integral activities of daily living (*i.e.*, managing money, cooking, grocery shopping)^[3-5]. Their physical symptoms, inability to independently care for themselves, and knowledge of their terminal disease often erodes their emotional and psychological well-being.

While it is clear that liver transplantation is essentially the only known cure for complications of end-stage liver disease, the ability to receive a transplant can be unpredictable: One in five individuals awaiting liver transplantation will die on the waitlist^[6]. While the process of listing individuals for liver transplantation is highly structured through formal medical, surgical, social, and psychological evaluations, there is no standard of care for the process to transition those who are deemed too sick for liver transplantation to comfort care. We aimed to evaluate current utilization of palliative care services in liver transplant candidates who

did not survive to liver transplant and understand which patient characteristics are associated with palliative care consultation in this population.

MATERIALS AND METHODS

All adult cirrhotic patients who were newly listed for liver transplant at a single, large volume United States liver transplant center from January 1, 2013 to December 31, 2014 and died prior to transplant or were delisted for being too ill for transplant were included in this study. We excluded patients delisted due to inadequate social support, medical non-adherence, active substance abuse, or those who were transplanted at another center.

Patient demographics (age, gender, ethnicity, language spoken), etiology of liver disease, Model for End-Stage Liver Disease (MELD) score at time of transplant listing, and education level were received from the United Network for Organ Sharing and Organ Procurement and Transplantation Network registries. Patients' MELD at delisting or death, Child Pugh Score at time of removal, presence of hepatocellular cancer (HCC), and insurance type were collected through review of the electronic health record. Details on the palliative care consultations were also manually reviewed from the electronic medical records.

Descriptive statistics were computed for all continuous variables (age at listing, candidate MELD lab score when being listed for transplant) including means, medians, and interquartile ranges. The rank sum test was used for these continuous variables. Pearson's χ^2 testing was used for the categorical values (candidate gender, ethnicity, highest education level, and diagnosis) to further compare the baseline characteristics of patients removed from the waiting list vs those who remained active. We employed univariable logistic regression to identify factors associated with palliative care consultation with a *P* value cut-off of 0.10. These factors were then evaluated for inclusion in the final multivariable logistic regression model using backwards stepwise elimination, using a *P* value cut-off of 0.05.

This study was approved by the Institutional Review Board of UCSF. Stata, version 12 (Stata Corp., College Station, TX) was used for statistical analyses.

RESULTS

There were 683 patients placed on the liver transplant list in 2013-2014, of which 107 (16%) ultimately dying (*n* = 62) or removed for clinical decompensation prior to liver transplant (*n* = 45). Median age was 58 years and majority (66%) was male. Majority of the patients who died or were de-listed were white (53.3%), followed by Hispanic (22.4%), Asian (12.1%), Black (9.3%), and other (2.8%). The etiology of cirrhosis was alcohol (11%), hepatitis C (41%), alcohol and hepatitis C (20%), and various other etiologies (30%). Majority of these patients had Child-Pugh Class C cirrhosis (60.7%)

Table 1 Baseline characteristics of 107 patients who died or were delisted for being too sick for transplant

	<i>n</i> = 107
Age at listing, yr	58 (53-63)
Male sex	71 (66%)
Ethnicity	
White	57 (53.3%)
Hispanic	24 (22.4%)
Asian	13 (12.1%)
Black	10 (9.3%)
Other	3 (2.8%)
MELD at time of listing	16 (12-23)
Etiology of cirrhosis	
Alcohol related	12 (11%)
Hepatitis C	44 (41%)
Alcohol + hepatitis C	21 (20%)
Other	30 (28%)
Child-pugh score at de-listing	
A	16 (15%)
B	26 (24.3%)
C	65 (60.7%)
HCC	56 (52%)
Education level	
College degree or less	100 (93.5%)
Graduate level degree	7 (6.5%)

MELD: Model for end-stage liver disease; HCC: Hepatocellular cancer.

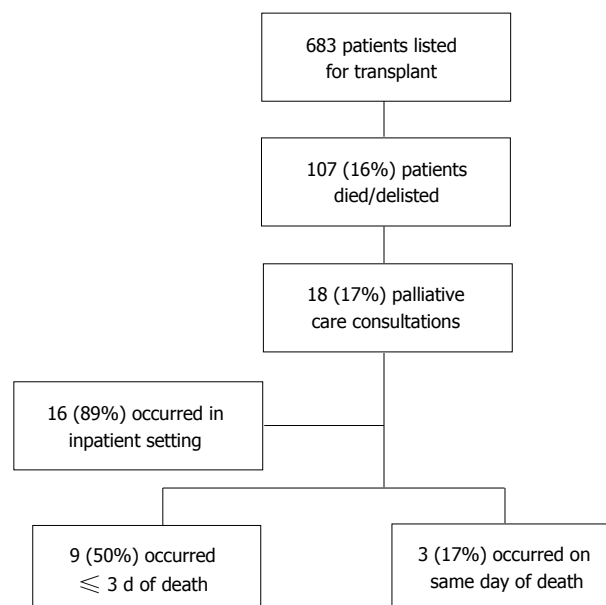
while 52% of these patients had HCC. In terms of education level, 93.5% had a college degree or less (Table 1).

Of these 107 patients who were delisted or died while awaiting transplant, 18 (17%) received a palliative care consult in the 2-year period, of which 89% occurred as inpatients. The median number of days (interquartile range) from palliative care consultation to death was 4 (1-11) d; half of these consultations occurred within 72 h of death and 17% on the same day as death (Figure 1). Reasons for palliative care consultation included aiding in transitioning to hospice in 78% of patients, goals of care without transition to comfort care in 11%, and symptom management including refractory ascites, pruritus, and pain in the remaining 11%. Even from the 26 patients with ESLD who were delisted for advanced HCC, just 12% had palliative care consultation.

Patient characteristics associated with palliative care consultation in univariate analysis included younger age (OR = 0.92; $P < 0.01$), white race (OR = 3.74; $P = 0.03$), and higher MELD at listing (OR = 1.06; $P = 0.02$) and at delisting (OR = 1.05; $P = 0.01$). Subsequent multivariable analysis revealed only younger age (OR = 0.92; $P = 0.02$) and white race (OR = 4.90; $P = 0.02$) remained associated with utilization of palliative care services (Table 2).

DISCUSSION

According to the World Health Organization, palliative care "improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable

**Figure 1** Palliative care consultations in patients who died or were delisted over a 24-mo period.

assessment and treatment of pain and other problems, physical, psychosocial, and spiritual^[7]. For patients with end-stage liver disease, early integration of palliative care into their routine medical care is particularly crucial to understanding patients' preferences for the end of life, as progressive hepatic encephalopathy often leads to impaired decision-making. Those on the liver transplant list, however, represent a unique sub-group of patients with a "terminal" condition - by virtue of having end-stage liver disease - but await the promise of a cure through liver transplantation. In this setting, palliative care, which traditionally has been considered only for those "at the end of life", may be perceived - by both the patient and providers alike - as unnecessary and unwelcome^[8-11].

Indeed, we observed very low utilization of palliative care services among liver transplant candidates who ultimately died or were delisted for being too sick for liver transplant. Among the 17% of these patients who received palliative care services, half of the consultations occurred within 72 h of death and one in five occurred on the day of death, hardly enough time to develop rapport and aid both patients and their caregivers in the transition to supportive care at the end of life^[12,13]. Importantly, we identified two factors - younger age and non-Hispanic white race - that were associated with palliative care consultation. This finding confirms a prior study evaluating barriers to palliative care among older adults that demonstrated that a terminal diagnosis in an elderly patient often is considered an "expectation" rather than a shock compared to that in a younger patient; it is also possible that younger patients at the end of life have more support networks/caretakers at this stage that advocate for improved quality of life^[14,15]. Cultural and language barriers likely contribute to underutilization of palliative care services in the non-white

Table 2 Factors associated with palliative care consultation, univariable and multivariable logistic regression analysis

Factor	Univariable analysis		Multivariable analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, per year	0.92 (0.87-0.98)	< 0.01	0.92 (0.87-0.98)	0.02
Male sex	0.76 (0.27-2.16)	0.61	-	-
White race	3.74 (1.14-12.26)	0.03	4.90 (1.30-18.30)	0.02
MELD at listing	1.06 (1.01-1.12)	0.02	1.00 (0.97-1.10)	0.39
MELD at delisting	1.05 (1.00-1.10)	0.01	1.00 (0.97-1.10)	0.34
Etiology of liver disease				
Alcohol related	Reference	Reference		
Hepatitis C	0.95 (0.17-5.28)	0.95	-	-
Alcohol + hepatitis C	1.56 (0.25-9.65)	0.63		
Other	0.77 (0.12-4.88)	0.78		
Child-pugh score at delisting				
A	Reference	Reference		
B	0.60 (0.03-10.30)	0.73	-	-
C	4.90 (0.60-40.10)	0.14		
HCC	0.89 (0.32-2.46)	0.83	-	-
College or lower level of education (<i>vs</i> graduate level)	1.23 (0.14-10.9)	0.85	-	-
Private insurance (<i>vs</i> government)	0.70 (0.25-1.97)	0.50	-	-
English language	0.93 (0.24-3.65)	0.92	-	-

MELD: Model for end-stage liver disease; HCC: Hepatocellular cancer.

population.

In addition, we find it interesting that more than three-fourths of palliative care consultations in our population were to assist with transition to comfort care and just 11% were for aid in symptom management. This depicts how transplant clinicians view palliative care, as a mode to help make patients comfortable at the end of life, but not to facilitate goals of care discussions or to help relieve pain and suffering in a patient population with a terminal condition without transplantation. We recognize the need for integration of palliative care and transplant hepatology teams in efforts to provide comprehensive care for our patients to meet their physical and psychosocial needs even when actively listed for liver transplantation.

We acknowledge that this study is limited by a relatively small sample size; however, it represents the *entire* eligible population at our liver transplant center during the study period, so is an unselected group. Another limitation is that we only evaluated those who died or were delisted rather than all patients on the liver transplant list. This was intentional, as we first wanted to evaluate the uptake of palliative care among those for whom death was certain.

Despite these limitations, this study represents one of the largest to date to evaluate palliative care consultation in the liver transplant population. Poonja *et al*^[16] reported their experience in the liver transplant population at the University of Alberta and noted that of the 102 patients removed from the waitlist or declined over a 5 year period, only 10% were referred to palliative care despite high levels of pain, nausea, and depression. As patients with ESLD have high burden of symptoms, we advocate for increased utilization of palliative care services - for both symptom management and discussions regarding goals of care - and integration of such services early in the liver

transplant listing process. Baumann *et al*^[17] confirmed that early palliative care utilization in patients listed for transplantation led to improved symptom management and well-being in this population.

While this study represents a critical first step towards developing interdisciplinary programs directed at providing palliative care to liver transplant candidates, future studies should focus on understanding barriers to early integration of palliative care in the liver transplant population among all ages and ethnicities, in both the inpatient and outpatient setting. In addition, in a prospective study, patient-centered outcomes can be obtained in efforts to show the direct impact of palliative care involvement on the physical and psychosocial well being of these patients. Ultimately the goal should be to facilitate collaboration and, perhaps, even co-management between transplant and palliative care providers for the care of these complex patients - even when the intention to treat is curative - to improve the quality of care and quality of life for patients with cirrhosis awaiting liver transplantation.

COMMENTS

Background

Patients with end stage liver disease have 50% 5-year mortality due to complications of cirrhosis and experience a large symptomatic burden including debilitating fatigue, muscle wasting, anorexia, and intractable pruritus. While it is clear that liver transplantation is essentially the only known cure for complications of end-stage liver disease, the ability to receive a transplant can be unpredictable. In patients who do not have access to liver transplant, palliative care services may aid in quality of life of patients and caretakers alike.

Research frontiers

Though the process of listing individuals for liver transplantation is highly structured, there is no standard of care for the process to transition those who are deemed too sick for liver transplantation to comfort care. Current utilization of palliative care services in liver transplant candidates who did not survive to liver transplant is not largely understood.

Innovations and breakthroughs

Authors aimed to understand the use of palliative care services in patients with end-stage liver disease who do not have access to liver transplant over a 2-year period and a large volume center. Palliative care services were consulted in less than 20% of patients who were died or removed from the transplant list, majority of which occurred while patients were already hospitalized. In univariable analysis, patients of younger age, white race, and higher model for end-stage liver disease (MELD) scores at time of listing and delisting were more likely to receive palliative care services. Only younger age and Caucasian race were still associated with integration of palliative care services through multivariable analysis. The authors recognize that palliative care services are grossly underutilized in patients who are not deemed transplant candidates.

Applications

While this study represents a critical first step towards developing interdisciplinary programs directed at providing palliative care to liver transplant candidates, future studies should focus on understanding barriers to early integration of palliative care in the liver transplant population. In addition, in a prospective study, patient-centered outcomes can be obtained to show the direct impact of palliative care involvement on the physical and psychosocial well being of these patients. Ultimately the goal should be to facilitate collaboration and, perhaps, even co-management between transplant and palliative care providers for the care of these complex patients - even when the intention to treat is curative - to improve the quality of care and quality of life for patients with cirrhosis awaiting liver transplantation.

Terminology

Palliative care services encompass more than aiding in transitioning to comfort care and assisting in goals of care discussions, and can be particularly helpful in symptom management, even in patients who are not terminally ill. The laboratory based MELD score accounts for patients' bilirubin, international normalized ratio (INR), and creatinine levels and was calculated both at time of transplant listing and delisting. The child pugh score entails a combination of laboratory (bilirubin, albumin, INR) and clinical (presence of ascites, encephalopathy) factors; though it was originally used to predict mortality in cirrhotics at the time of surgery, it can also aid in understanding the severity of liver disease.

Peer-review

This is an integral single center retrospective study that aims to shed light on the need for integration of palliative care into the liver transplant population, even when the intent to treat is curative, in efforts to improve their quality of life.

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Retrospective Study

Evaluating twenty-years of follow-up after orthotopic liver transplantation, best practice for donor-recipient matching: What can we learn from the past era?

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Abstract

AIM

To characterize major determinants of 20-year survival after liver transplantation (LT).

METHODS

This longitudinal single-institution study includes 313 consecutive patients who received a LT between 1988 and 1992. Pretransplant clinical characteristics and laboratory values were assessed and compared between 20-year survivors and non-survivors. Particular attention was paid to the Model for End-Stage Liver Disease (labMELD)-score and the Eurotransplant Donor Risk Index (ET-DRI) to unravel their impact on 20-year survival after LT.

RESULTS

Twenty-year survivors were significantly younger (44 *vs* 50 years, $P = 0.001$), more likely to be female (49% *vs* 36%, $P = 0.03$) and less likely to be obese at the time of LT (19% *vs* 32%, $P = 0.011$). Mean labMELD-score ($P = 0.156$), rate of high-urgency LT ($P = 0.210$), cold-ischemia time ($P = 0.994$), rate of retransplantation ($P = 0.12$) and average donor age (28 *vs* 33 years, $P = 0.099$) were not statistically different. The mean estimated glomerular filtration rate was higher among survivors ($P = 0.007$). ET-DRI > 1.4 ($P = 0.020$) and donor age ≥ 30 years ($P < 0.022$) had significant influence on 20-year survival. The overall survival was not significantly impacted by labMELD-score categories ($P = 0.263$).

CONCLUSION

LT offers excellent long-term results in case of optimal donor and recipient conditions. However, mainly due to the current organ shortage, these ideal circumstances are rarely given; thus algorithms for donor-recipient matching need to be refined, in order to enable a maximum benefit for the recipients of high quality as well as marginal organs.

Key words: Liver transplantation; Long-term outcome; Ideal recipient; Recipient characteristics; Donor-recipient matching

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Core tip: We compare characteristics of 20-year survivors and non-survivors after liver transplantation. The lab model for end-stage liver disease-score seems not to be an adequate tool for predicting long-term (20 years) outcome. The Eurotransplant Donor Risk Index (ET-DRI) has a significant impact on long-term survival. While close to 60% of patients that received a donor organ with an ET-DRI < 1.2 survived for 20 years and longer, only less than 40% of the patients with an ET-DRI > 1.4 survived the same number of years. Only about 20% survivors had overweight before transplantation, compared to about 33% non-survivors. The mean estimated glomerular filtration rate was higher among survivors.

Buescher N, Seehofer D, Helbig M, Andreou A, Bahra M, Pascher A, Pratschke J, Schoening W. Evaluating twenty-years of follow-up after orthotopic liver transplantation, best practice for donor-recipient matching: What can we learn from the past era? *World J Transplant* 2016; 6(3): 599-607 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i3/599.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i3.599>

INTRODUCTION

Over the last three decades, liver transplantation (LT) has become the standard therapeutic treatment for

patients with terminal liver failure^[1-4]. Short- and long-term results have improved, resulting in dramatic prolongation of recipients' life expectancy^[5]. Surgical techniques, pharmaceutical regimens, and intensive care management were continuously refined^[6,7]. Equally as important, LT centers have gained invaluable experience regarding the long-term management of LT patients^[3,4,8]. Many obstacles resulting in patient and graft loss have been identified, and means to overcome them have been developed. This has led to a broad increase in the number of potential LT recipients^[9].

However, with growing waiting lists and an increasing number of LT-centers, the LT community is now facing the issue of fair organ allocation. The limited amount of donor organs led to the implementation of different liver allocation policies^[10,11] and a more liberal acceptance of extended criteria donor (ECD) organs^[12,13]. The implementation of Model for end-stage liver disease (MELD) allocation in 2006 within the Eurotransplant area has reduced waiting list mortality to about 10%^[14], but has also increased the one-year mortality in many European centers, *e.g.*, at our center from 8.2% to about 17.4%^[15]. Donor-recipient-matching has become crucial to achieving reasonable one year mortality^[16] and acceptable waiting list mortality, especially when allocating marginal organs to progressively sicker recipients.

With this study, we aim to evaluate the influence of pretransplant labMELD and Eurotransplant Donor Risk Index (ET-DRI) on the long-term survival of a cohort of LT-recipients. Furthermore, we compared the pretransplant characteristics of recipients who survived ≥ 20 years after their LT to those who died within the 20-year observation period.

MATERIALS AND METHODS

Study design

A longitudinal single-institution study was performed to characterize 20-year LT survivors. Institutional Review Board approval was obtained for this study.

Patients

The cohort has been described previously^[17]. Indications for primary transplants are presented in Table 1. Patients were divided into groups with regards to their underlying disease: Cholestatic/autoimmune comprises all patients with primary ($n = 19$) or secondary ($n = 3$) sclerosing cholangitis, primary ($n = 29$) or secondary ($n = 1$) biliary cirrhosis and autoimmune hepatitis ($n = 12$). The group hepatobiliary malignancy includes all cases of hepatocellular carcinomas (HCC, $n = 27$), cholangiocarcinomas ($n = 5$) as well as Klatskin tumors ($n = 4$), while virus-related cirrhosis includes all patients with hepatitis B ($n = 47$), hepatitis C ($n = 32$), hepatitis B and C ($n = 3$) and hepatitis B and D ($n = 10$) virus cirrhosis. Overall, virus-related cirrhosis (29.4%), cholestatic/autoimmune liver disease (20.4%), alcoholic cirrhosis (16.0%), hepatobiliary malignancy (11.5%), cryptogenic cirrhosis (9.3%) and acute liver failure

Table 1 Indications of primary liver transplant

	All patients <i>n</i> = 313 (100%)	20-yr survivors <i>n</i> = 157 (50%)	20-yr non- survivors <i>n</i> = 141 (45%)	Ratio ¹	Lost <i>n</i> = 15 (5%)
Virus-related cirrhosis	92 (29.4%)	46 (29.30%)	39 (27.70%)	1.18	7
Hepatitis B	47 (15.0%)	26 (16.6%)	19 (13.5%)		
Hepatitis C	32 (10.2%)	13 (8.3%)	17 (12.1%)		
Hepatitis B and D	10 (3.2%)	5 (3.2%)	2 (1.4%)		
Hepatitis B and C	3 (1.0%)	2 (1.3%)	1 (0.7%)		
Cholestatic/autoimmune	64 (20.4%)	38 (24.2%)	20 (14.2%)	1.90	6
Alcoholic cirrhosis	50 (16.0%)	23 (14.6%)	27 (19.1%)	0.85	
Hepatobiliary malignancy	36 (11.5%)	7 (4.5%)	28 (19.9%)	0.25	1
HCC	27 (8.6%)	6 (3.8%)	20 (14.2%)		
CCC	5 (1.6%)	0 (0.0%)	5 (3.5%)		
Klatskin tumor	4 (1.3%)	1 (0.6%)	3 (2.1%)		
Cryptogenic cirrhosis	29 (9.3%)	15 (9.6%)	13 (9.2%)	1.15	1
Acute liver failure	23 (7.3%)	16 (10.2%)	7 (5.0%)	2.29	
Others	19 (6.1%)	13 (8.3%)	6 (4.3%)	2.20	

¹ratio of survivors/non-survivors in the respective indication category.
HCC: Hepatocellular carcinomas; CCC: Cholangiocellular carcinoma.

(7.3%) were the most common indications for primary LT. Of the twenty-seven HCC patients, seven did not fall under the later defined Milan criteria.

Characteristics of donors and recipients are depicted in Table 2. In summary, the cohort consists of 313 consecutive patients who received a primary LT at the Charité, Campus Virchow-Klinikum, between 1988 and 1992. During the twenty-year follow-up those patients received a total of 365 livers including 54 retransplantations (46 first retransplantations). There were 178 male and 135 female recipients. At the date of primary LT, median patient age was 47 (14-66) years including two patients who were minors at the age of 14 and 16, while median donor age was 30 (9-64) years. Mean labMELD-Score was 18.6 ± 7.6 and mean ET-DRI was 1.35 ± 0.2 .

Patients were observed until their death, loss to follow-up, or graft loss. Data were censored at time of patients' death, loss to follow-up, graft loss or at 20 years after transplantation, respectively. A graft survival analysis was performed in which labMELD-scores, pre-transplant laboratory values (median 0 d before LT, range 0-84 d), clinical characteristics and ET-DRI were evaluated for the primary LT as well as for the primary graft, in order to compare characteristics of 20 year-survivors and non-survivors.

MELD-score calculations

LabMELD-scores were retrospectively calculated using the pretransplant serum bilirubin level, serum creatinine

Table 2 Pretransplant characteristics

	All patients <i>n</i> = 313	20-yr- survivors <i>n</i> = 157	20-yr- non- survivors <i>n</i> = 141	<i>P</i>
Recipients				
Age (yr)	47 (14-66)	44 (14-66)	50 (25-65)	0.001
Age < 18, <i>n</i> (%)	2 (0.6)	2 (1.3)	0 (0)	0.06
Age > 55, <i>n</i> (%)	57 (18)	19 (12)	36 (26)	0.03
Gender, <i>n</i> (%) female	135 (43)	77 (49)	51 (36)	0.03
labMELD-score	18.6 (± 7.6)	19.4 (± 8.3)	18.1 (± 7.0)	0.156
Urgent LT, <i>n</i> (%)	23 (7)	15 (10)	8 (6)	0.21
BMI (kg/m ²)	23.0 \pm 3.3	22.7 \pm 3.0	23.5 \pm 3.7	0.037
HBMI, <i>n</i> (%)	78 (25%)	30 (19%)	45 (32%)	0.011
HLIP, <i>n</i> (%)	45 (14%)	20 (15%)	23 (19%)	0.376
Donors				
Donor age (yr)	30 (9-64)	28 (14-64)	33 (9-60)	0.099
ET-DRI	1.35 (± 0.2)	1.32 (± 0.2)	1.37 (± 0.2)	0.121
Transplant				
Cold ischemia time, h	10.6 (± 4)	10.6 (± 4)	10.7 (± 4)	0.994
Retransplantation, <i>n</i> (%)	46 (15)	18 (11)	25 (18)	0.120
Liver function				
tBili	8.1 \pm 11.9	9.0 \pm 12.6	7.7 \pm 11.6	0.363
AST	115 \pm 460	124 \pm 486	111 \pm 454	0.820
ALT	102 \pm 233	102 \pm 177	108 \pm 286	0.849
INR	1.76 \pm 0.8	1.82 \pm 0.8	1.7 \pm 0.8	0.226
Clinical characteristics				
Systolic BP (mmHg)	120 \pm 20	119 \pm 20	122 \pm 21	0.340
Diastolic BP (mmHg)	71 \pm 11	71 \pm 12	72 \pm 11	0.353
Laboratory parameters				
Glucose (mg/dL)	120 \pm 58	116 \pm 46	126 \pm 70	0.174
Cholesterol (mg/dL)	134 \pm 72	129 \pm 55	138 \pm 86	0.311
Triglycerides (mg/dL)	95 \pm 67	91 \pm 56	100 \pm 80	0.326
Creatinine (mg/dL)	1.0 \pm 0.8	1.06 \pm 1.0	0.95 \pm 0.4	0.247
eGFR (mL/min per 1.73 m ²)	98 \pm 59	106 \pm 70	88 \pm 39	0.007

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HLIP: Hyperlipidemia; HBMI: Overweight; MELD: Model for end-stage liver disease; ET-DRI: Eurotransplant donor-risk-index; INR: International normalized ratio.

level, and INR according to Kamath *et al.*^[18]

Given Quick values were converted into INR with the help of the corresponding batch numbers. Serum bilirubin, INR, or serum creatinine values of less than 1.0 were set to 1.0 to preclude negative scores. Serum creatinine level was capped at 4.0. MELD-scores were capped at 40. We were able to retrieve MELD-scores for 308 patients. For the compilation of Kaplan-Meier curves, recipients were grouped into three different categories: MELD ≤ 15 (*n* = 126), MELD = 16-25 (*n* = 134) and MELD > 25 (*n* = 48).

ET-DRI calculations

The ET-DRI was assessed using the required donor and transplant factors according to Braat *et al.*^[19]

We were able to calculate the corresponding ET-DRI for 179 patients (57%). For the remaining donors the latest GGT level was unknown, which is an essential factor for ET-DRI calculation. Ninety-four of these recipients were 20-year survivors, 85 were non-survivors. For Kaplan-Meier estimates, the grafts were divided into three groups: ET-DRI < 1.21 (*n* = 54), 1.21-1.40 (*n* = 61) and > 1.4 (*n* = 64).

Laboratory parameters

Laboratory parameters were obtained after a fasting period of at least 12 h and included serum levels of total cholesterol, triglycerides, creatinine, Quick-value, total bilirubin (tBili), aspartate aminotransferase, alanine aminotransferase and glucose.

Variables

Overweight (HBMI) was defined as body-mass-index (BMI = weight/height²) above 25. Blood cholesterol levels of more than 200 mg/dL, triglyceride levels above 175 mg/dL, or statin treatment were considered "hyperlipidemia" (HLIP). The MDRD-formula was used to estimate glomerular filtration rate (eGFR). An eGFR < 60 mL/min per 1.73 m² was considered moderately impaired renal function (MIRF), while rates < 30 mL/min per 1.73 m² were defined as severely impaired renal function (SIRF)^[20].

Statistical analysis

Categorical variables were compared by the χ^2 test and summarized as percentages and frequencies. Continuous variables were compared using unpaired *t* test and summarized as median and range, or mean \pm SD. A *P* value of less than 0.05 was interpreted as statistically significant. Kaplan-Meier estimates were used to calculate survival curves. Differences in survival curves were compared using log-rank statistics. All calculations were done using the SPSS software package (version 22.0 for Windows, SPSS Inc., Chicago, IL).

RESULTS

After a median follow-up of 233 mo (0-260), 157 patients were alive (141 with complete sets of data, 16 with incomplete sets of data) and 141 had died (27 patients within 6 mo after LT) while 15 patients were lost to follow-up 99 to 243 mo after LT.

Recipients' characteristics

Table 1 depicts the distribution of primary indication for LT among survivors and non-survivors. The most common indications among survivors were virus-related cirrhosis (29.3%), cholestatic/autoimmune liver disease (24.2%), and alcoholic cirrhosis (14.6%), while among non-survivors virus-related cirrhosis (27.7%), hepatobiliary malignancy (19.9%) and alcoholic cirrhosis were the most frequent. The ratio of survivors/non-survivors was lowest for hepatobiliary malignancies (0.25) and highest for cholestatic/autoimmune liver disease (1.90) and acute liver failure (2.29).

As shown in Table 2, median age of 20-year-survivors and non-survivors was 44 (14-66) and 50 (25-65) years, respectively (*P* = 0.001). Both minors (primary indication PSC and ALF) were alive after twenty years of follow-up. The group of non-survivors includes significantly more LT recipients over the age of 55 (26% compared

to 12% of the survivors, *P* = 0.03) while the group of survivors has a significantly larger amount of female recipients (49% compared to 36% of the non-survivors, *P* = 0.03). Mean BMI for survivors and non-survivors was 22.7 \pm 3.0 and 23.5 \pm 3.7 kg/m², respectively (*P* = 0.037). There were no significant differences for survivors and non-survivors regarding pretransplant labMELD-score (19.4 \pm 8.3 and 18.1 \pm 7.0, *P* = 0.156), rate of high-urgent LT (10% and 6%, *P* = 0.210), cold-ischemia time (10.6 \pm 4 and 10.7 \pm 4 h, *P* = 0.994) and rate of retransplantation (11% and 18%, *P* = 0.12).

Donors' characteristics

Among survivors, median donor age was 28 years (14-64) compared to a median donor age of 33 years (9-60) among non-survivors (*P* = 0.099). Mean ET-DRI for survivors and non-survivors was 1.32 \pm 0.2 and 1.37 \pm 0.2, respectively (*P* = 0.121).

Patient and graft survival

The overall actuarial patient survival rates at 1, 10 and 20 years were 88.4%, 72.7% and 52.5%, respectively. The overall graft survival rates were 83.7%, 64.7% and 46.6% after 1, 10 and 20 years, respectively.

Liver function tests

None of the liver function tests that were compared showed a statistically significant difference between survivors and non-survivors (Table 2). Prior to LT, mean total bilirubin was 9.0 \pm 12.6 mg/dL for survivors and 7.7 \pm 11.6 mg/dL for non-survivors (*P* = 0.363). Mean aspartate aminotransferase was 124 \pm 486 U/L for survivors and 111 \pm 454 U/L for non-survivors (*P* = 0.820). Mean pretransplant alanine aminotransferase was 102 \pm 177 U/L for survivors and 108 \pm 286 U/L for non-survivors (*P* = 0.849).

Clinical and laboratory parameters

Systolic BP and diastolic BP were not significantly different between survivors and non-survivors. 20-year survivors' mean blood glucose was 116 \pm 46 mg/dL compared to 126 \pm 70 mg/dL among non-survivors (*P* = 0.174). Cholesterol (129 \pm 55 and 138 \pm 86, *P* = 0.311) and triglycerides (91 \pm 56 and 100 \pm 80, *P* = 0.326) values did not differ significantly between survivors and non-survivors. Regarding the renal function, mean eGFR of 106 \pm 70 mL/min per 1.73 m² in survivors was significantly higher than mean eGFR of 88 \pm 39 mL/min per 1.73 m² in non-survivors (*P* = 0.007). Detailed data are presented in Table 2, where the percentages relate to the amount of patients with complete data in the specific category.

Nineteen percent of the twenty-year survivors had HBMI before transplantation, while 32% of the non-survivors had HBMI (*P* = 0.016). Comparing survivors and non-survivors, prevalence of HLIP (15% and 19%, *P* = 0.407), MIRF (20% and 21%, *P* = 0.886) and SIRF (5% and 3%, *P* = 0.547) did not show a significant

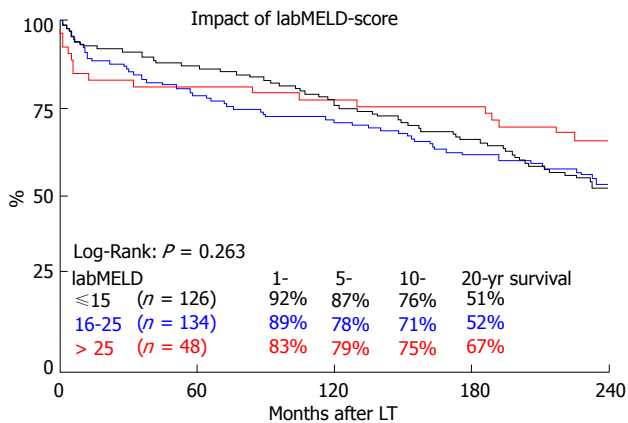


Figure 1 The impact of lab model for end-stage liver disease categories on 20 year survival. MELD: Model for end-stage liver disease; LT: Liver transplantation.

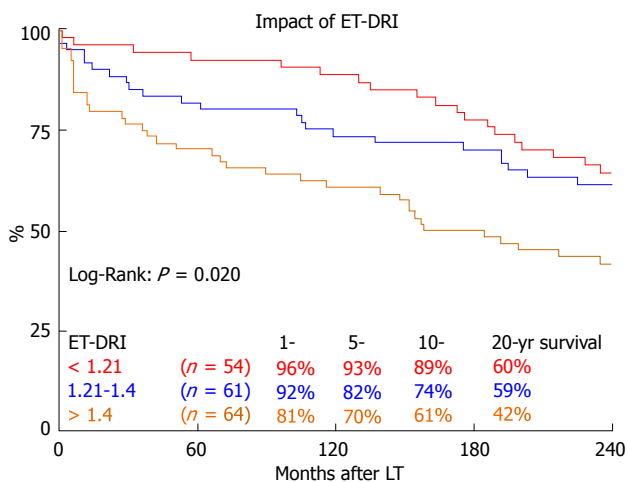


Figure 2 The impact of eurotransplant donor risk index categories on 20 year survival. LT: Liver transplantation; ET-DRI: Eurotransplant donor-risk-index.

difference.

To further analyze the impact of renal function, patients were split up into separate groups, based on their eGFR before transplantation (Table 3). Eighty percent of the survivors and 79% of the non-survivors had an eGFR > 60 ($P = 0.860$), pointing to normal renal function. The groups that comprise eGFR values of 60 to 69 and 70 to 79 contain significantly more non-survivors than survivors (20.0% and 15.7% compared to 6.5% and 6.5%, $P = 0.001$ and $P = 0.011$, respectively), while 30.3% of the survivors had an eGFR > 120 compared to 20.0% of the non-survivors ($P = 0.042$).

A subgroup analysis was performed to assess the underlying diseases among those patients who later developed MIRF and SIRF. The most common indications for primary LT among patients with MIRF at 20 years after LT ($n = 85$) were virus-related cirrhosis ($n = 32$), CD/AIH ($n = 18$) and alcoholic liver disease ($n = 15$). Among patients who later developed SIRF ($n = 10$), the most common primary indications were CD/AIH ($n = 4$), virus-related cirrhosis ($n = 3$) and

Table 3 Pretransplant renal function n (%)

	20-yr survivors $n = 155$	20-yr non-survivors $n = 140$	P
eGFR > 60	126 (80%)	112 (79%)	0.860
MIRF	31 (20%)	29 (21%)	0.879
SIRF	7 (4.5%)	4 (2.9%)	0.453
eGFR 30-39	10 (6.5%)	5 (3.6%)	0.261
eGFR 40-49	8 (5.2%)	7 (5.0%)	0.950
eGFR 50-59	8 (5.2%)	13 (9.3%)	0.169
eGFR 60-69	10 (6.5%)	28 (20%)	0.001
eGFR 70-79	10 (6.5%)	22 (15.7%)	0.011
eGFR 80-89	22 (14.2%)	13 (9.3%)	0.193
eGFR 90-99	16 (10.3%)	14 (10.0%)	0.927
eGFR 100-109	15 (9.7%)	9 (6.4%)	0.308
eGFR 110-119	10 (6.5%)	7 (5.0%)	0.593
eGFR > 120	47 (30.3%)	28 (20.0%)	0.042

LT: Liver transplantation; eGFR: Estimated glomerular filtration rate; MIRF: Moderately impaired renal function; SIRF: Severely impaired renal function.

polycystic liver disease ($n = 2$).

Kaplan-Meier estimates

As shown in Figure 1, the overall survival at 1, 5, 10 and 20 years for the three different groups of labMELD-Scores, was 92.1%, 86.5%, 76.2% and 51.3% for group 1 (labMELD ≤ 15), 88.8%, 77.6%, 70.9% and 51.9% for group 2 (labMELD = 16-25) and 83.3%, 79.2%, 75.0% and 66.7% for group 3 (labMELD > 25). The 20-year survival did not differ significantly ($P = 0.263$). This was also true for 0.5- ($P = 0.226$), 1- ($P = 0.293$), 5- ($P = 0.293$), 10- ($P = 0.522$) and 15-year ($P = 0.241$) survival. Survival of recipients with labMELD > 25 was not significantly worse compared to all others at 6 mo after LT, ($P = 0.095$), also not at 1-year ($P = 0.158$), 5-year ($P = 0.704$) and 10-year ($P = 0.726$). At 15-year ($P = 0.143$) and 20-year ($P = 0.107$), recipients with MELD > 25 showed better overall survival, but this difference was not statistically significant.

Long-term survival was significantly influenced by ET-DRI ($P = 0.020$, Figure 2). Comparing only two groups, ET-DRI ≤ 1.4 and >1.4, the survival outcome showed a significant difference as well ($P = 0.011$) (data not shown). Looking at the donor age separately (< vs ≥ 30 years), we also found a significant impact on long-term survival as shown in Figure 3 ($P < 0.014$). A more detailed analysis of donor and recipient age based on a recipient age of < and ≥ 55 years and a donor age of < vs ≥ 30 years revealed a highly significant impact on long term outcome in the comparison of these four categories ($P < 0.0001$, Figure 4).

In a sub-analysis of patients with the best long-term survival^[17] (CD/AIH and ALF) the effect of donor quality (ET-DRI) was even more pronounced: Transplanting an ET-DRI < 1.21 organ resulted in an 20 year survival of 79% compared to 39% for an ET-DRI > 1.4 organ (Figure 5).

Figure 6 shows the impact of the BMI on the long-term outcome after LT. Patients without pretransplant

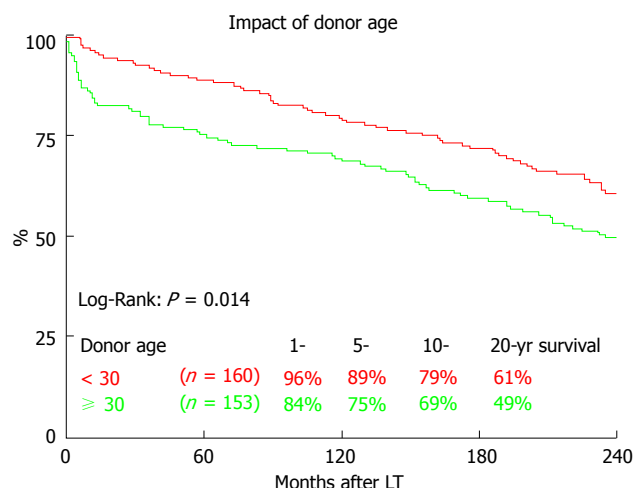


Figure 3 The impact of donor age on 20-year survival. LT: Liver transplantation.

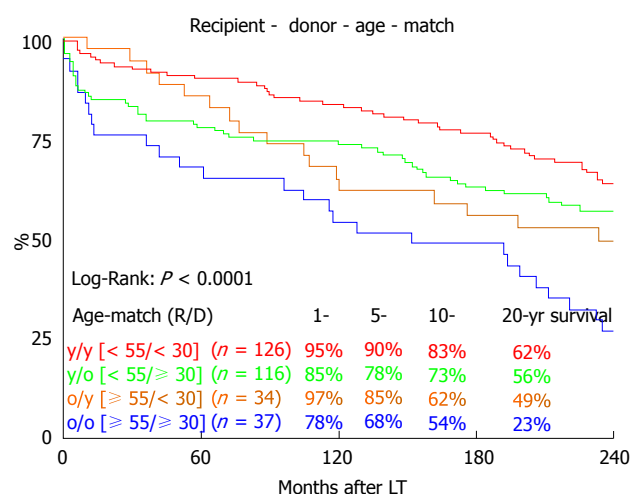


Figure 4 The influence of recipient-donor age match on 20-year survival. LT: Liver transplantation.

HBMI (< 25) showed significantly better overall 20-year survival (60.4% vs 40.6%, $P = 0.003$). HBMI did not significantly impact 1 year (90.0% vs 90.6%, $P = 0.703$), 5 year (80.0% vs 82.8%, $P = 0.471$) or 10 year (70.0% vs 75.5%, $P = 0.191$) survival.

Presence of MIRF and SIRC before transplantation did not significantly influence the overall 20-year survival ($P = 0.936$ and 0.387 , respectively) (data not shown).

Causes of death

As we have previously published^[17], the most common causes of death overall were recurrence of primary disease (21.3%), infection (20.6%) and *de-novo* malignancy (19.9%). While recurrent disease was most common in the first decade after LT, followed by infection and *de novo* malignancy, *de novo* malignancy was the most common cause of death during the second decade after LT, followed by infection and cardiovascular events. Recurrence of primary disease

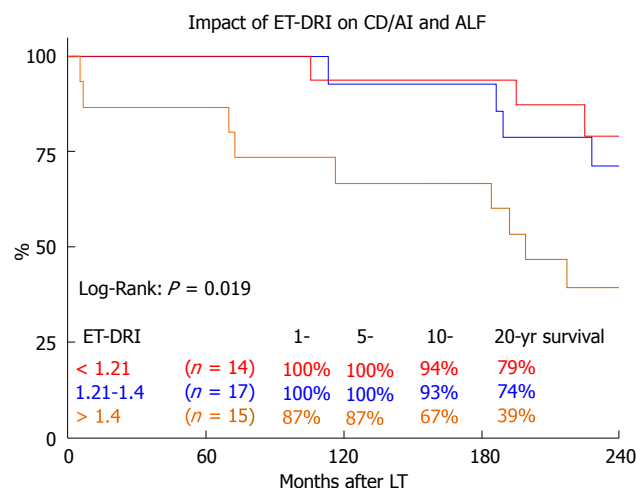


Figure 5 The impact of eurotransplant donor risk index categories on 20-year survival of recipients with cholestatic diseases, autoimmune hepatitis and acute liver failure. LT: Liver transplantation; ET-DRI: Eurotransplant donor-risk-index.

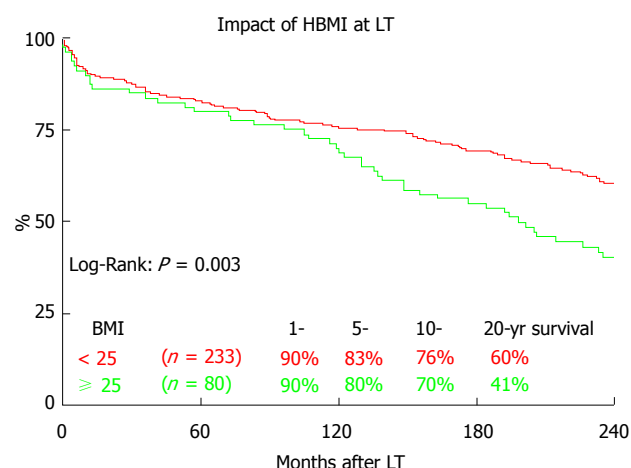


Figure 6 The impact of overweight (overweight, body-mass-index > 25) at time of liver transplantation on 20-year survival. LT: Liver transplantation; HBMI: High body mass index (> 25).

was especially common in patients with hepatobiliary malignancy and virus-related cirrhosis. Among the *de-novo* malignancies, squamous-cell carcinomas were most common. Pneumonia and sepsis were the most common infections.

DISCUSSION

Recently, our center published the first European single-institution 20-year survival data and the most promising long-term outcomes worldwide to this point^[17]. More than half of our cohort survived for two decades after LT. With the present study, we aimed to compare the characteristics of 20-year survivors and 20-year non-survivors in order to characterize those patients who achieved outstanding long-term survival.

Not surprisingly, on average 20-year survivors were significantly younger and predominantly female. Pre-

vious studies have also found that survival for female recipients is slightly higher compared to male recipients. The prevalence of cardiovascular risk factors, as well as cardiovascular events, is higher in male long-term survivors, which may explain this finding^[17,21].

The Kaplan-Meier analyses of the long-term survival in this cohort show that the greatest disparity in outcome based on ET-DRI categories (Figure 2) seems to occur within the first year after LT; after this there is little divergence in the Kaplan-Meier curves according to donor risk. Thus, after the short-term post-transplant period has passed, the underlying disease and further recipient characteristics seem to play a more important role than the initial graft quality. Long-term outcome studies, such as this one, are valuable in identifying such recipient characteristics. One example is the fact that in our cohort, presence of HBMI does not become a significant prognostic factor until 10 years after LT.

As far as the distribution of primary indications for LT goes, we found that hepatobiliary malignancies had a particularly low survival rate^[17]. In this cohort, the ratio of survivors/non-survivors for patients with hepatobiliary malignancy was 0.25; several patients in this group presented at an advanced stage. Due to the high prevalence of recurrent disease among patients with HCC far beyond the Milan criteria^[22] and advanced cholangiocellular carcinomas^[23], they are no longer eligible for LT. The European Liver Transplant Registry states 20-year patient survival rates of 27% for primary liver tumors, which make up for 14% of the total indications for LT^[24]. On the other hand, patients with autoimmune and cholestatic liver disease (ratio 1.9) as well as patients with acute liver failure (ratio 2.29), made up a significant part of the 20-year survivors, which is in line with the findings of the European Liver Transplant Registry, which lists 20-year patient survival rates of 44% for cholestatic disease, 55% for autoimmune liver disease and 47% for acute hepatic failure, which make up for a total of 21% of all indications^[24].

Unexpectedly, the labMELD-score did not significantly influence 20-year survival in our cohort. Our study supports the findings of previous studies^[25] showing that the labMELD score is particularly relevant during the first couple years after LT. LabMELD categories showed a strong trend regarding the differences in 1-year survival, even if not statistically significant. After ten years, these differences evened out. Most surprisingly, after 20-years, recipients with labMELD > 25 showed the best overall survival. Even though the labMELD-score is able to predict waiting list mortality, it does not seem to be an adequate tool for predicting long-term outcome and thus survival benefit^[26]. With a mean labMELD-score of 18.6, the patients in our cohort can be considered relatively healthy compared to German patients receiving transplants in the current era, with an average matchMELD of 34^[14]. Also, the mean ET-DRI of 1.35 suggests excellent donor organ quality. In summary excellent overall conditions for transplantation, which

are hardly realized under the current LT conditions. This makes it difficult to interpret the impact of our data on the era of MELD-allocation with ECD organs. The MELD-score has contributed to reduce the waiting list mortality^[27] and decrease the waiting time for LT^[28]. However, there are several weaknesses: Interlaboratory variability of creatinine, bilirubin and INR causes a lack of objectivity^[29,30]. Secondly, the score does not adequately represent the necessity for LT for many indications, making it necessary to assign priority-based extra-points, which have seen a rather arbitrary up- and down-regulation^[31,32]. Most importantly, the MELD score neglects all donor characteristics in the allocation process whatsoever. Therefore, organ allocation according to a MELD-based policy is not true donor-recipient matching at all. Our findings suggest that, depending on the quality of a given donor organ, the underlying disease, the recipients' age and many other factors, a similar MELD value may result in very different long-term outcomes.

Another unexpected finding was the lack of significant impact of an impaired renal function prior to transplantation on long-term survival. The significant difference in mean eGFR between survivors and non-survivors (106 ± 70 mL/min per 1.73 m^2 vs 88 ± 39 mL/min per 1.73 m^2 , respectively, $P = 0.007$) is most likely due to the large amount of survivors with eGFR > 120 mL/min per 1.73 m^2 (30% vs 20%) and the fact that the MDRD-formula does not adequately represent the renal function for patients without impairment^[33]. In our previous publication mentioned above, we showed that a moderately or severely impaired renal function at 6 mo after LT was an independent risk factor for long-term survival in this cohort^[17]. However, in this study, neither patients with pretransplant MIRF nor those with SIRF showed significantly lower overall survival. This is contrary to what other authors have described^[34-36]. What was striking was the high number of non-survivors that had an eGFR that was just above 60, making these patients barely off the limit for an impaired renal function. Possibly, a number of non-survivors were pushed into renal impairment just after their LT. Ojo *et al.*^[36] found that the 5-year incidence of SIRF after LT was 18.1%, resulting in a 4.55-fold increased risk of death and Sanchez *et al.*^[35] described that the lower the initial GFR after LT, the earlier renal failure develops within the next 5 years, emphasizing the importance of a well-controlled post-transplant renal function.

Only about one in five survivors had HBMI before transplantation, compared to every third non-survivor ($P = 0.011$). Obese patients with terminal liver failure are not only at increased risk for perioperative morbidity and mortality^[37], but also for experiencing cardiovascular events^[38], which make up for a major proportion of deaths after LT^[3,17,39].

We found a significant impact of ET-DRI on long-term survival. While close to 60% of patients that received a donor organ with an ET-DRI < 1.2 survived for two

decades and longer, only less than 40% of the patients with an ET-DRI > 1.4 survived for twenty years. In recent years, more than 60% of all LT donor organs in Germany have an ET-DRI of > 1.5^[14], a number that is likely to increase even more with decreasing rates of organ donation. The impact of donor age by itself, which is one of the factors of the ET-DRI, on long-term survival was also significant. Regarding the recipient-donor age match it seems that “older” livers may be suitable for younger recipients, but the benefit of younger organs for elderly recipients evens out 10 years after transplant.

Schaubel *et al.*^[40] described that regardless of the organ quality, higher labMELD recipients have a significant survival benefit from LT, whereas lower labMELD candidates who receive higher ET-DRI organs demonstrate higher mortality and no significant survival benefit. According to that particular study, 2000 life-years could be saved per year if benefit-based allocation was implemented.

Our data suggest that the ideal LT recipient is a young woman with acute liver failure or CD/AIH, who has a BMI < 25, a normal kidney function and no dyslipidemia. Such a patient would benefit the most from a donor organ < 30 years old with an ET-DRI of < 1.2. Since this combination of characteristics may hardly be found in recent years, it is even more important to match a specific donor organ to an adequate recipient, based on benefit-based allocation.

COMMENTS

Background

With major improvements in outcomes after liver transplantation and growing experience regarding transplant management, both the indications for liver transplantation (LT) and donor criteria have been expanded over the years. Shortage of donor organs has led to changes in liver allocation policies and the use of marginal organs.

Research frontiers

Very long-term outcome data (20 years) after LT are scarce. In the presented cohort the best 20-year survival published ever so far was described. This retrospective analysis focuses on donor and recipient characteristics of survivors and non-survivors to elucidate factors that may be predictive of long-term survival.

Innovations and breakthroughs

Several factors influencing long-term survival after liver transplantation could be identified. It seems that “older” livers may be suitable for younger recipients, but the benefit of younger organs for elderly recipients evens out 10 years after transplant. The labMELD score seems not to be an adequate tool in prediction of long term survival. HBMI becomes predictive only ten years after transplant. A high number of non-survivors had an estimated glomerular-filtration-rate that was just above 60, making these recipients barely off the limit for an impaired renal function. Possibly, a number of non-survivors were pushed into renal impairment just after their LT. Immunosuppressive regimens should take this into account and may be adapted accordingly.

Applications

This study gives valuable insights in donor-recipient matching, when trying to achieve excellent long-term outcome, especially when allocating marginal organs to progressively sicker recipients.

Terminology

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BP: Blood pressure; COD: Cause of death; DCD: Donation after cardiac death; ECD: Extended-criteria donor; ET-DRI: Eurotransplant Donor Risk Index; eGFR: Estimated glomerular-filtration-rate; HBMI: Overweight; HLIP: Hyperlipidemia; HCC: Hepatocellular carcinoma; INR: International normalized ratio; LT: Liver transplantation; MELD: Model for end-stage liver disease; MIRF: Moderately impaired renal function; SIRF: Severely impaired renal function; tBili: Total bilirubin.

Peer-review

This retrospective study concerning characteristics of more than 20 years survivors after LT is very interesting and useful.

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Biology of chronic graft-*vs*-host disease: Immune mechanisms and progress in biomarker discovery

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Abstract

Chronic graft-*vs*-host disease (cGVHD) is the leading

cause of long-term morbidity and mortality following allogeneic hematopoietic stem cell transplantation. It presents as a chronic inflammatory and sclerotic auto-immune-like condition that most frequently affects the skin, oral mucosa, liver, eyes and gastrointestinal tract. Both clinical and animal studies have shown that multiple T cell subsets including Th1, Th2, Th17, T follicular helper cells and regulatory T-cells play some role in cGVHD development and progression; B cells also play an important role in the disease including the production of antibodies to HY and nuclear antigens that can cause serious tissue damage. An array of cytokines and chemokines produced by different types of immune cells also mediate tissue inflammation and damage of cGVHD target tissues such as the skin and oral cavity. Many of these same immune regulators have been studied as candidate cGVHD biomarkers. Recent studies suggest that some of these biomarkers may be useful for determining disease prognosis and planning long-term clinical follow-up of cGVHD patients.

Key words: Chronic graft-*vs*-host disease; Biomarker; Allogeneic hematopoietic stem cell transplantation; Cytokine

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Core tip: Chronic graft-*vs*-host disease (cGVHD) is a frequent long-term medical complication of allogeneic hematopoietic stem cell transplantation which can have a devastating impact on overall health and quality of life. This immune-mediated disorder manifests as an inflammatory and autoimmune-like disorder that can affect multiple tissues in an individual patient. Both clinical and animal studies demonstrate that multiple T cell subsets, as well as B cells, and their secreted cytokines play important roles in cGVHD initiation and progression. In the last decade many molecular biomarkers have been identified that correlate with cGVHD onset and/or progression, and some might have applications clinically in the near future.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is utilized primarily as a curative treatment for both hematological and non-hematological malignancies^[1], although it has been used successfully in small-scale clinical trials as a stem cell therapy for some inherited diseases such as Recessive Dystrophic Epidermolysis Bullosa^[2]. In the case of hematologic malignancies, the graft-vs-leukemia or graft-vs-tumor (GVL or GVT) effect mediated by donor-derived T cells helps to eliminate malignant cells in the transplant recipient^[3]. However, a major long-term complication of allo-HSCT is chronic graft-vs-host disease (cGVHD), which occurs in 30%-70% of patients, with adults more frequently affected than pediatric patients^[4]. Chronic GVHD manifests as an autoimmune-like inflammatory disease that can affect a single organ, but more typically it presents as a multi-organ disease affecting the skin (75% of patients), oral mucosa (51%-63% of patients), liver, eyes and gastrointestinal tract (22%-51% of patients)^[4]. Oral mucosal disease can include salivary gland pathology or sclerosis of the lamina propria or submucosa. Other tissues including the lung, esophagus, joints, muscles and genitalia can also be involved (Table 1). cGVHD is often preceded by acute GVHD, which typically occurs within 100 d after transplantation, although the acute form can persist longer.

In allo-HSCT patients, cGVHD is the most common cause of non-relapse mortality (NRM, which refers to mortality not related to the primary malignancy or disease) among patients surviving more than two years^[5]. Other important contributing factors to patient mortality are viral or bacterial infection and secondary malignancies (Figure 1)^[4,6]. A recent analysis by the Center for International Blood and Marrow Transplant Research (CIBMTR) of more than 26000 allo-HSCT patients demonstrated that the incidence of cGVHD is increasing worldwide, making it imperative that we fully understand the etiology of this disease^[7].

This review will focus on the pathobiology of cGVHD, which has features of both alloimmune and autoimmune disease and involves altered activities and function of various T cell populations [T helper (Th) 1, Th2, Th17, T follicular helper cells and regulatory T-cells] as well as of B cells. Equally important are the various cytokines and chemokines produced by immune cells and their target tissues, which cause inflammation and tissue damage. A second productive area of cGVHD research is biomarker discovery; high-throughput approaches including mass spectrometry have led to the identification of a number

of molecular markers from blood and saliva that correlate with active disease. Not surprisingly, many of these markers are associated with altered host immunity and/or tissue inflammation. This review will not discuss current primary and secondary therapeutic strategies for cGVHD; for an in-depth discussion of this topic, the reader is referred elsewhere^[8-10].

CLINICAL FEATURES

According to the NIH consensus criteria published in 2005, cGVHD can be subclassified into: (1) Classic cGVHD presenting with manifestations that can be ascribed only to cGVHD; and (2) Overlap syndrome that has diagnostic or distinctive cGVHD manifestations together with features typical of acute GVHD^[11]. Acute GVHD occurs in 40%-60% of patients receiving allo-HSCT and is one of the major risk factors for subsequent cGVHD. To improve cGVHD classification, the NIH severity score was developed which documents the number of organs involved and numerically scores the degree of functional impairment. Generally, patients are assessed as having mild, moderate or severe disease on a scale of 1 to 4 for each tissue^[11-13]. However, clinical symptoms of cGVHD often overlap with other autoimmune diseases such as lichen planus and scleroderma and the degree of organ involvement is highly variable, which can make diagnosis challenging^[8,14]. Table 1 lists signs and symptoms that are considered to be diagnostic of cGVHD as well as some of the commonly observed clinical features that are considered to be insufficient for disease diagnosis. As many as three or more tissues can be affected in a single patient, as reflected in the NIH global severity classification of cGVHD^[13]. Skin manifestations that are considered diagnostic include poikiloderma (altered pigmentation with erythema), lichen planus-like lesions, sclerosis and morphea-like features (Table 1). Distinctive features (often observed in skin cGVHD but not sufficient for diagnosis) include depigmentation, papulosquamous lesions, ichthyosis and pruritis. Skin appendages are often targeted as well but these signs are not considered diagnostic: Symptoms can include scalp hair thinning or alopecia, sweat impairment and nail dystrophy or onycholysis (nail loss)^[11,13].

Cutaneous cGVHD can occur in two forms termed lichenoid and sclerodermatous^[15]. Lichenoid lesions usually occur early in the course of the disease, presenting as erythematous papules or plaques, with a squamous surface. Typical affected sites include the face, ears, palms and soles. Sclerodermatous cGVHD, which generally develops as a later complication, appear as sclerotic, shiny, white or yellow plaques with patchy hyperpigmentation or a poikilodermal appearance^[15]. Sclerodermatous cGVHD can be localized or generalized and affect underlying tissues including the fascia, ligaments and peripheral nerves, causing pain and morbidity for the affected patient.

Oral symptoms vary but commonly involve lichenoid changes, xerostomia as a result of salivary gland damage,

Table 1 Signs, symptoms and prevalence of chronic graft-*vs*-host disease in selected organs and tissues

Organ or tissue	Prevalence, % ¹	Diagnostic features ²	Distinctive features ³
Skin	75%	Poikiloderma Lichen planus-like features Sclerosis	Depigmentation Papulosquamous lesions
Mouth	51%-63%	Morphea-like features Lichen planus-like features	Xerostomia Mucocoeles Mucosal atrophy Ulcers Pseudomembranes
Liver	29%-51%	None ⁴	None ⁴
Eye	22%-33%		Dry, gritty or painful eyes Cicatricial conjunctivitis Keratoconjunctivitis-Sicca syndrome
GI tract and esophagus	7%-45%	Esophageal web Strictures or stenosis in upper esophagus	
Lung	4%-19%	Bronchiolitis obliterans	Air trapping and bronchiectasis on chest CT scan
Muscles, fascia and joints	6%	Fascitis Sclerosis	Myositis or polymyositis
Genitalia	1%	Joint stiffness or contractures Lichen planus-like features Lichen sclerosus-like features	Erosions, Fissures, Ulcers

¹Frequency of tissue involvement at initial cGVHD diagnosis (from Lee *et al*^[4]); ²Clinical symptoms that are sufficient for cGVHD diagnosis. Information adapted from references 8 and 13; ³Clinical symptoms that are frequently seen in cGVHD, but insufficient for cGVHD diagnosis. Information adapted from references 8 and 13; ⁴While no diagnostic or distinctive features have been identified for liver cGVHD, hepatitis is often seen (and also sometimes in acute GVHD) with elevated serum levels of bilirubin, alkaline phosphatase and alanine aminotransferase (ALT)^[13]. cGVHD: Chronic graft-*vs*-host disease; CT: Computed tomography.

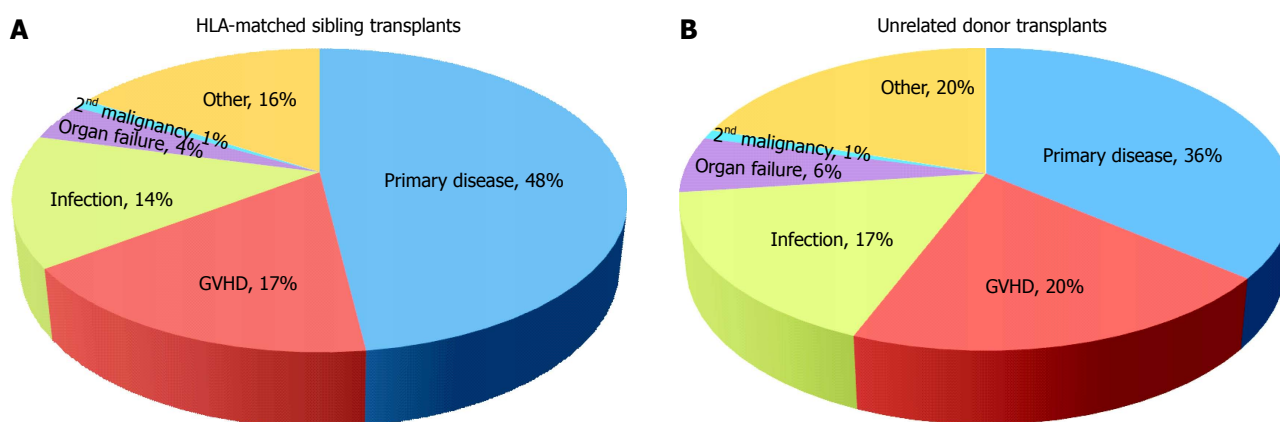


Figure 1 Causes of death among allogeneic hematopoietic stem cell transplantation patients. Pie charts show causes of death among patients who received a cell graft from (A) an HLA-matched sibling or (B) an unrelated donor. Data is from the Center for International Blood and Marrow Transplant Research, for allogeneic hematopoietic stem cell transplants performed in 2012-13^[88] (Available from: URL: <http://www.cibmtr.org/Data/Resources/pages/index.aspx>). GVHD: Graft-*vs*-host disease.

mucositis, erythema, mucocoeles and restricted mouth opening (trismus) due primarily to sclerosis^[16-18]. However, under current guidelines only lichen planus-like features are considered to be diagnostic (Table 1)^[13]. Oral sensitivity and pain are often observed, which in more severe cases manifests as dysphagia (difficulty with swallowing) and weight loss. In one recent study of 210 cGVHD patients, 29% of cases were classified as malnourished by measurement using the Patient-Generated Subjective Global Assessment tool. Malnutrition was correlated with a lower body-mass index and poorer overall survival^[19]. Gingivitis and tooth decay also occur because of xerostomia and altered oral immunity

related to immunosuppression and reduced salivary IgG production^[20].

Clinical symptoms seen in other involved tissues such as the liver, eyes, gastrointestinal tract, lungs, muscles/fascia and genitalia are summarized in Table 1, and have been reviewed extensively elsewhere^[9,13]. Neurological manifestations of cGVHD are rare, but when present can include Myositis and Myasthenia gravis that affect the peripheral nervous system, and less commonly, various complications that affect the central nervous system^[21]. Clinical features of cGVHD do not seem to vary with patient age, graft source (typically either bone marrow or PBSCs) and type of pre-transplant conditioning^[4,9].

Most cases of cGVHD occur 4-6 mo after allo-HSCT, but 5%-10% of patients are diagnosed more than one year following allogeneic transplantation.

RISK FACTORS

The best documented risk factors for cGVHD are a history of acute GVHD (seen in 40%-60% of cGVHD patients), the use of PBSCs for grafting, a female donor-male recipient combination, older patient age and the use of HLA-mismatched or unrelated donors^[13,22,23]. The increasing use of PBSCs (which contain more T cells compared to aspirated bone marrow) is one factor that influences the incidence and severity of cGVHD, since alloreactive T cells are a major player in cGVHD pathobiology^[7]. These risk factors appear to largely explain the increasing incidence of cGVHD worldwide in allo-HSCT; however, additionally, a significant decline in early NRM appears to be contributing to the increased incidence of cGVHD in long-term survivors^[7]. Notably, the frequency of GVHD-associated mortality is similar in HLA-matched sibling transplants compared to transplants performed using an unrelated donor (Figure 1).

A number of studies have also implicated certain genetic polymorphisms in addition to HLA antigen disparity between donor and recipient in the risk of GVHD risk (reviewed in Pidalá *et al*^[24]). For example, polymorphisms in a considerable number of genes that encode cytokines, chemokines or their receptors are associated with increased risk of cGVHD. These include genetic variants in the donor and/or recipient *IL-10* genes^[25-27], donor *IL-1 α* gene^[28], recipient *IL-6* gene^[29], recipient MHC class I-related chain A (*MICA*) gene (Val allele)^[30], and donor and recipient *IL-1* receptor antagonist (*IL-1ra*) genes^[25,28]. For *MICA*, which acts as an activating ligand for the NKG2D receptor on certain types of T-cells, cGVHD incidence was positively correlated with serum *MICA* levels in patients post-HSCT; on the other hand, the presence of *MICA* antibodies prior to transplantation conferred protection against cGVHD^[30]. A smaller number of genetic polymorphisms have been associated with decreased risk of cGVHD^[24].

Baron *et al*^[31] utilized gene expression profiling of donor CD4⁺ and CD8⁺ T cells to develop a "GVHD-predictive signature", demonstrating the central importance of the TGF- β signaling pathway in regulating donor T cell function^[31]. Remarkably, the so-called "dangerous donor" trait derived from T cell gene expression profiling not only predicted early (acute) GVHD, but also cGVHD occurrence in the recipient at one year post-transplantation. These observations reinforces other studies in humans and mice showing that the growth factor TGF- β has pleiotropic effects on T cells, including inhibition of Th1 cell differentiation and promoting expansion of regulatory T cells that are protective against cGVHD^[9,32,33]. It also suggests that the grafted stem cells can have a long-term, dominant influence on the transplant recipient's T cell profile and consequently the overall health of the patient.

PATHOBIOLOGY OF CGVHD: ROLE OF T CELLS, B CELLS AND THEIR CYTOKINES

T cells

While the mechanisms that cause the inflammation and tissue damage of acute GVHD are now quite well understood, the pathobiology of cGVHD is more complex and less well understood. Many investigators believe that the destructive immunological and autoimmune mechanisms that cause cGVHD are distinct from acute GVHD, irrespective of whether or not the cGVHD evolves from acute GVHD^[6,34]. Activated donor T cells are the most important cell population in cGVHD, since T cell depletion from the graft prevents cGVHD in both human and animal studies^[35]. The use of rabbit anti-thymocyte globulin (ATG) in conditioning regimens prior to transplant reduces the risk of subsequent acute and cGVHD, either by depleting donor T cells or by interfering with their activation by recipient alloantigens^[22,36]. The major T cell subsets proposed to be involved in cGVHD include CD4⁺ T cells, CD4⁺ regulatory T cells (Tregs) and CD8⁺ T cells (Table 2).

Th1, Th2 and Th17 cells: Alloreactive CD4⁺ T cells that react to foreign (donor-derived) antigens include several Th cell subsets, primarily Th1, Th2 and Th17 cells. A central role for Th1 cells in acute GVHD is well established^[6]; however, the importance of Th1 (and Th2) cells in cGVHD is still a matter of debate, even though Th1 cytokines such as interferon- γ (IFN- γ) can be found in skin and other tissues of affected patients^[37]. Infusion of murine IFN- γ -null donor T cells reduced cGVHD symptoms in skin and salivary glands, indicating a role for Th1 cells in certain tissues^[38]. A role for Th2 cells has been suggested because of the role of Th2 cytokines such as IL-4 and IL-13 (Table 2) in the production of antibodies to both self and non-self-antigens in patients; murine studies support the involvement of the Th2 cytokines IL-4 and IL-10 in stimulating B cell expansion in cGVHD^[39].

Th17 cells produce several cytokines including IL-17, IL-21 and IL-22, which have potent pro-inflammatory functions in cGVHD^[40]. IL-17A, produced mainly by CD8⁺ T cells, stimulates scleroderma which is an important feature of cutaneous cGVHD; however, current data suggests that co-expressed Th1 cytokines such as tumor necrosis factor- α (TNF- α) contribute to the observed pathology^[41]. Improvement in cGVHD symptoms correlates with a reduction in Th-17 cell numbers in peripheral blood^[42]. In liver cGVHD, there are increased numbers of Th17 cells and an increased Th17/Treg ratio observed in liver biopsies, suggesting that Th17 cells are an important driver of clinical liver disease^[43].

T follicular helper cells: T follicular helper (TFH) cells promote differentiation of naïve B cells into memory B

Table 2 Immune cell types and their function in chronic graft-*vs*-host disease

Cell type	Subtypes	Key cytokines or markers	Brief summary of disease involvement
CD4 ⁺ T cells	Th1	IFN- γ , TNF- α	Pro-inflammatory. Important in acute GVHD, but role in cGVHD unclear
	Th2	IL-4, IL-13	Stimulate antibody production. Role in clinical cGVHD poorly defined
	Th17	IL-17; also IL-21, IL-22, TNF- α	Pro-inflammatory. IL-17 levels correlate with disease severity; IL-17 induces scleroderma of skin and lung
	Tregs	TGF- β , required for Treg proliferation and (differentiation)	Produced mostly in thymus. Suppress autoreactive T cells. Lower levels of Tregs present in cGVHD patients, associated with thymic damage and loss of self-tolerance in cGVHD
	T follicular helper cells ¹	Express CCR5, PD-1 and ICOS	Promote abnormal B cell maturation into long-lived active plasma cells, and IgG secretion
CD8 ⁺ T cells		CXCL9, CXCL10	Mediate graft- <i>vs</i> -tumor effect of transplant. Serum CXCL9 levels elevated in cGVHD patients
B cells (total)		Increased BAFF/B-cell ratio, elevated serum BAFF levels	Decreased in active cGVHD. Remaining B cells are resistant to apoptosis
Naïve and transitional B cells		CD19	Decreased in active cGVHD
Memory B cells (total)		CD19, CD27	Decreased in active cGVHD. Cells essential for a normal immune response to bacterial pathogens or opportunistic infections
Regulatory B cells		IL-10	Decreased in active cGVHD. Function to maintain tolerance and help prevent autoimmune disease
Plasma cells		CD27, CD38	Increased in active cGVHD. Cells secrete immunoglobulins including IgGs and are resistant to apoptosis

¹Mainly classified into Th2 and Th17 subtypes^[44]. cGVHD: Chronic graft-*vs*-host disease; BAFF: B-cell activating factor; CD3: Cluster of Differentiation molecule 13, 19, 27 and 38; CCR5: Chemokine (C-C Motif) receptor 5; CXCL: Chemokine (C-X-C motif) ligand; ICOS: Inducible T-Cell Co-Stimulator; IFN- γ : interferon gamma; IL: Interleukin; PD-1: Programmed cell death 1; Th: T helper cell; TGF- β : Transforming growth factor beta; TNF- α : Tumor necrosis factor alpha.

cells and class switching of *IgG* genes in the germinal center, within secondary lymphoid organs. A recent study showed that TFH cells were unusually active with prolonged survival in cGVHD patients, which correlates with the aberrant survival of B cells and hypersecretion of immunoglobulins^[44]. The increased survival of Th2- and Th17-type TFH cells was correlated with increased cellular expression of the pro-survival marker Bcl-2. Overall, the study by Forcade *et al*^[44] suggests that aberrant B cell activity including production of antibodies is driven, at least in part, by abnormal TFH cell activity. Studies using a murine model have confirmed the importance of TFH cells in cGVHD pathogenesis, particularly for the development of bronchiolitis obliterans syndrome which is a signature feature of lung cGVHD^[45] (Table 1).

Tregs: Tregs, which are CD4⁺ CD25⁺ and also express the transcription factor FOXP3, suppress autoreactive T cells and are important for immune system homeostasis. Specifically, Tregs are essential for the establishment and maintenance of tolerance after allo-HSCT^[46]. Tregs are depleted in both acute and cGVHD, demonstrating their importance as suppressors of inflammation and disease development^[23]. Impaired Treg production and function has been linked to thymic damage as a result of CD4⁺ lymphopenia following allo-HSCT, at least in myeloablative patients^[47]. The presence of Tregs in the skin and oral mucosa of cGVHD patients in a functional (*e.g.*, CXCR3⁺)

state suggests they may play a role in limiting tissue damage by alloreactive T cells^[48]. Pharmacological approaches used to treat steroid-refractory cGVHD that increased Treg cell numbers have shown promise clinically in treating cutaneous cGVHD^[49]. Further, in a study of allo-HSCT patients with acute leukemia, direct infusion of Tregs together with conventional T cells protected against GVHD in almost 90% of engrafted patients, while still maintaining the GVT anti-tumor effect conferred by conventional T cells^[50]. These studies suggest that manipulation of Tregs might be a feasible approach to reducing or preventing GVHD without compromising the anti-tumor surveillance capacity of the patient's immune system.

CD8⁺ T cells: CD8⁺ T cells are another immune cell population present in tissues affected by cGVHD, including the skin and oral mucosa^[17]. Donor CD8⁺ cells mediate the GVT effect of allo-HSCT that typically results in the eradication of malignant cells from the patient. Among the cytokines produced by CD8⁺ cells are CXCL9 and CXCL10; CXCL9 is elevated in the serum of early-stage cGVHD patients, with CXCL9 levels being correlated with disease severity^[51] (Tables 2 and 3).

B cells

In addition to T cells, there is increasing evidence that B cells play a number of important roles in cGVHD pathogenesis^[52]. Patients with active cGVHD consistently have

Table 3 Candidate biomarkers of chronic graft-*vs*-host disease¹

Gene/protein	Function	Biofluid ³	Ref.
BAFF, soluble; BAFF/B cell ratio	Growth factor, promotes B cell expansion and activation	Blood	[59,60,77]
CXCL9 ²	Chemokine produced by activated T cells	Blood	[51,79]
CD-13, soluble	Antigen presentation	Blood	[59]
C-reactive protein ³	Acute phase protein	Blood	[12]
Cystatin B	Inhibitor of cathepsin proteases	Saliva	[80]
IL-1ra	Inhibitor of IL-1 receptor signaling	Saliva	[80]
IL-2R, soluble	IL-2 receptor, marker of activated T cells	Blood	[59,76]
IL-6	Pro-inflammatory Th2 cytokine	Blood	[42,75]
IL-10	Th2 cytokine	Blood	[73]
IL-15	Enhances anti-tumor function of CD8 ⁺ T cells	Blood	[78]
Lactoperoxidase	Anti-microbial enzyme	Saliva	[81]
Lactoferrin	Iron-binding glycoprotein	Saliva	[81]
MICA, soluble	Stimulates T cell activity <i>via</i> NKG2D receptor	Blood	[30]
TGF- β	Anti-inflammatory cytokine; stimulates activity of Tregs	Blood	[33]
TNF- α	Pro-inflammatory Th1 cytokine	Blood	[73-75]

¹This table only includes proteins identified in human biofluids. Antibodies are discussed in the text; ²Blood markers were measured in either plasma or serum isolated from peripheral blood, depending on the study. For saliva, whole unstimulated saliva collected from oral cGVHD patients was used; ³Increased CRP levels were especially associated with joint/fascia and skin involvement, compared to the non-cGVHD control group. cGVHD: Chronic graft-*vs*-host disease; BAFF: B-cell activating factor; CXCL: Chemokine (C-X-C motif) ligand; CD-13: Cluster of Differentiation molecule 13 (or aminopeptidase N); IL-1ra: Interleukin 1 receptor antagonist; IL-2R: Interleukin 2 receptor; MICA: MHC class I-related chain A; TGF- β : Transforming growth factor beta; TNF- α : Tumor necrosis factor alpha; CRP: C-reactive protein.

lower numbers of naïve and transitional B cells as well as total B cells^[53,54] (Table 3). Regulatory B cells that secrete the anti-inflammatory cytokine IL-10 (and form a subpopulation within the transitional and memory B cell compartments) were also less frequent in cGVHD patients and displayed a deficiency in IL-10 production^[55]. Together with Tregs, regulatory B cells play a central role in graft tolerance and the prevention of autoimmune disease and hence represent a topic worthy of further investigation in relation to cGVHD^[56]. CGVHD patients are susceptible to pneumococcal infection which can cause severe or fatal infections in long term transplant survivors^[57]. This susceptibility to infections is associated with the abnormal B cell profile, including decreased numbers of memory B cells that are critical for a normal immune response including IgG production^[53,58]. Like many other autoimmune conditions, cGVHD patients frequently produce allo- and auto-antibodies to DNA and/or other antigens such as male HY antigen, which can correlate with disease onset and severity (see below). Activated B cells secrete an array of Th1 and Th2 cytokines that can regulate the function of T cell populations including Tregs. Levels of B cell activation factor (BAFF), a cytokine that promotes the survival and differentiation of activated B cells, are consistently increased relative to B cell numbers in patients with cGVHD^[59,60]. As discussed above, the increased activity of TFH cells appears to play a significant role in producing the abnormal B cell profile characteristic of cGVHD^[44].

Perhaps the best evidence that B cells are functionally important in human cGVHD are the numerous clinical observations with Rituximab, a humanized monoclonal antibody that targets the membrane protein CD20 of B cells, causing their cell death. Rituximab (and other anti-CD20 drugs) are effective in the treatment of steroid-

refractory cGVHD, resulting in rapid and selective depletion of B cells and diminished activation of cytotoxic T cells; concurrently, the number and activity of Tregs are elevated^[52,61,62]. In one study, the prophylactic use of Rituximab after allo-HSCT significantly reduced the incidence of both acute and cGVHD as well as NRM^[63]. Hence, inhibiting B cell function has profound effects on both B and T cell homeostasis, with significant benefits to cGVHD patients especially in cases where other primary and/or secondary treatments have been unsatisfactory.

ANIMAL MODELS OF CGVHD

Several types of murine models have been utilized for studies of GVHD pathobiology including: (1) a bone marrow transplantation (BMT) model involving lethal radiation (total body irradiation, TBI) and transplantation of syngeneic marrow into treated mice^[64]; (2) a parent-into-F1 model where donor spleen cells are infused into non-irradiated mice^[39]; and (3) a transgenic model utilizing a self-antigen, membrane-associated chick ovalbumin, expressed under the control of the K14 promoter (K14-mOVA), where autoreactive skin disease is promoted by adoptive transfer of CD8 T cells from a second mouse strain, OT-1, that has an engineered T cell receptor specific for an ovalbumin peptide^[65,66]. These animal models each demonstrate one or more manifestations of clinical cGVHD including the presence of anti-DNA antibodies, sclerosis, weight loss and chronic inflammation of skin and mucosal tissues associated with elevated Th1, Th2 and/or Th17 cytokines. The K14-mOVA adoptive transfer model has been used to test the efficacy of novel anti-inflammatory biologics that target the Janus kinase (JAK)^[67] and Histone Deacetylase 6^[68] enzymes, which were shown to be effective at suppressing and/or

reversing cutaneous disease. Tofacitinib, the JAK inhibitor, blocked the expansion and activation of CD8⁺ cells thereby reducing IFN- γ secretion by CD8⁺ cells and keratinocytes as well as preventing the downstream consequences of interferon signaling such as chemokine production and keratinocyte apoptosis^[67]. Another JAK inhibitor, Ruxolitinib (INCB018424), reduced murine GVHD (acute GVHD) symptoms and the levels of pro-inflammatory cytokines by both impairing differentiation of CD4⁺ T cells into IFN- γ and IL-17-producing cells, and by promoting the production of protective Tregs^[69]. Notably this JAK 1/2 inhibitor reduced GVHD symptoms and improved overall animal survival while still maintaining the anti-tumor (GVT) effect^[70].

While these pre-clinical models have been valuable in defining the immune mediators of cGVHD, the animal models do not typically parallel the evolution of the human disease, especially the common clinical presentation of classic cGVHD^[8]. Additionally, in mice receiving intensive conditioning regimens (especially radiation), there is a well characterized scenario of inflammatory cytokine release, T cell activation and homing to target organs where tissue destruction occurs through the action of PBMCs and their associated cytokines. However, in humans the preparative regimen is only one factor involved in GVHD initiation, and its influence may be diminished in patients who now receive reduced-intensity conditioning prior to allo-HSCT^[8]. Some recently described animal models exhibit systemic disease with multi-organ involvement including the lung, which appears to more closely resemble human cGVHD^[71]. Despite some weaknesses, animal models will undoubtedly continue to provide insight into specific aspects of cGVHD pathobiology and will be essential for preclinical testing of new therapies for acute and cGVHD.

CGVHD BIOMARKERS

An emerging area of cGVHD research involves the discovery and validation of biomarkers that might eventually be used in clinical diagnosis or treatment planning. To date, most studies have focused on protein and immune cell biomarkers, even though RNAs (including mRNAs and micro RNAs) might also have utility as disease biomarkers^[54,60]. As defined at the first meeting of the NIH Biomarker Working Group in 2006, cGVHD biomarkers could be used in disease management or clinical trials to: (1) predict response to therapy; (2) measure disease activity; (3) predict the risk of developing cGVHD; (4) diagnose cGVHD or predict prognosis; and (5) serve as a surrogate end point for therapeutic response^[37].

To date, researchers have utilized mass spectrometry-based discovery approaches as well as Luminex and antibody arrays to screen clinical samples for potential serum and saliva protein biomarkers. Biomarkers identified to date can be broadly divided into proteins that function as cytokines and chemokines, immune (*e.g.*, cytokine) receptors and other types of immune or non-immune proteins (Paczesny *et al*^[72] for a recent review).

Identified serum biomarkers that might indicate overall disease (and/or altered immune cell) activity include B cell activation factor (BAFF), MICA and anti-MICA antibodies, TNF- α , IL-15 and Chemokine (C-X-C motif) ligand 9 (CXCL9). Salivary biomarkers, associated mainly with oral cGVHD, include IL-1ra, cystatin B, lactotransferrin and lactoperoxidase (Table 3). Cellular markers primarily comprise immune cell populations that are altered in cGVHD (Table 2).

Serum biomarkers

Chronic cGVHD onset and/or persistence is associated with increased levels of TNF- α , BAFF, IL-6, sIL-2R (soluble IL-2 receptor alpha), and IL-10, and decreased levels of TGF- β and IL-15 (Table 3). Several studies have reported elevated levels of the pro-inflammatory cytokine TNF- α in acute and cGVHD, with measured levels correlating with cGVHD severity^[73-75]. IL-6 shows a similar trend and correlation with cGVHD severity^[42,75]. Soluble IL-2 receptor alpha (sIL-2R α) is another example of a serum marker that is increased in pediatric and adult patients with cGVHD^[59,76]. BAFF, a growth factor that promotes B cell differentiation and immunoglobulin production, is increased in both pediatric and adult patients with cGVHD; levels of this growth factor are often reported relative to the number of B cells in blood samples^[51,59,60,77]. High levels of BAFF protein were present in allo-HSCT patients who subsequently developed cGVHD, confirming its role in alloimmunity^[77]. CXCL9 levels are also elevated in newly diagnosed cGVHD patients and were correlated with disease severity in three different cohorts studied at two transplant centers^[51].

Other markers besides BAFF and CXCL9 have been shown to have potential predictive value in allo-HSCT patients for determining future disease. For example, elevated levels of soluble MICA protein post-allo-HSCT were associated with an increased risk of cGVHD (by contrast, as stated above, the presence of MICA antibodies before transplantation conferred some protection from cGVHD)^[30]. Similarly, Pratt *et al*^[78] have shown that patients with low serum levels of IL-15 at day 7 post-transplant had 3-fold higher risk of developing cGVHD subsequently. IL-15 levels were observed to be inversely correlated with CD8 T cell levels, which are important for the GVT effect but also influence the development of cGVHD (see above).

In addition to intrinsic, host-dependent (*e.g.*, immune) factors, the levels of biomarkers such as BAFF and CXCL9 can be modified by extrinsic factors including immunosuppressive drugs such as corticosteroids^[51,54]. Hence, as recognized by many investigators, independent validation of promising biomarker candidates is essential. CXCL9 was recently validated as a cGVHD biomarker in a multicenter United States study of allo-HSCT patients^[79].

Salivary biomarkers

Two recent studies utilizing mass spectrometry approaches identified a total of 82 and 102 salivary proteins, re-

spectively, that showed altered expression in oral cGVHD^[80,81]. IL-1 receptor antagonist (IL-1ra) exhibited reduced expression in patients with oral cGVHD^[80]. The changes in IL-1ra expression coupled with higher levels of IL-1 family cytokines^[16] in saliva likely enhance oral inflammation and subsequent tissue damage. In particular, IL-6 levels have been shown to correlate with oral cGVHD severity^[16]. Changes in expression of salivary lactoperoxidase and lactotransferrin have also been reported, indicative of impaired innate immunity in oral cGVHD^[81] (Table 3). The alterations in the salivary proteome among proteins involved in innate and acquired immunity are consistent with the clinical features of oral cGVHD, in particular patient susceptibility to bacterial and viral infections^[4,18]. Changes in inorganic salivary components, especially Na⁺ and Cl⁻ ions and inorganic phosphate, also occur in concert with cGVHD onset, correlating with hyposalivation and damage to the salivary glands^[82,83].

Other biofluids

Certain Th2 and Th17 cytokines, in particular IL-6, IL-10, IL-17A and TNF- α are elevated in the tear fluid of cGVHD patients and correlated with systemic cGVHD regardless of ocular symptoms; levels of three of these cytokines (IL-6, IL-10 and TNF- α) also were significantly correlated with ocular cGVHD parameters^[84].

Cellular biomarkers

In addition to protein biomarkers, a large number of immune cell populations have been studied as potential cGVHD biomarkers. Some of the best studied are listed in Table 2. As discussed in the Pathobiology section above, CD4⁺ IL-17⁺ Th17 cells are elevated in active cGVHD while Tregs that express the markers CD4, CD25 and FoxP3 are typically decreased^[42,47]. There are also complex changes in the B cell population of cGVHD patients. Overall, total B cell counts are decreased in cGVHD patients as are the levels of naïve, transitional and regulatory B cells. In contrast, differentiated CD38⁺ CD27⁺ IgG-secreting plasma cells are increased in patients with active cGVHD (Table 2)^[53,54].

Antibodies

Antibodies including autoantibodies are another group of well-studied potential biomarkers that are produced in cGVHD patients by an aberrant B cell population. Up to 80% of allogeneic transplants involving a female donor-male recipient combination produce antibodies against Y-chromosome-encoded HY proteins, and these antibodies appear to predict the development of cGVHD^[8,37]. Antibodies to Platelet-derived Growth Factor (PDGF) Receptor, double stranded DNA and anti-nuclear antibody (ANA) are also common in cGVHD patients^[59]. Anti-PDGF receptor antibodies cause accumulation of reactive oxygen species and stimulate type I collagen expression, suggesting a role for these antibodies in skin and lung fibrosis^[85,86]. Patients with classic cGVHD were found

to have higher levels of ANA and anti-DNA antibodies compared to patients who had a prior history of acute GVHD where B cells have limited involvement^[59].

CONCLUSION

CGVHD is a chronic inflammatory and autoimmune-like condition that involves a complex interplay between the immune systems of the transplant donor and recipient. Despite significant progress in understanding the risk factors, and the development of effective second-line treatments for steroid-refractory cGVHD, the incidence of cGVHD is increasing worldwide^[7]. While donor-derived T cells are still considered to be the preeminent mediators of cGVHD, aberrant B cells clearly play a significant role in promoting autoimmunity and inflammation, and conferring susceptibility to serious, often life-threatening infections. The enhanced activity of T follicular helper cells in cGVHD also appears to play a key role in the aberrant B cell activity and the resulting autoimmune-like features of cGVHD, including the presence of antibodies that target HY and nuclear proteins^[44].

In addition to the significant progress in our understanding of cGVHD immunobiology and pathobiology, guidelines for biomarker development and validation were recently updated. The updated guidelines include recommendations for biomarker identification, verification, qualification, and application with terminology based on Food and Drug Administration and European Medicines Agency guidelines^[72]. Suggested areas of focus for validation include biomarkers that are prognostic, stratify cGVHD risk or are predictive of future disease. Biobank repositories that can serially collect peripheral blood and cell samples from allo-HSCT patients in a standardized format will also be an important tool for pre-clinical biomarker validation^[72]. The French National Cryostem Project is one example of such a national effort^[87,88] which, together with multicenter collaborations^[51,79], should enable protein biomarkers to be added to the clinician's toolkit for cGVHD patient care in the not-too-distant future.

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Organ transplantation and drug eluting stents: Perioperative challenges

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Abstract

Patients listed for organ transplant frequently have severe coronary artery disease (CAD), which may be treated with drug eluting stents (DES). Everolimus and zotarolimus eluting stents are commonly used. Newer generation

biolimus and novolimus eluting biodegradable stents are becoming increasingly popular. Patients undergoing transplant surgery soon after the placement of DES are at increased risk of stent thrombosis (ST) in the perioperative period. Dual antiplatelet therapy (DAPT) with aspirin and a P2Y₁₂ inhibitor such as clopidogrel, prasugrel and ticagrelor is instated post stenting to decrease the incident of ST. Cangrelor has recently been approved by Food and Drug Administration and can be used as a bridging antiplatelet drug. The risk of ischemia *vs* bleeding must be considered when discontinuing or continuing DAPT for surgery. Though living donor transplant surgery is an elective procedure and can be optimally timed, cadaveric organ availability is unpredictable, therefore, discontinuation of antiplatelet medication cannot be optimally timed. The type of stent and timing of transplant surgery can be of utmost importance. Many platelet function point of care tests such as Light Transmittance Aggregrometry, Thromboelastography Platelet Mapping, VerifyNow, Multiple Electrode Aggregrometry are used to assess bleeding risk and guide perioperative platelet transfusion. Response to allogenic platelet transfusion to control severe intraoperative bleeding may differ with the antiplatelet drug. In stent thrombosis is an emergency where management with either a drug eluting balloon or a DES has shown superior outcomes. Post-transplant complications often involved stenosis of an important vessel that may need revascularization. DES are now used for endovascular interventions for transplant orthotropic heart CAD, hepatic artery stenosis post liver transplantation, transplant renal artery stenosis following kidney transplantation, *etc*. Several antiproliferative drugs used in the DES are inhibitors of mammalian target of rapamycin. Thus they are used for post-transplant immunosuppression to prevent acute rejection in recipients with heart, liver, lung and kidney transplantation. This article describes in detail the various perioperative challenges encountered in organ transplantation surgery and patients with drug eluting stents.

Key words: Drug eluting stents; Cangrelor; Stent thro-

mbosis; Organ transplant; Antiplatelet medication; Platelet function assays; Mammalian target of rapamycin inhibitors; Post-transplant immunosuppression; Post-transplant endovascular inhibition; Ticagrelor; Thromboelastograms platelet mapping; Novolimus; Biolimus A9

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Core tip: Patients undergoing transplant surgery soon after the placement of drug eluting stents (DES) are at increased risk of stent thrombosis (ST) in the perioperative period. Dual antiplatelet therapy with aspirin and a P2Y12 inhibitor is instated post stenting to decrease the incident of ST. Cadaveric organ availability is unpredictable, therefore, discontinuation of antiplatelet medication cannot be optimally timed. Many platelet function point of care tests are used to assess bleeding risk and guide perioperative platelet transfusion. Response to allogenic platelet transfusion to control severe intraoperative bleeding may differ with the antiplatelet drug. DES are now used for endovascular interventions for post-transplant orthotopic heart coronary artery disease, hepatic artery stenosis post liver transplantation, *etc.* Antiproliferative drugs used in DES are also used for post-transplant immunosuppression.

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INTRODUCTION

Percutaneous coronary intervention (PCI) is presently the most frequent revascularization procedure used for treating coronary artery disease (CAD). It surpasses coronary artery bypass grafting. Balloon angioplasty and coronary stenting are the most common percutaneous coronary interventions.

Angioplasty is complicated by vessel spasm, recoil, and abrupt closure. Coronary stenting with bare metal stents (BMS) may prevent these complications, however, they are associated with restenosis rates of 25%-30%^[1]. Studies on stent thrombosis (ST) with BMS show that clinical consequences of angiographic ST includes a 64.4% incidence of death or myocardial infarction at the time of ST and a six-month mortality of 8.9%^[2]. For clinically defined ST events, the associated six-month mortality is as high as 20.8%. Due to such high risk of death following ST, it must be prevented at all costs. The angiographic outcome yielded by primary percutaneous intervention (PPCI) by drug eluting balloons (DEB)-only in selected patients was comparable to those stented by BMS alone and when DEB insertion was followed by stenting with BMS. If the patient has potential contraindications to DES, then DEB-only is a good alternative^[3].

When the stented coronary artery is narrowed due

to the development of neo-intimal hyperplasia within the stent, it is termed as restenosis. An inflammatory reaction, both acute and chronic, results when there is arterial trauma and a foreign body response. Smooth muscle migration and proliferation result in scar tissue formation within the stent, thus narrowing the vessel lumen. This process generally begins to occur in first six to eight weeks after stenting, but can be seen beyond one year after stent placement.

DES was introduced to reduce the rate of restenosis. The antiproliferative drug eluted inhibits smooth muscle and endothelial cell proliferation^[4], thus delaying the inflammatory response. The layering of endothelial cells over the stent is slower paced than with BMS. When the stent is endothelialized, it becomes incorporated into the artery. Complete healing of first generation DES may take upto two years^[5]. The drug is held and released by a biocompatible polymer coating^[6]. However, endothelialization of the stent may also be delayed. This increases the risk of subacute ST. Risk of after DES implantation is related to stent length, stenting across branch ostia, disruption of adjacent vulnerable plaques, and plaque prolapse^[7]. Failure to form a complete neo-intimal layer over stent struts or impaired healing makes the stent more susceptible to thrombosis^[8]. Premature interruption of DAPT, renal failure, cardiac compromise with low ejection fraction (EF), bifurcation stenting and diabetes contribute to the risk of thrombotic events in DES^[9].

DES

The type of stent can have significant implications on the perioperative management of a transplant recipient (Table 1).

First generation DES

Coronary first generation drug eluting stents were coated with antiproliferative drugs sirolimus and paclitaxel. First generation stents used were Paclitaxel eluting TAXUS (Boston Scientific, Natick, MA) stent (PES) and sirolimus eluting CYPHER (Cordis, Miami, FL) stent (SES). Paclitaxel, which is derived from a Pacific Yew Tree (*Taxus Brevifolia*), is a cytotoxic anti-neoplastic drug which causes cell-cycle arrest in the G2/M phase transition^[10,11]. PES, have a bimodal release that is completed in approximately two weeks^[12]. Sirolimus is a macrolide antibiotic with potent antifungal, immunosuppressive, and anti-mitotic activities, and is produced by the fungus *Streptomyces hygroscopicus*^[11]. Sirolimus is cytostatic, and produces cell-cycle arrest in the G1/S phase transition. Sirolimus eluting stents (SES) slowly elute over a time frame of four to six weeks.

Second generation stents

Everolimus and zotarolimus are drugs used in second generation durable polymer stents. Second generation stents commonly used are zotarolimus eluting stent

Table 1 Types of stents

Generation of DES	Drug eluted	Some commercially available products	Features
First generation	Sirolimus, Paclitaxel	TAXUS, CYPHER	High Incidence of stent thrombosis, subacute as well as late thrombosis
Second generation	Zotarolimus, Everolimus	ENDEAVOR, XIENCE V	Safer and more efficacious as compared to first generation stents
Third generation	Novolimus, Biolimus A9	SYNERGY, BIOMATRIX, NOBORI, DESyne	Newer generation biodegradable stents which have shown superior outcomes

DES: Drug eluting stents.

(ZES) ENDEAVOR (Medtronic Inc. NJ) and everolimus eluting stent (EES), XIENCE V (Guidant Corporation, IN). Everolimus is a derived from sirolimus. Everolimus has a shorter half-life, and a greater bioavailability. It also has different blood metabolite patterns, as compared to sirolimus^[13].

Third generation stents

Newer generation biodegradable drug-eluting stents are designed to manage the longer side effects of residual durable polymer which persist after the drug has been completely eluted. The biodegradable polymer is applied to the abluminal side or outside surface only. Thus the inner or luminal side is free from the drug. After 3-4 mo of implantation, this stent loses most of its coating, acquiring a profile which is similar to that of a BMS^[14,15]. Novolimus and Biolimus A9 have been used in the third generation biodegradable stents. Biolimus A9 is a highly lipophilic analogue of sirolimus. The uptake by the coronary vessel wall is much better, thus the risk of systemic immunosuppression and toxicity is reduced^[16]. Novolimus is an active metabolite of sirolimus. It provides efficacy at lower dose (85 mcg of novolimus vs 140 mcg of sirolimus) and a lower polymer load^[17]. Recent ones introduced are the SYNERGY, BioMatrix, Nobori and DESyne stents^[18]. The NOBORI is a biodegradable biolimus eluting stent. Third generation stents with bioresorbable scaffolds such as the Abbott's BVS®, an everolimus-eluting device with a poly-L-lactic acid (PLLA)-base, is now seeing increasing clinical use. Elixir's DESolve®, a PLLA-based novolimus-eluting device is another device used clinically. Biotronik's DREAMS®, a metallic magnesium-based paclitaxel-eluting device, is a third device that has been deployed^[19]. The drug attaches directly, without polymer to the textured stent surfaces, in stents such as the BioFreedom stents and Yukon Choice stents^[18]. Coatings which are non-pharmacological, such as carbon, silicon carbide and titanium-nitride-oxide provide better outcomes than BMS. Gene eluting stents such as the Genous stent, function by promoting the attachment of endothelial progenitor cells^[18].

A meta-analysis of 51 trials that included a total of 52158 randomized patients concluded that all DES have demonstrated superior efficacy when compared with BMS^[20]. First generation stents have a high incidence of stent thrombosis, both subacute as well as late thrombosis^[9]. Among DES, second-generation devices are

substantially safer and more efficacious when compared with first-generation devices^[20]. These second generation stents are now being used to revascularize blocked left main coronary artery and are clearly superior to CABG. RESOLUTE all-comers (Randomized Comparison of a Zotarolimus-Eluting Stent with an Everolimus-Eluting Stent for Percutaneous Coronary Intervention) trial showed that ZES was noninferior to EES at 12-mo for the primary end point of target lesion failure^[21]. The NOBLE (Coronary Artery Bypass Grafting vs Drug Eluting Stent Percutaneous Coronary Angioplasty in the Treatment of Unprotected Left Main Stenosis) and EXCEL (Evaluation of XIENCE Everolimus Eluting Stent vs Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization) trials were conducted to compare PCI vs CABG. The EXCEL trial concluded that there was an equipoise for long-term mortality between CABG and PCI in subjects with unprotected left main coronary artery (ULMCA) disease up to an intermediate anatomical complexity. The anatomical and clinical characteristics impacted the decision making between CABG and PCI, and also in prediction of the long term mortality^[22]. Clinical characteristics which shifted long-term mortality predictions in favor of PCI was COPD, male gender and old age. Reduced left ventricular ejection fraction, lower creatinine clearance, younger age and female gender favored CABG^[22]. Thus PCI of the ULMCA with drug-eluting stents is safe and effective when performed in high volume centers with expertise^[23]. The SYNERGY bioresorbable polymer everolimus-eluting stent was noninferior to the PROMUS Element Plus everolimus-eluting stent with respect to 1-year target lesion failure^[24]. In a large meta-analysis, bioresorbable polymer based biolimus eluting stents (BP-DES) were associated with superior clinical outcomes compared with BMS and first generation DES and similar rates of death/MI, MI and target vessel revascularization (TVR) compared with second generation durable polymer DES. However, there were higher rates of ST compared with cobalt chromium EES^[25]. The novolimus eluting coronary stent DeSyne was found to be superior to ZES at a five year follow up^[26].

Various strategies have been employed to reduce the adverse effects associated with the drug eluting stents. A novel curcumin loaded nanoparticles (Cur-NP) preparation administered intravenously after stent implantation recovered endothelium function by accelerating endothelial cells restoration^[27].

Combretastatin CA4 inhibits the SMC cycle more

effectively than paclitaxel and sirolimus. It may be a newer antiproliferative drug which can be used for drug-eluting stents^[28]. Another drug called MiR-21 modulates the post stenting inflammatory response. This may have a therapeutic potential to clinical efficacy of stenting^[29].

ANTIPLATELET MEDICATION

Antiplatelet medications prevent thrombus formation till the stent is completely endothelialized. Intraluminal thrombus formation may lead to vascular occlusion, transient ischemia, or infarction^[27]. Antiplatelet drugs interfere with platelet adhesion, release and/or aggregation^[30].

Aspirin

Aspirin binds to enzyme cyclo-oxygenase preventing conversion of arachidonic acid to thromboxane, thus interfering with platelet action. Aspirin alone has little or no effect on angiographic or clinical restenosis. Lower doses of aspirin, 75-100 mg, are used in combination with other antiplatelet agents. Higher dose of aspirin is associated with increased risk of bleeding when used along with clopidogrel without any added benefit^[31].

Aspirin irreversibly inhibits platelets. Therefore, its action lasts until a significant number of platelets have been synthesized. By day 3, complete recovery of platelet aggregation may occur in 50% of cases. By day 4, complete recovery occurs in approximately 80% of cases^[32]. Reduced aspirin responsiveness can be measured by impedance platelet aggregometry^[33]. Some of the potential causes of reduced aspirin responsiveness include non-compliant intake, genetic polymorphisms of COX-1, increased platelet turnover and drug interactions^[34].

Clopidogrel

Clopidogrel has an active metabolite which irreversibly inhibits the acts on the ADP P2Y₁₂ receptor. The P2Y₁₂ receptor plays a vital role in the formation of a thrombus since it amplifies and completes the ADP response to thromboxane, thrombin and collagen^[35], and completes the activation of GP IIb/IIIa and GP I a/IIa for further stabilization of platelet aggregates^[36,37]. At steady state, the average inhibition level observed with a dose of 75 mg of clopidogrel per day is between 40%-60%. The prevalence of reduced clopidogrel response in patients is evaluated between 5% and 44%^[38] and is termed as high on treatment platelet reactivity (HTPR). Some of the causes of clopidogrel HTPR include genetic polymorphisms of the P2Y₁₂ receptor and of CYP3As, accrued release of adenosine phosphate, and up-regulation of other platelet activation pathways^[35].

Ticagrelor

It is a direct-acting, oral, newer reversible P2Y₁₂ receptor antagonist, and has a faster onset, and is more predictable and potent than clopidogrel. It binds allosterically to the

platelet ADP P2Y₁₂ receptor, thus, the binding does not cause a conformational change in the P2Y₁₂ receptor. It has a short offset time. It does need metabolic activation. It has a superior safety profile as compared to clopidogrel or prasugrel as seen in the PLATO (Platelet Inhibition and Patient Outcomes) study^[39]. It has been proven superior than clopidogrel in patients with chronic kidney disease. However, it should be avoided in patients with moderate-to-severe hepatic impairment and high bleeding risk^[40]. Complications include lung injury and dyspnea due to endogenous adenosine release^[41].

Prasugrel

Prasugrel is an oral irreversible inhibitor of the P2Y₁₂ receptor. Current European Society of Cardiology guidelines recommend prasugrel or ticagrelor over clopidogrel in patients with acute coronary syndromes (ACS) after PCI^[42]. If clopidogrel is used as a first line antiplatelet agent, then a platelet function assay should be performed, and a switch to prasugrel or ticagrelor is recommended for those with HTPR^[43]. The advantage of prasugrel is that it has a 5%-6% or low percentage of non-responders^[43].

Cangrelor

Cangrelor is an intravenous short-acting (half-life 3-6 min) P2Y₁₂ inhibitor, which is directly reversible. It does not require metabolic conversion. Intravenous cangrelor can produce rapid platelet aggregation with almost full recovery of platelet activity within 60-90 min of withdrawal^[44]. When cangrelor is administered intravenously to patients with CAD, the risk of MACE and stent thrombosis is reduced. There are however, increased events of minor bleeding^[44]. Additionally, cangrelor plays an important role in cases where cardiologist is not comfortable preloading a patient with antiplatelet therapy before an angiography, when it is uncertain that the patient may need urgent surgery. It has been recently approved by the FDA in June 2015^[45].

It is useful as a "bridging therapy" in patients with stents or acute coronary syndrome who need surgery, since they are increased risk for stent thrombosis when oral P2Y₁₂ therapy is temporarily stopped^[46].

The optimal duration of dual antiplatelet therapy has been a topic of debate. Most trials which compare antiplatelet strategies after PCI in a population state that the risk of bleeding and ischemia are average. Unfortunately, the information to recommend choices based on individual patient risks is scarce, especially beyond 1 year of DES placement and DAPT. There are many common risk factors associated with individual patient risks of ischemia and bleeding^[47].

A trial compared 6 wk of clopidogrel, aspirin and oral anticoagulation medications with 6 mo of clopidogrel therapy. However, there were no superior outcomes with the 6 wk triple therapy^[48]. Another study determined when permanent DAPT is discontinued before 30 d post cobalt chromium everolimus-eluting stent implantation, there was a strong association with ST. If the DAPT was discontinued after 90 d, it was safer^[49]. A large multicenter

study determined that the safety and efficacy of a 6-mo DAPT post implantation of new-generation DES was noninferior to that of a 12-mo DAPT^[50].

There is a lot of debate regarding short term dual antiplatelet therapy vs extended dual antiplatelet therapy. A study concluded that extended DAPT is associated with 8 fewer myocardial infarctions per 1000 treated patients per year. But unfortunately, there were 6 more major bleeding events than shorter-duration DAPT. Thus the duration of the DAPT should ideally be optimized taking into account the patient's values and preferences^[51]. A meta-analysis concluded that among selected patients undergoing DES implantation, a short duration (3-6 mo) of DAPT appears as the safest strategy. An extended duration (24-36 mo) of DAPT reduces thrombotic complications but with an excess in major bleeding complications^[52-54]. The duration of DAPT is challenging to adjust in those patients with an increased bleeding or thrombotic risk. These patients need a personalized DAPT duration, which is tailored to patients's, not stent's, characteristics^[55].

Two large studies, the Patient Related Outcomes With Endeavor vs Cypher Stenting Trial (PROTECT), and PROTECT US, determined that at a median follow-up of 4.1 years, major bleeding occurred in 2.8% subjects and ischemic events in 6.3%^[47]. There was no difference in mortality or stroke^[56].

The SECURITY trial which studied 6 mo vs 12 mo dual antiplatelet therapy following second generation DES implantation concluded that in a low-risk population, the 6 mo of DAPT following second-generation DES implantation was acceptable for the incidence of death, MI and stroke^[57]. The OPTIMIZE trial results stated that in patients with stable coronary artery disease or low-risk ACS treated with zotarolimus-eluting stents, 3 mo of DAPT was noninferior to 12 mo for NACCE, (NACCE; a composite of all-cause death, myocardial infarction (MI), stroke, or major bleeding) without significantly increasing the risk of stent thrombosis^[58].

The 2014 ACC/AHA current guidelines^[59] recommend 12 mo of DAPT post DES implantation. As the result of several randomized clinical trials showing the safety of a shorter duration of DAPT, the European Heart Society altered their recommendations to 6-12 mo of DAPT post DES implantation^[42].

PERIOPERATIVE MANAGEMENT

Transplant organ recipients usually have end stage organ disease and other comorbidities, and can be assigned the American Society of Anesthesiologists Grade 4 status. Furthermore, all transplant surgery can be classified as high risk. Thus, potential transplant recipients with drug eluting stents require extensive workup and evaluation. It is essential that the transplant anesthesiologist, surgeon and cardiologist be a part of the multidisciplinary team to help determine the optimal management for surgery in these patients. Such patients also need to be

screened carefully by the Transplant Center's Selection Committee prior to UNOS listing as a potential organ recipient. Major considerations would be whether the recipient would tolerate such a high risk associated with the transplant surgery and whether the organ is being optimally allocated (Table 2).

Living donor transplant surgery is an elective procedure and can be optimally timed so that the risk of intraoperative bleeding and ischemia is minimized in a drug eluting stent recipient. On the other hand, cadaveric organ availability is unpredictable, therefore, the discontinuation of antiplatelet therapy cannot be optimally planned. Discontinuation of anti-platelet medication for transplant surgery can pose a significant challenge for perioperative management. Patients undergoing transplant surgery soon after the placement of coronary stents are at increased risk of ST in the perioperative period. The risk of perioperative ischemia is higher if the stent were originally inserted for ACS rather than stable coronary artery disease (SCAD). When antiplatelet therapy is discontinued due to risk of bleeding, the risk of ST is clearly elevated, especially during surgery, which is generally a hypercoagulable state due to increased fibrin formation. If the antiplatelet therapy is continued, there may be bleeding, which in turn leads to hypotension. Hypotension may slow the blood through the stent resulting in ST. Thus risk of ST will be elevated in the perioperative period regardless of whether the antiplatelet therapy is continued or not. If the patient is on top of the Transplant Center's recipient list, one may discontinue oral antiplatelet medication and use a bridging therapy till a cadaveric organ is obtained. However, such a strategy may have inherent risks and would need meticulous monitoring.

ACC/AHA guidelines state in patients undergoing urgent noncardiac surgery during the first 4 to 6 wk after BMS or DES implantation, dual antiplatelet therapy should be continued unless the relative risk of bleeding outweighs the benefit of the prevention of stent thrombosis. In patients who have received coronary stents and must undergo surgical procedures that mandate the discontinuation of P2Y12 platelet receptor-inhibitor therapy, it is recommended that aspirin be continued if possible and the P2Y12 platelet receptor-inhibitor be restarted as soon as possible after surgery. Perioperative management of antiplatelet therapy should be formulated by a team of the surgeon, anesthesiologist, cardiologist, and patient, who should weigh the relative risk of bleeding with that of stent thrombosis^[59].

Aspirin is usually continued throughout the surgical procedure. The 2014 European Society of Cardiology and European Association for Cardiothoracic Surgery guidelines on myocardial revascularization support the 5 d clopidogrel withdrawal period before CABG. These guidelines also add that platelet function testing should be used to guide antiplatelet therapy interruption rather than a specified arbitrary time period^[42]. Recent studies state that patients on aspirin and clopidogrel < 5 d before CABG who had preoperative ADP-induced platelet aggregation \geq

Table 2 Antiplatelet drugs

Drug	Mechanism of action	Duration of action	Platelet responsiveness	Features
Aspirin	Aspirin binds to enzyme cyclo-oxygenase preventing conversion of arachidonic acid to thromboxane	Effect of aspirin lasts until a significant pool of new platelets is synthesized	Reduced aspirin responsiveness can be measured by impedance platelet aggregometry	Aspirin alone has little or no effect on angiographic or clinical restenosis
Clopidogrel	Irreversibly inhibits the ADP P2Y12 receptor	At steady state, the average inhibition level observed with a dose of 75 mg of clopidogrel per day is between 40%-60%	The prevalence of reduced clopidogrel response in patients is evaluated between 5% and 44% and is termed as HTPR	Some of the causes of clopidogrel HTPR include genetic polymorphisms of the P2Y12 receptor and of CYP3As, accrued release of adenosine phosphate, and up-regulation of other platelet activation pathways
Ticagrelor	Direct-acting, oral, newer reversible P2Y12 receptor antagonist	It binds allosterically to the platelet ADP P2Y12 receptor, thus, the binding does not cause a conformational change in the P2Y12 receptor. It has a short offset time	More predictable and potent than clopidogrel	Should be avoided in patients with moderate-to-severe hepatic impairment and high bleeding risk. Complications include lung injury and dyspnea due to endogenous adenosine release
Prasugrel	Oral irreversible inhibitor of the P2Y12 receptor	Effect of prasugrel lasts until a significant pool of new platelets is synthesized	Better inhibition for those with high HTPR	A 5%-6% or low percentage of non-responders
Cangrelor	Intravenous directly reversible P2Y12 inhibitor	Half-life 3-6 min	Rapid platelet aggregation with almost full recovery of platelet activity within 60-90 min of withdrawal	Useful to preload with antiplatelet therapy before the angiography should the patient's anatomy require urgent surgery

HTPR: High on treatment platelet reactivity.

50% have bleeding risk similar to those receiving aspirin monotherapy, thus a 5 d clopidogrel discontinuation period may not always be necessary^[60]. Guidelines also recommend the discontinuation of ticagrelor 5 d prior to surgery and recommencing therapy as soon as it is safe to do so. Since prasugrel has more prolonged and effective platelet inhibition than clopidogrel, it should be stopped 7 d prior to surgery^[42].

The risk of stent thrombosis is associated with stent type and time from stenting to surgery. It will be highest if BMS or DES is inserted within 30 d of the transplant surgery. The risk is high when the surgery is carried out < 1 mo after BMS and < 6 mo after DES, is intermediate if performed between 1-6 mo after BMS and 6-12 mo after DES, and low if performed > 6 mo after BMS and > 12 mo after DES^[61].

A study involving over 12000 patients with previous coronary stenting who underwent over 17000 surgical procedures stated that cardiac death occurred in 2.5%, myocardial infarction in 1.5%, and serious bleeding event in 6.4%. Surgery increased 1.58 × the risk of cardiac death during follow-up. Older generation stents were associated with higher risk of adverse events as compared to BMS > 12 mo before surgery. Newer DES showed similar safety as BMS > 12 mo and between 6 and 12 mo. They also trended to be safer between 0 and 6 mo^[61].

European Guidelines state that most surgical procedures can be performed on DAPT or ASA alone with acceptable rates of bleeding^[42]. The timing of surgery mattered most during the first 6 mo after PCI, with

respect to MACE events. There was no association of the stent type (BMS vs DES) with MACE after surgery. The guidelines further state that whenever possible, the elective non cardiac surgery should be postponed till the completion of the full course of DAPT ideally, 6 mo in SCAD and 1 year in acute coronary artery syndrome (ACS) patients, and that surgery be performed without discontinuation of aspirin^[42]. Shorter duration of DAPT may be justifiable if surgery cannot be delayed. In very high risk patients, 5 d prior to surgery, patient maybe switched from clopidogrel to a reversible antiplatelet agent with a short half-life such as IV tirofiban or eptifibatide, and stop the infusion 4 h prior to surgery^[42]. The substitution of DAPT with LMWH or UFH is ineffective. In surgical procedures with low-to-moderate bleeding risk, surgeons should be encouraged to operate while maintaining DAPT^[42].

Various Platelet Function Assays for P2Y12 Receptor Antagonisms are Light Transmittance Aggregometry, (LTA), vasodilator stimulated phosphoprotein (VASP), VerifyNow, TEG Plateletmapping and Multiple Electrode Aggregometry (MEA)^[62]. The LTA uses plasma and optically measures platelet aggregation, and is considered the gold standard. The VASP uses whole blood and flow cytometry to specifically measure P2Y12 activity, as it is the only assay which is not affected by the ADP's effect on the P2Y1 receptor, and thus is specific for P2Y12 inhibition. The VerifyNow P2Y12 assay uses whole blood, and optically measures platelet aggregation. Advantages of VerifyNow is that it is readily available in clinical settings and is a point of care assay^[62]. The Assessment

of Dual AntiPlatelet Therapy with Drug-Eluting Stents (ADAPT-DES) trial is a very large observational platelet function study. It stated that upto 50% of 30-d post-PCI ST could be attributed to HTPR, which was defined as a P2Y12 reaction unit value of > 208 with VerifyNow® test^[63]. Point of care platelet function testing can also be done with TEG Plateletmapping (TEG-PM). It measures the degree of platelet inhibition resulting from aspirin or ADP receptor antagonists and correlates well with light transmission aggregometry^[64]. TEG-PM can measure the percentage adenosine 5'-diphosphate platelet receptor inhibition (ADP-PRI) by clopidogrel prior to urgent transplant surgery. An ADP PRI of 30% or more can be classified as high bleeding risk. Another study was conducted to predicted risk of bleeding and adverse outcomes by TEM-PM in patients taking clopidogrel within 7 d of non-cardiac surgery. Interestingly, there was no correlation between duration of clopidogrel omission and percentage ADP-PRI^[65].

Excessive bleeding can be treated by allogenic platelet transfusions (PT) in patients on P2Y12 receptor inhibitors. Though the American Association of Blood Banks 2015 clinical practice guidelines suggests prophylactic platelet transfusion for patients having major elective nonneuraxial surgery with a platelet count less than 50×10^9 cells/L, there is no recommendation for platelet transfusions for patients on dual antiplatelet therapy^[66]. In the APTITUDE-Coronary Artery Bypass Graft (APTITUDE- CABG) study, VASP reactivity index, was assessed before and after *in vivo* PT administered for excessive bleeding in patients undergoing cardiac surgery while on a maintenance dose of aspirin and clopidogrel ($n = 45$), prasugrel ($n = 6$), or ticagrelor ($n = 3$). When compared with baseline, there was a significant relative increase of 23.1% in platelet activation after PT transfusion. PT restores platelet reactivity in patients with ACS/PCI and in patients undergoing cardiac surgery on P2Y12 RI while bleeding with a less effect with increasing potency of P2Y12 inhibition^[67]. A recent study stated that clopidogrel had no effect on donor PLT function. Prasugrel has mild effect on donor platelet function. Ticagrelor completely abolished ADP mediated PLT activation in all assays tested. The observed effects were due to Ticagrelor and not elevated adenosine concentrations in the patient's plasma. A modified multiple electrode aggregometry (MEA) assay can be used to determine whether the patient would be likely to benefit from platelet (PLT) transfusions^[68].

The BRIDGE trial was a pharmacodynamic study evaluating platelet reactivity of cangrelor vs placebo in ACS and/or patients with a stent who were at increased risk of thrombotic events because of discontinuation of an oral P2Y12 inhibitor before cardiac surgery^[46]. The primary efficacy end point [percentage of patients with all samples during the infusion achieving platelet reactivity unit (PRU) < 240 as determined by VerifyNow P2Y12 assay, Accumetrics, San Diego, CA] was met in 98.8% of cangrelor- treated patients compared to 19.0%

of placebo-treated patients. After discontinuation of cangrelor, platelet reactivity was similar for both cangrelor and placebo groups^[55]. Cangrelor has been approved by the FDA in June 2015^[45]. When cangrelor occupies the P2 Y12 receptor, the active metabolite of clopidogrel is unable to bind to it. However, this reaction is avoided when clopidogrel is given at the end of the cangrelor infusion. Earlier administration increases the recovery of platelet function. Antiplatelet effects of prasugrel were apparent when prasugrel was administered 0.5 h before cangrelor was stopped^[69,70].

In the Drug Eluting Stent Event Registry of Thrombosis (DESERT)^[71], the largest case- control registry of late/very late thrombosis after DES, 75% of ST events occurred after 1 year, similar to the 60% rate observed in a study^[71]. Furthermore, the clinical presentation of late/very late ST events in DESERT was mainly ST-segment-elevation myocardial infarction (67%). More than half of all ST-related MIs were Q-wave MIs, and subsequent mortality was increased 8-fold after an ST-related MI, the greatest hazard of any MI type^[71].

In stent restenosis can be managed with BMS, brachytherapy, rotational atherectomy and cutting balloons, DEB and DES. A meta-analysis concludes that for treatment of any type of coronary in-stent restenosis (ISR), PCI with everolimus-eluting stents is optimal, because of the best angiographic and clinical outcomes. Use of drug coated balloons (DCB) is also favored, because of its ability to provide favorable results without adding a new stent layer^[73]. Additionally, when DES are implanted to treat BMS restenosis, at 6 mo, struts coverage is more complete when compared with DES implanted in atherosclerotic lesions^[74]. In patients with DES-ISR, EES were superior, both clinically, as well as angiographically, when compared with DEB^[75].

POST TRANSPLANT IMMUNOSUPPRESSION

The drugs sirolimus, everolimus, biolimus and novolimus are inhibitors of the mammalian target of rapamycin (mTOR). After organ organ transplantation, the mTORs are used along with calcineurin inhibitors (CNIs) to provide immunosuppression. They are also used as proliferation signal inhibitors coated on DES. Their use in cancer therapy bears the same mechanism. Everolimus antagonizes the negative effects of CNIs kidney cell and neuronal metabolism and stimulates mitochondrial oxidation, thus reducing the vascular inflammation^[13]. In transplantation, everolimus has been used post-transplant in heart, liver, lung and kidney transplant recipients to prevent acute rejection. In kidney transplant patients, everolimus may minimize or remove calcineurin inhibitors^[76]. Interestingly, renal transplant patients with DES had a low rate of ST, probably related to the immunosuppressants given to prevent kidney rejection^[77]. Everolimus has also been approved by the FDA for use in liver transplantation (LT), and is safe for use with tacrolimus within the first month

after LT^[78].

POST TRANSPLANT ENDOVASCULAR INTERVENTION WITH DES

DES has been successfully used to stent stenotic lesions post-transplant surgery. Transplant coronary artery disease (TCAD) is a major cause of morbidity and mortality after the first year after orthotopic heart transplantation (OHT). OHT patients with ISR have poor long-term prognosis^[79]. EES used on OHT patients with TCAD is associated with a low incidence of target vessel revascularization (TVR) and target lesion revascularization (TLR)^[80]. Unfortunately, long-term mortality remains high in orthotopic heart transplantation (OHT) recipients after PCI with either DES or BMS^[81].

Transplant renal artery stenosis (TRAS) following kidney transplantation has an incidence rate ranging from 6% to 23%. Endovascular intervention with DES improves blood pressure control and allograft function^[82]. ISR occurs in as many as 13% of patients after PTA and stent insertion. A case report describes three such patients, of which, in two patients, the transplant renal artery remained patent after insertion of PES, and one patient required balloon angioplasty 7 mo after the DES was inserted^[83]. BMS have been used to treat lung transplant related pulmonary artery stenosis^[84]. DES have been placed into the pulmonary veins as a bridge to heart lung transplantation in a patient with extensive and recurrent congenital pulmonary vein stenosis^[85]. DES have been safely used and may prevent ISR in patients who undergo intracoronary bone marrow mononuclear cell transplantation post coronary stenting^[86]. Orthotopic liver transplantation (OLT) is commonly complicated by hepatic artery stenosis (HAS). It can lead to hepatic artery thrombosis, with subsequent liver failure in 30% of the patients. Though traditionally this was managed with either surgical revascularization or retransplantation, use of DES has resulted in high technical success and provided for excellent patency. Avoidance of hepatic artery thrombosis is possible in > 95% of patients with endovascular treatment and close follow-up^[87]. Paclitaxel eluting balloon has been employed successfully to treat biliary anastomotic strictures after liver transplantation^[88]. Stents have also been used to manage stenosis in the hepatic veins and/or inferior vena cava above hepatic venous anastomosis to relieve an outflow venous block following living donor liver transplantation^[89].

CONCLUSION

Though several perioperative challenges encountered in organ transplantation surgery and patients with drug eluting stents, these can be optimally managed with proper planning and teamwork, ensuring patient safety.

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Complement related kidney diseases: Recurrence after transplantation

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Abstract

The recurrence of renal disease after renal transplantation is becoming one of the main causes of graft loss after

kidney transplantation. This principally concerns some of the original diseases as the atypical hemolytic uremic syndrome (HUS), the membranoproliferative glomerulonephritis (MPGN), in particular the MPGN now called C3 glomerulopathy. Both this groups of renal diseases are characterized by congenital (genetic) or acquired (auto-antibodies) modifications of the alternative pathway of complement. These abnormalities often remain after transplantation because they are constitutional and poorly influenced by the immunosuppression. This fact justifies the high recurrence rate of these diseases. Early diagnosis of recurrence is essential for an optimal therapeutically approach, whenever possible. Patients affected by end stage renal disease due to C3 glomerulopathies or to atypical HUS, may be transplanted with extreme caution. Living donor donation from relatives is not recommended because members of the same family may be affected by the same gene mutation. Different therapeutically approaches have been attempted either for recurrence prevention and treatment. The most promising approach is represented by complement inhibitors. Eculizumab, a monoclonal antibody against C5 convertase is the most promising drug, even if to date is not known how long the therapy should be continued and which are the best dosing. These facts face the high costs of the treatment. Eculizumab resistant patients have been described. They could benefit by a C3 convertase inhibitor, but this class of drugs is by now the object of randomized controlled trials.

Key words: Kidney disease recurrence; Complement dysregulation; Atypical hemolytic uremic syndrome; C3 glomerulopathies; Dense deposit disease; Plasma therapy; Eculizumab; C3 glomerulonephritis

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Core tip: Complement cascade is an important pathway of several kidney diseases. A distinction should be made between kidney diseases with complement overactivation

and those with complement dysregulation. The latter are related to congenital or acquired abnormalities of complement factors. These diseases are linked to constitutional abnormalities of the patients, have high recurrence rate after renal transplantation and represent an important cause of graft loss. Diagnosis and treatment are not easy to be made. Just in the last decade a growing knowledge in the field of genetic and biology allowed the complement inhibitors to be the first class drug in the treatment.

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INTRODUCTION

The aim of this review is to highlight the relevance of recurrent diseases after kidney transplantation and, in particular, to discuss the frequency and severity of recurrence of two groups of renal diseases strictly related to each other: C3 glomerulopathy (C3G) and thrombotic microangiopathy (TMA).

Along with the improved control of acute rejections and infections, the recurrence of primary nephropathy has become the most important cause of graft loss principally for patients who have glomerulonephritis (GN) as the primary disease^[1,2]. In some series, recurrence of the original disease was reported to be the principal cause of graft loss more than one year after transplantation^[2] (Table 1). Some renal diseases have a higher risk of recurrence and recurrence-related graft loss. Hariharan *et al*^[3] observed, in a total of 4913 renal transplants, that the greatest relative risk (RR) for graft failure was related to the recurrence of hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) (5.36), membranoproliferative glomerulonephritis (MPGN) (2.77) and focal segmental glomerulosclerosis (FSGS) (2.25). Interestingly, HUS/TTP and many cases of MPGN were related to complement cascade dysregulation and were ascribed to genetic abnormalities or to acquired abnormalities of components of the complement system^[4].

ROLE OF COMPLEMENT IN KIDNEY DISEASES

The complement proteins may be seen in the biopsies of all forms of GN, and all three activation pathways have been documented in different kidney diseases and may be activated by different triggers^[5] (Figure 1). Indeed, the complement system is activated by three pathways (the alternative, classical and lectin pathways) that generate a common terminal pathway. The classical (CP) and lectin pathways (LP) are triggered by the

recognition of pathogens or damaged cell surfaces by antibodies and recognition molecules^[6]. Many glomerular diseases, such as membranous nephropathy, IgA nephropathy and lupus nephropathy, involve these pathways. The activation of the alternative pathway (AP) is relatively complex. The AP undergoes continuous low-grade activation in the fluid phase by spontaneous C3 hydrolysis that is responsible for the deposition of a low amount of C3b onto cell surfaces (Figure 2). Self-surfaces are protected from complement damage by several regulators that are either membrane-anchored or in the fluid phase. Perturbation of the balance between complement activators and regulators provides the basis for aHUS and MPGN/C3G^[7].

As mentioned above, the complement system is involved in the vast majority of kidney diseases. Two broad categories of kidney diseases should be distinguished. The first category is associated with complement over-activation and characterizes diseases such as lupus nephritis, membranous nephropathy, immune complex-associated MPGN and IgA nephropathy. The second category is related to complement dysregulation and characterizes diseases such as aHUS and C3G. In the former category, complement is activated by other factors, including immune-complex formation and deposition. After transplantation, the original disease may recur but is also more easily controlled by the immunosuppression needed to support the transplanted kidney. In the latter disease, complement activation may occur spontaneously and is often related to abnormalities of complement regulating factors. These nephropathies often recur after renal transplantation because the diseases are related to a constitutional and often genetically determined abnormality of the complement proteins. These abnormalities are not corrected either by the transplant itself or by the immunosuppressive therapy.

Tremendous advances are being made in our understanding of both aHUS/TMA and C3G. With the improvement of our understanding of genetics and biology, it has become increasingly clear that different disease mechanisms may cause the disease formerly called TTP/HUS. Furthermore, these mechanisms may deeply influence the recurrence rate after transplantation^[8].

Similarly, the role of complement in C3G has been better defined^[9], thus allowing us to move from a histologically based classification of the MPGNs to a new classification based on pathophysiology^[10,11].

To date, the term aHUS applies to a heterogeneous group of diseases that have in common a TMA associated with some degree of renal failure. Frequently, aHUS patients have a complement abnormality (a genetic mutation or an autoantibody to complement factors) as the primary etiology. As a consequence, they are affected by a complement mediated TMA (Figure 3)^[8].

Similarly, after reclassification, the MPGNs are distinguished into immune-complex-mediated MPGNs and C3Gs. The latter have clear signs of C3 staining with little

Table 1 Causes of graft loss (living kidney transplantation)

	< 1 yr		> 1 yr	
	Non identical	Identical	Non identical	Identical
Acute rejection	5 (41.7%)	0 (0%)		
CAN with/without CR	2 (16.7%)	0 (0%)	16 (31.4%)	5 (23.8%)
CNI nephrotoxicity	0 (0%)	0 (0%)	2 (3.9%)	1 (4.8%)
Recurrence of original disease	1 (8.3%)	0 (0%)	10 (19.6%)	6 (28.6%)
Death with functioning graft	2 (16.7%)	1 (50%)	19 (37.3%)	7 (33.3%)
Discontinuation of immunosuppressant	0 (0%)	1 (50%)	4 (7.8%)	1 (4.8%)
Non-compliance	1 (8.3%)	0 (0%)	0 (0%)	1 (4.8%)
Others	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)
P	0.2002		0.6158	

CAN: Chronic allograft nephropathy; CR: Chronic rejection; CNI: Calcineurin inhibitor.

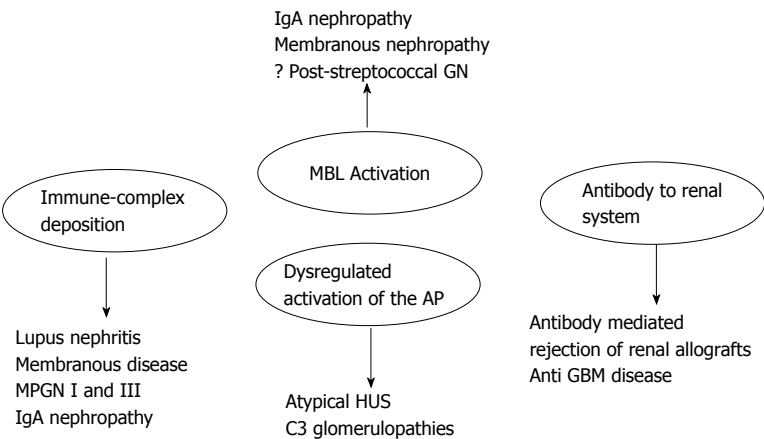


Figure 1 Mechanisms of complement activation in kidney disease. HUS: Hemolytic uremic syndrome; MBL: Mannose binding lectin; IgA: Immunoglobulin A; MPGN: Membranoproliferative glomerulonephritis; GBM: Glomerular basement membrane; AP: Alternative pathway.

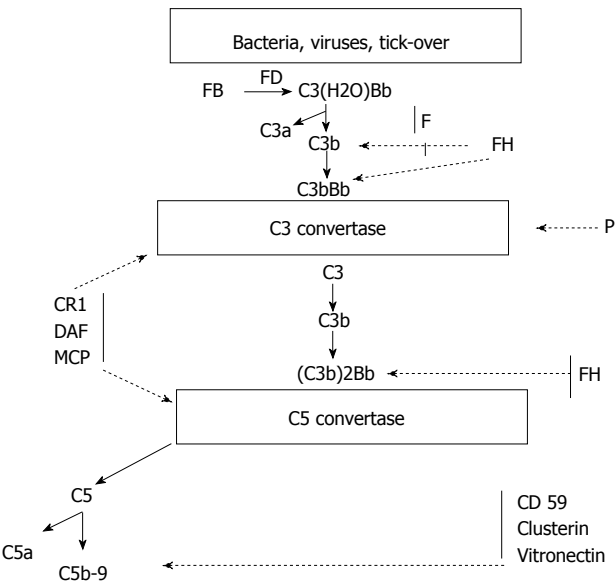


Figure 2 The C3 complement alternative pathway. C3(H2O) Bb: Alternative pathway initiation convertase; FB: Complement factor B; FD: Complement factor D; FH: Complement factor H; FI: Complement factor I; CR1: Complement receptor 1; DAF: Decay accelerating factor; MCP: Membrane cofactor protein; P: Properdin.

or no immunoglobulin deposition evident on renal biopsy. C3Gs are further divided into dense deposit diseases (DDD) and the recently recognized entity C3GN^[11].

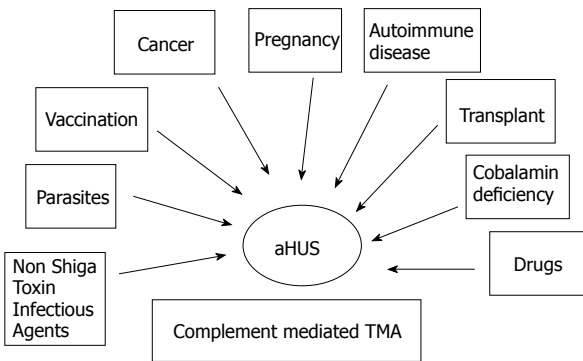


Figure 3 Heterogeneity of atypical hemolytic uremic syndrome. TMA: Thrombotic microangiopathy; HUS: Hemolytic uremic syndrome.

EPIDEMIOLOGY

GNs that occur in the transplanted kidney may be caused either by recurrent or *de novo* disease. In clinical and in the epidemiological studies is necessary to distinguish between these conditions. True recurrence occurs when: (1) post-transplant proteinuria or hematuria or elevated serum creatinine is found after transplantation; (2) biopsy-proven kidney disease is diagnosed in the native kidneys; or (3) the same disease is proven by biopsy in the transplanted kidney^[12]. Challenges to the diagnosis of recurrent diseases are manifold. They include: (1)

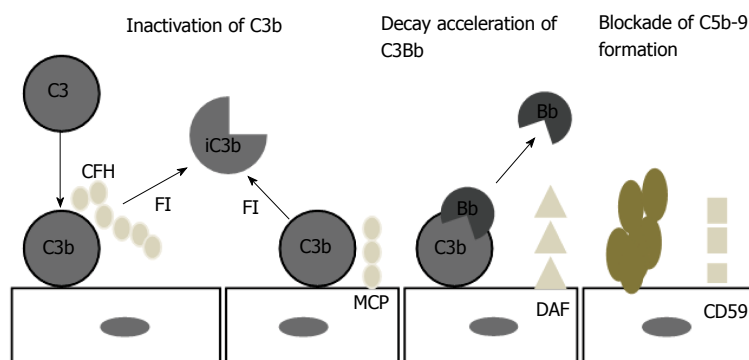


Figure 4 Regulation of complement on surfaces. CFH: Complement factor H; FI: Complement factor I; iC3b: Inactivated C3b; MCP: Membrane cofactor protein; DAF: Decay accelerating factor; CD59: MAC inhibitor protein.

misdiagnosis or mislabeling of native kidney disease; (2) lack of a unified approach to using diagnostic tools for the diagnosis of recurrent disease; and (3) difficulties in differentiating recurrent disease from other causes of renal damage such as drug toxicity and chronic rejection^[3,13].

There are still other potential biases occurring among registries dealing with recurrences of renal diseases mediated by complement dysregulation. For example Shiga toxin-related HUS combined with aHUS in many registries. Additionally, the vast majority of registries or networks report data by using the classification of MPGNs, which precedes the results of the consensus report on C3G^[14] and the recent consensus report and reclassification of GNs^[10,11].

Because of the above-mentioned factors, the data reported by different registries as the North American Pediatric Renal Transplant Collaborative Study (NAPRTCS), the Australia New Zealand Dialysis Transplant Data System (ANZDATA), the Renal Allograft Disease Registry (RADR) and the United States Renal Data System (USRDS) differ significantly in reporting the prevalence of recurrent GNs after transplantation^[3,12,15-18]. A study by Shimmura *et al.*^[2] on 266 living kidney transplants clearly documents that recurrence of the original disease is the third leading cause of graft loss after one year from transplantation (Table 1). The aforementioned study by Hariharan^[3] documents the highest RR for graft failure for HUS/TTP and MPGN.

Two other studies on pediatric patients^[19,20] report high rates of recurrence for aHUS and type I and II MPGN according to the old classification, although there is a wide range of rates among the studies.

Series related to the early 2000s indicated that the risk of post-transplant recurrence for aHUS was 20% in pediatric patients and 50% in adult patients^[21]. Recently, in 280 patients with aHUS screened for CFH, FI or MCP mutations, post-transplant aHUS recurrence was reported in 33%^[22], 37%^[23] and 60%^[24], respectively.

Fewer data are available regarding the epidemiology of MPGN recurrence according to the new classification. Indeed, many registries are still using the old classification. According to these data, MPGN type I recurs in 20%-30% of patients, whereas MPGN type II recurs in 80%-100% of patients^[25].

More recently, Kasiske *et al.*^[26], observing 1574 MP

GNs in 140109 transplant patients recorded in the USRDS an observation that the true recurrence rate of MPGN increased over time, with the most frequent recurrences of GN between 1995 and 2003.

After the reclassification^[10,11], the most interesting and recent data on C3G recurrence are those reported by Zand *et al.*^[27]. According to these data, the recurrence rate of C3GN is 66.7%, and graft failure occurs in 50% of patients with recurrence.

PATHOPHYSIOLOGY OF TMA AND ITS RECURRENCE

As mentioned above, the complement AP is constitutively active. After the generation of C3b, it binds either to either pathogens or the host cells. This necessitates the prompt and tight control of its activity. In turn, C3b may generate new C3 convertases (C3bBb) that act as an auto-amplifier by creating new C3b molecules. The same enzymes may also generate the C5 convertases that activate C5, the anaphylatoxins C5a and C5b and activate the membrane attack complex (MAC) C5b-C9^[28]. In normal conditions, the AP may be spontaneously activated by the process called tick over. Cell surfaces are protected from auto-activation by several factors both in the fluid phase and anchored to the cell membranes.

The principal inhibiting factor is complement factor H (CFH), which acts both in the fluid phase and on cell surfaces. Factor H also act as a co-factor to complement factor I (CFI)^[29-31]. Cell surfaces are also protected by at least 4 specific membrane regulators: (1) complement receptor 1 (CR1/CD 35); (2) membrane cofactor protein (MCP/CD46); (3) decay accelerating factor (DAF/CD55); and (4) protectin (CD59), which blocks MAC formation (Figure 4)^[32,33].

The loss of this complex regulation results in complement activation, with consequent cell damage^[34,35]. The role of complement dysregulation is increasingly recognized as the principal cause of TMAs. It may be caused by genetic mutations or by autoantibodies. Additionally, a triggering factor, often from the environment, is needed.

Figure 5 represents the whole spectrum of TMAs. In this figure, three different conditions are possible: (1) complement-driven TMA (*i.e.*, aHUS), where there is an underlying complement defect; (2) complement-

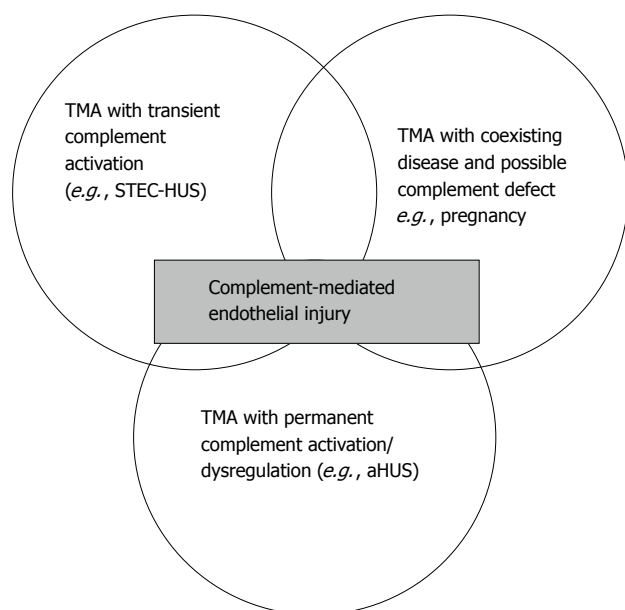


Figure 5 Spectrum of thrombotic microangiopathies. TMA: Thrombotic microangiopathies; STEC-HUS: Shiga toxin-producing *Escherichia coli*-hemolytic uremic syndrome.

enhanced TMA, as in Shiga-toxin-producing *Escherichia coli*-HUS (STEC-HUS); and (3) TMA with coexisting disease (e.g., pregnancy, lupus). The most frequently mutated gene in aHUS is *CFH*. Mutations have been identified in 25% of sporadic cases and 40% of familial cases^[6,36,37]. In addition to *CFH*, the *CFH* gene family includes five other complement factor H-related genes (*CFHR1-5*). Deletion, duplications and hybrid proteins occur^[38]. As a result, the loss of cell surface protection may occur and aHUS may develop^[36,39].

The *CFHR3-CFHR1* deletion is the most frequent deletion associated with aHUS. Recently a new role for the FHR protein was identified by Goicochea de Jorge *et al.*^[40]. According to these findings, FHRs could act as competitive antagonists to FH. *MCP* (CD46) mutations are found in 5%-9% of aHUS patients^[36]. The protein is membrane-anchored and serves as a co-factor of FI. *FI* mutations lead to a lack of complement control accounting for 4%-8% of aHUS^[36,37].

In addition to mutations in regulatory genes, gain of function mutations of effector proteins (C3 and factor B) has been reported. Factor B mutations are reported in < 4% cases of aHUS^[41-43]. Factor B mutations enhance the C3bB formation. Gain of function mutations in C3 are reported in 2%-8% of aHUS^[36,37]. These mutations impair MCP and confer resistance to cleavage by CFI^[44,45].

Mutations in the thrombomodulin gene (*THBD*) have been reported in up to 5% of aHUS patients^[36]. Few data have been reported on this mutation. It is probable that the *THBD* mutation may influence disease severity in association with other mutations^[46].

Autoantibodies against different complement factors, independent of genetic mutations or in association with genetic abnormalities, may cause aHUS. Antibodies to anti-complement factor H (FHAA) account for 3%-8%

of aHUS and are often found in association with *FHR1* deficiency or deletion^[36]. Recently, in India, an association of FHAA and aHUS was reported in approximately 60% of the pediatric population^[47]. Auto antibodies to anti-factor I have been reported only in three patients^[48]. Their role is not yet clear.

In many cases, the development of aHUS in patients who are affected by complement abnormalities needs a triggering factor. Among these well-known factors are pregnancy, drugs, and autoimmune disorders. Complement abnormalities were identified in 83% of patients with pregnancy associated aHUS^[49]. *CFH* mutations have been reported in 4 patients with aHUS associated with ticlopidine^[50]. Further 4% of patients affected by lupus disease documented an aHUS related to complement abnormalities^[51,52]. Complement abnormalities strongly influence aHUS recurrence after renal transplantation. Indeed, aHUS recurrence was reported at rates from 20% to 50% in the era when genetic analysis of complement proteins was not available^[53-56]. Recently, several studies have highlighted the risk of aHUS recurrence according to different genes abnormalities^[53] (Table 2). Complement abnormalities have also been found in conditions that should not be affected by recurrence.

STEC-HUS of the native kidneys should be protected from recurrence. However, two patients with a history of STEC-HUS were recently diagnosed with post-transplant recurrence^[57]. Both patients were recognized to be affected by complement abnormalities; one had a heterozygous *CFI* mutation, and the other had a heterozygous *MCP* mutation.

Patients affected by *MCP* mutations rarely have aHUS recurrence after transplantation^[58]. Recently, however, transplant failures due to aHUS recurrence have been observed in patients affected by *MCP* mutations^[59]. Almost all of these patients were affected by combined *MCP* and *CFH* or *CFI* mutations.

Patients affected by *CFH* mutations are at a high risk of recurrence. In two French case series, recurrences were observed in 80% of children^[22] and 75% of adults^[60]. The graft failure rates in the case of recurrence are approximately 86%.

Interestingly, the location of *CFH* mutation impacts the recurrence risk^[61]. Indeed, mutations involving the C-terminal domain of *CFH* confer higher risk and have a worse prognosis^[49]. This finding is consistent with the critical role of the *CFH* C-terminal domain in binding to the endothelium and exerting the protective role of the endothelial cells^[62].

The majority of FHAA is directed against the C-terminal domain of factor H. As a consequence, a higher risk of recurrence should be expected due to FHAA. However, this does not seem to be the case, because aHUS recurrence due to FHAA is uncommon^[63].

Nonetheless, the recurrence risk due to FHAA is not easily understood because 40% of patients with FHAA are also affected by mutations in the complement genes^[64]. Additionally, a reduction of FHAA is achievable with the immunosuppressant therapy, thereby reducing

Table 2 Risk of atypical hemolytic uremic syndrome recurrence according to the implicated genetic abnormality

Gene	Protein location	Functional impact	Mutation frequency in aHUS (%)	Recurrence frequency after transplantation (%)
Mutation				
<i>CFH</i>	Plasma	Loss	20-30	75-90
<i>CFI</i>	Plasma	Loss	2-12	45-80
<i>CFB</i>	Plasma	Gain	1-2	100
<i>C3</i>	Plasma	Gain	5-10	40-70
<i>MCP</i>	Membrane	Loss	10-15	15-20
<i>THBD</i>	Membrane	Loss	5	1 case
Genetic polymorphism (frequency in control population)				
Homozygous <i>CFHR1del</i> (3%-8%)	Circulating	Undetermined	14-23 (> 90% in patients with anti-CFH antibodies)	NA

aHUS: Atypical hemolytic uremic syndrome; C3: Complement C3; CFH: Complement factor H; CFI: Complement factor I; CFB: Complement factor B; MCP: Membrane cofactor protein; THBD: Thrombomodulin; CFHR1: Complement factor H receptor 1; NA: Not available.

the risk of aHUS recurrence.

In summary, the risk of recurrence is 4 times higher in patients with mutations in the *CFH* gene or carriers of the hybrid gene between *CFH/CFHR1*. In a recent study by Le Quintrec^[65], patients with the hybrid gene lost their grafts due to early recurrence.

The relevance of *CFI* mutations on aHUS recurrence has discordant results and interpretations. The first studies to *CFI* mutations reported a high recurrence rate and graft loss^[10,31,53,58,66,67]. A study by Bienaimé *et al.*^[68] in 2010 reported that patients with *CFI* mutations do not seem to carry a higher risk of recurrence. These data were more recently confirmed by the study mentioned above by Le Quintrec *et al.*^[65].

MCP mutations rarely affect aHUS recurrence because the endothelial cell surfaces of the transplanted kidney normally express MCP. Only three recurrences have been reported in the literature^[69,70]. In these patients, recurrence might be ascribed to combined complement gene mutations^[59] or microchimerism from the recipient endothelial cells^[70].

Data on the role of THBD are scarce. aHUS recurrence due to *THBD* mutations should not occur because the molecule is membrane-anchored as MCP. Additionally, a small proportion of THBD is present in soluble form. Nonetheless, sporadic cases of recurrence due to THBD have been reported^[71,72]. In one patient the recurrence occurred early post-transplantation during the ischemia-reperfusion phase. During this phase, the soluble form of THBD might be not adequate to protect from recurrence.

Patients affected by gain of function mutations (*CFB*, *C3*) are also exposed to the risk of recurrence. To date, four patient carriers of the *CFB* mutation have been reported to have aHUS recurrences and consequent graft loss^[73,74]. Data on recurrence in patients affected by *C3* mutations are discordant. Le Quintrec^[65] reported a high recurrence rate, with 4 recurrences in 5 grafts. Previously, Noris *et al.*^[75] reported only two recurrences in 7 transplanted patients. In an attempt to explain the difference, Zuber *et al.*^[53] speculated that for some patients, the intra-graft production of normal C3 might occur and might be protective.

Several environmental triggers might act to damage the graft endothelium and to facilitate aHUS recurrence on already damaged cells in patients with genetic abnormalities.

Anti-HLA antibodies^[76], ischemia-reperfusion events^[77], immunosuppressant drugs^[78] and viral infections^[79], either isolated or in association, might play a relevant role and favor aHUS recurrence in genetically predisposed patients.

Le Quintrec *et al.*^[65] attempted to identify the risk factors for aHUS recurrence. Low C3 levels and the presence of a mutation were significant in the univariate analysis. In a multivariate analysis of mutations, a mammalian target of rapamycin (mTOR) inhibitor regimen and recipient age were significantly associated with increased aHUS recurrence rates.

PATHOPHYSIOLOGY AND RECURRENCE OF C3G

After the reclassification of MPGNs, as mentioned above^[10,14], C3Gs included the MPGNs caused by complement dysregulation rather than the MPGN immune-complex-related disorders^[80]. As a consequence, C3Gs include the GNs for which immunofluorescence microscopy is C3-positive and immunoglobulin-negative.

C3Gs may be sub-divided into DDD and C3GN based on electron microscopy, even if, in some cases the distinction is challenging^[14,81]. Recently, advances toward an improved understanding of the characteristics of C3 deposits have been made through proteomic analysis and laser microdissection (LMD)^[82]. Laser dissection and mass spectrometry of glomeruli from patients with C3G documented an accumulation of the AP and the terminal complement complex proteins, thus confirming that C3G results from abnormalities of the AP, which lead to glomerular damage^[81].

The pathophysiology of AP pathway activation in C3GN and DDD is very similar, with fluid phase dysregulation due to gene mutations or autoantibodies occurring in both disorders. Indeed, as for aHUS, the complement abnormalities in C3Gs may occur on a genetic basis or as acquired factors as autoantibodies.

The most common acquired complement defect is represented by the presence of an antibody called the C3 nephritic factor (C3NeF), which blocks CFH-mediated decay and stabilizes C3 convertase^[81,83]. In particular, C3NeF binds to C3 convertase and inhibits the action of factor H, CR1 and DAF, blocking the dissociation of the convertase. C3NeF enhances C3 convertase activity 10 fold^[9,84,85]. The frequency of C3NeF is high in C3G, ranging from 50% to 80% of patients^[83]. C3NeF may also be associated with genetic mutations. Recently, other auto antibodies have been found in C3Gs. These auto antibodies are directed against C3 convertase, factor B^[86] or anti-factor H^[87,88]. AP dysregulation in DDD is more frequently autoantibody-induced with respect to TMA. Genetic abnormalities also have been encountered. Few patients have been identified with genetic mutations of factor I, MCP, C3, factor B and factor H^[83,89]. In an extensive study by Servais *et al.*^[83], only 5.3% of the patients affected by C3GN had *CFI* mutations, and 1.8% had *MCP* mutations.

In 2010, Martinez-Barricarte *et al.*^[90] identified a mutant C3 protein resistant to factor H inactivation in a patient affected by DDD. More recently, a different C3 mutation has been identified.

Mutations in factor H have been reported more frequently among patients affected by C3Gs. Mutations may result in a defective protein or a complete lack of protein H. Mutations may occur in a homozygous or heterozygous manner^[91,92] and may be associated with C3NeF, thus documenting the association of different risk factors.

In recent years genetic mutations of the *CFHR* gene cluster have been reported among patients with C3G^[93]. *CFHR* family gene mutations^[94], deletions^[95], duplication^[96] and hybrid genes^[97] have been reported in patients with C3Gs either in either isolated patients or family groups.

For example, Gale *et al.*^[96] reported two Cypriot families whose members were affected by a *CFHR5* mutation. The protein produced by the mutated gene was poorly effective in binding to C3b on cell surfaces and thus led to the deregulation of the fluid phase of the AP. The disease was called *CFHR5* nephropathy.

Recently, Malik *et al.*^[98] reported patients from the same family affected by C3G due to abnormal copies in the *CFHR3* and *CFHR1* loci. The finding of familial cases of C3G highlights the genetic origin of several C3Gs and the related complement AP dysregulation.

In summary the specific cause of C3G is inadequate regulation of the complement system. The causes of complement dysregulation may be divided into genetic and acquired factors. Among the former are changes in many of the complement genes: Among the latter are specific antibodies called C3 nephritic factors or C3NeFs that impair normal regulation of the complement system. It appears that patients with DDD are more likely to have C3NeFs, while patients with C3GN are more likely to have abnormalities in a group of proteins called the "Complement Factor H-Related" proteins.

Additionally, genetic defects may represent the basis of either C3G or aHUS (Table 3). Indeed, in recent years, a large number of genetic studies have established a strong association between the factor H-related proteins and different diseases involving complement dysregulation. This association, together with the recent functional data on factor H-related proteins such as FH competitors and complement deregulators, has gained the attention of the complement scientific community^[99].

From the pathophysiological point of view, many cases of C3Gs and TMA are associated with defective control of the AP. The inevitable questions are whether C3G and TMA are the other sides of the same coin and which factors determine whether a patient develops one disease instead of the other^[5].

Animal models highlight that C3G may be the consequence of prevalent dysregulation of fluid phase complement activation, whereas TMA is principally related to complement activation on the capillary wall. The same studies determined that an absolute deficiency of factor H favors fluid phase complement activation and C3G, whereas the absence or abnormality of the binding region of factor H favors TMA^[100]. It has also been hypothesized that *CFH* and *CFH/CFHR* mutations induce aHUS to inhibit the CFH binding to most cell surfaces, whereas C3G-associated mutant *CFHRs* do not inhibit CFH binding to endothelial cell surfaces^[6].

Concerning C3G recurrence after transplantation, the finding of familial cases of C3GN highlights the genetic origin and the related complement AP dysregulation of the vast majority of C3GN. These data form the basis of its recurrence after transplantation. However, fewer data are available on C3G recurrence compared to TMA. Indeed, C3G is a rare disease and principally, its pathogenesis and its complement-dependent nature have been recognized only recently. More data are available on DDD recurrence. Indeed, this disease was identified a long time ago based on its characteristic microscopic aspects. This finding occurred long before our understanding of its pathogenesis. In a retrospective analysis of 75 children, the 5-year graft survival rate was only 50%^[101]. Almost all adult patients had recurrences after transplantation and up to 25% lost their graft^[19].

In a large, retrospective cohort study of 80 adults and children affected by C3G, Medjeral-Thomas *et al.*^[102] reported a histological recurrence following renal transplantation in all 6 DDD patients. Recurrence was associated with graft loss in 50% of patients. Similarly, four of seven C3GN patients transplanted had histological recurrences. Graft loss occurred in 3 patients. A UNOS review reported a 10-year graft survival rate of 57.5% for patients affected by DDD recurrence^[103]. In different studies, the reported rate of DDD recurrence is variable ranging from 18% to 100%^[104,105].

Considering only those patients whose diagnosis was made by renal biopsy, the recurrence rate was over 70%^[106,107]. Disease recurrence may occur suddenly after transplantation. However, cases of recurrence many years later are also described^[107]. The risk factors for

Table 3 Overview of mutations in complement factor H related protein genes

Genetic defect	Phenotypic expression
Duplication in the <i>CFHR5</i> gene	C3 glomerulopathy (CFHR5 nephropathy)
Duplication in the <i>CFHR1</i> gene	C3 glomerulopathy
Hybrid <i>CFHR3/CFHR1</i> gene	C3 glomerulopathy
Hybrid <i>CFHR2/CFHR5</i> gene	C3 glomerulopathy
Hybrid <i>CFH/CFHR1</i> gene	aHUS
Hybrid <i>CFH/CFHR3</i> gene	aHUS

CFHR: Complement factor H related; aHUS: Atypical hemolytic uremic syndrome.

recurrence and graft loss for DDD are not well defined. No relationship with preTx disease presentation or C3 serum levels has been found. Additionally, the C3NeF levels do not correlate with the risk of recurrence^[108]. The presence of heavy proteinuria seems to be the only risk factor related to recurrence.

The different genetic variants responsible of C3GN have been already described. Overall, C3GN recurs in two-thirds of transplanted patients and graft loss is common^[27,81,83]. Histologically, it recurs with a membranoproliferative pattern. Risk factors for recurrence are still now debated. According to some studies^[25], they include the severity of histological lesions in the native kidneys, HLA-B8 DR3, living related donors and previous graft loss for recurrence^[109]. To date, our understanding of C3GN recurrence is only based on case reports. Furthermore, the broadest study on C3GN outcomes after recurrence by Zand *et al*^[27] was unable to find any risk factor for recurrence. The multiple defects in complement regulatory proteins causing C3GN likely impair the establishment of any well-defined recurrence risk.

Eleven patients affected by CFHR5 nephropathy were successfully transplanted^[110]; however protocol biopsies have documented recurrence^[111]. The recurrence may be early after transplantation and demonstrates that renal-derived CFHR5 protein cannot prevent the development of CFHR5 graft nephropathy. Very recently Wong *et al*^[112] described a high recurrence rate in 5 patients affected by hybrid *CFHR3 1* gene-associated C3GN.

DIAGNOSIS OF RECURRENCE

Diagnosis of recurrence may be easy if the clinical history of the recipient is known and the diagnosis of C3G/aHUS of the native kidneys has been made after an etiological workup and a kidney biopsy. Unfortunately, the clinical history of the recipient and a renal biopsy of the native kidneys are often not available.

In such patients, if the graft is not doing well, a renal biopsy should be promptly performed and examined by light microscopy, immunofluorescence and electron microscopy. When the diagnosis of C3G/aHUS is suspected, a complete workup should be undertaken. The diagnostic approach should include a comprehensive biochemical,

genetic and pathologic analysis of the complement AP. This approach should include complement factors and complement regulatory protein levels, measurement of MCP on peripheral blood leukocytes as well as screening for anti-CFH antibodies and C3NeFs. Additionally, the genetic investigation should include mutation screening of CFH, CFI, MCP, C3 and CFB. The screening requires an extensive sequencing of all coding exons. Additionally, a study of recombination in the CFHR region should be made^[113]. The genetic studies are not easy to perform because the spectrum of genes currently known to be involved is rapidly expanding^[114]. Nonetheless, such studies are vital because the importance of genetic mutation screening to determine the outcome of retransplantation following a failed kidney allograft from a patient with recurrent aHUS has recently been documented^[115]. In other words, not all mutations have the similar detrimental effects. The absence of a more severe genotype could facilitate the successful treatment of the recurrence.

RECOMMENDATIONS, PREVENTION AND TREATMENT OF POST-TRANSPLANT aHUS AND C3Gs RECURRENCE

The vast majority of data are available for aHUS because C3G has been only recently defined and data on prevention and treatment rely more on case reports than on evidence-based medicine.

Recommendations

Patients with aHUS as a primary disease and patients with suspected aHUS and with STEC-HUS should be screened for all complement factors and regulating proteins. Additionally, a genotyping for *CFH*, *CFHR*, *CFI*, *MCP*, *CFB* and *C3* should be performed^[114].

Patients with a suspected diagnosis of C3G should also be screened for C3NeF and for other autoantibodies that are known to be involved in this disease.

Living donor renal transplantation, even in the eculizumab era, is not indicated for patients with mutations in *CFH*, *CFI*, *C3* and *CFB*. In patients with aHUS due to a mutation in *MCP*, donation may be safe after exclusion of other mutations often associated with *MCP* mutation. However, increased evidence for a polygenic pattern for aHUS and C3G and the still-unknown polymorphisms should always consider a living donation with extreme caution^[53].

Patients affected by aHUS but with no identified mutations should be recommended to proceed with transplantation combined with intensive plasma exchange (PE)^[21].

Prevention

To date, there is limited evidence for preventing C3G recurrence after transplantation. The more validated experience refers to the use of eculizumab to prevent aHUS recurrence^[80]. Whether these strategies may be

recommended to prevent C3G will be subject to future research.

More data are available concerning aHUS prevention. The avoidance of any possible endothelial insult has been highlighted^[113]. Post-transplant conditions that may cause endothelial insult include ischemia-reperfusion injury, infections, and immunosuppressive drugs. All of these factors could act as triggers to activate the AP in predisposed patients.

An association between calcineurin inhibitors (CNIs) and aHUS recurrence has been hypothesized^[7]. Other studies do not confirm this association and note that mTOR inhibitors are frequently used to avoid CNIs and may, *per se*, induce aHUS^[116,117]. PE has been used to prevent aHUS recurrence^[53]. However, PE has several drawbacks.

First, in some cases, PE fails to prevent aHUS^[118]. Second, there is a risk of recurrence when PE is interrupted. Third, the evidence of subclinical recurrent aHUS in patients still under treatment indicates that in some cases, PE does not control complement activation^[118].

Pre-transplant rituximab administration has been effective for patients with anti-CFH antibodies^[40,119,120]. In these patients, the association of PE may improve the treatment efficacy. The anti-C5 monoclonal antibody (eculizumab) has been used to prevent post-transplant aHUS in several patients. Among the reported patients, nine had either *CFH* mutations or a *CFH/CFHR1* hybrid gene. Another patient had a C3 mutation^[118,121-124]. All of these patients had a complement genetic abnormality with a risk of aHUS recurrence greater than 80%. Only one patient lost the graft due to an arterial thrombosis. All other patients had a successful recurrence-free post-transplant course, even if, to our knowledge, they are still undergoing eculizumab treatment^[116].

Treatment

In a retrospective study, Zand *et al.*^[27] reviewed the outcomes of 14 patients diagnosed with a C3G recurrence after transplantation. Ten patients did not receive any additional treatment. Three patients received rituximab treatment, but the overall outcome was poor.

Another study reported the beneficial effect of plasma infusions (PI) in patients with a genetic mutation in factor H^[125]. Case reports documented the efficacy of eculizumab in patients with DDD recurrence^[106] and patients with C3GN recurrence^[126], although the patient with C3GN repeat allograft biopsies showed progression of the disease. Other studies^[127,128] reported eculizumab efficacy for the treatment of recurrent DDD and C3GN. A randomized clinical trial to evaluate the efficacy of eculizumab in patients with C3G is ongoing^[129].

An exciting new approach to C3G treatment is the soluble complement receptor 1 (CR1), which promotes the breakdown of active C3b. The infusion of soluble CR1 was reported to improve C3 and serum MAC levels in a patient with DDD recurrence^[97].

Before the eculizumab era, patients affected by aHUS

recurrence were extensively treated with PE. In the French survey, the outcomes of aHUS recurrence were not different among patients, regardless of treatment with PE^[116]. PE combined with belatacept was effective for one patient, as reported by Midvedt *et al.*^[130]. Eculizumab has been reported to be effective in a recent study by Matar *et al.*^[131], regardless of concomitant PE treatment. The largest experience in treating recurrent aHUS with eculizumab was reported by Zuber *et al.*^[118].

According to their findings, eculizumab was efficient in treating aHUS recurrence after transplantation. The treatment should be started as early as possible, and the treatment tolerance is excellent. Interestingly, two patients who received a single dose regimen experienced a delayed relapse^[132]. Two attempts of eculizumab discontinuation were followed by new relapses^[133].

Overall, these experiences suggest that a high risk of relapse may persist after a first recurrence. This fact suggests caution in withdrawing eculizumab in this setting.

Additionally, active HUS lesions have been observed in patients with a documented C5 blockade receiving eculizumab regularly^[118]. Whether a C3 convertase blocker could more efficiently treat these patients is currently unknown.

Two additional studies have documented eculizumab efficacy in plasma therapy resistant or dependent patients with recurrent aHUS^[134,135]. More than 80% of the patients achieved TMA-free status.

The efficacy of eculizumab has changed our approach to aHUS and C3G recurrence after transplantation. However several questions remain to be answered, including: (1) Do complement investigations impact therapeutic decisions? (2) For how long should patients with recurrent aHUS or C3G be given eculizumab? and (3) Does eculizumab change our indications for renal transplant for patients on dialysis for aHUS or C3G?^[136].

It is crucial to explore the most appropriate dose, dosing intervals and duration of treatment to reduce the enormous financial burden of eculizumab therapy^[137].

CONCLUSION

Recurrence of primary disease after renal transplantation is currently one of the most important causes of graft loss.

Recurrence is principally common for those diseases, often glomerulonephritis, caused by constitutional abnormalities of the patient, not kidney related. Among these abnormalities are diseases caused by complement dysregulation such as aHUS and C3Gs. To date, aHUS and C3Gs often represent a contraindication to renal transplantation due to the frequency and severity of recurrent disease. The clinical use of the anti-C5 inhibitor, eculizumab, seems to overcome the limitations to kidney transplantation for selected patients. However, we have highlighted the drawbacks of this therapy, principally represented by the high costs of lifelong therapy. The main perspectives in the field of renal transplantation

of avoiding or treating recurrences are either diagnostic and therapeutic. An improved understanding of genetics and biology will allow an improved knowledge of gene mutations and the possibility of opening new methods in the field of living donor transplantation; Future therapeutic approaches are represented by the availability of purified deficient gene products and the availability of C3 convertase inhibitors. In addition to CR1 as mentioned above, the current targets of research include the compstatin analog Cp40, which can block C3b^[138]. Similarly, another research target is a monoclonal antibody able to inhibit the C3 convertase induced by C3NeF^[139].

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Face transplantation: Anesthetic challenges

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Abstract

Face transplantation is a complex vascular composite allotransplantation (VCA) surgery. It involves multiple types of tissue, such as bone, muscles, blood vessels, nerves to be transferred from the donor to the recipient as one unit. VCAs were added to the definition of organs covered by the Organ Procurement and Transplantation Network

Final Rule and National Organ Transplant Act. Prior to harvest of the face from the donor, a tracheostomy is usually performed. The osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss often requiring transfusion of blood products. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity. The recipient airway management most commonly used is primary intubation of an existing tracheostoma with a flexometallic endotracheal tube. The recipient surgery usually averages to 19-20 h. Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase. Prior to reperfusion, often, after one sided anastomosis of the graft, the contralateral side is allowed to bleed to get rid of the preservation solution and other additives. Intraoperative product replacement should be guided by laboratory values and point of care testing for coagulation and hemostasis. In face transplantation, bolus doses of pressors or pressor infusions have been used intraoperatively in several patients to manage hypotension. This article reviews the anesthetic considerations for management for face transplantation, and some of the perioperative challenges faced.

Key words: Face transplantation; Vascular composite allotransplantation; Organ harvest; Facial reconstruction

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Core tip: Face transplantation is a complex vascular composite allotransplantation surgery. During donor harvest, osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss often requiring transfusion of blood products. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity. The recipient surgery usually averages to 19-20 h. Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase, requiring use of pressors. This article reviews the anesthetic considerations for management

for face transplantation, and some of the perioperative challenges faced.

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INTRODUCTION

Face transplantation is a complex vascular composite allotransplantation (VCA) surgery. VCA involves multiple types of tissue, such as bone, muscles, blood vessels, nerves to be transferred from the donor to the recipient as one unit^[1]. It is a rapidly evolving field which has benefited tremendously from the advances in microsurgery, transplantation, and immunologic techniques. Complex facial defects can be corrected, both functionally and cosmetically. Restoration involves availability of sufficient blood supply, esthetic unit match, nerve function, and integration into the recipients surrounding structures.

The first face transplant was performed in France in 2005^[1] and the first near total face transplant was performed in the United States by the Cleveland Clinic Foundation in 2008^[2]. Till date (Jan 2016) there have been 37 (20 partial and 17 full face) transplants done in the world. In Europe, face transplants have been done in France, Spain, Belgium, Turkey and Poland. China has been the only Asian Country to venture in this field^[3-5]. There have been five patient deaths reported so far^[5].

VCAs were added to the definition of organs covered by federal regulation [the Organ Procurement and Transplantation Network (OPTN) Final Rule] and legislation (the National Organ Transplant Act). The designation went into effect on July 3, 2014^[6]. The United Network of Organ Sharing (UNOS) was assigned to oversee all face and hand transplants and take responsibility for developing all relevant policies and byelaws in this field. Thus a special VCA Transplantation committee was formed by UNOS in 2014, to develop aspects of VCA policies such as refining allocation policy, defining criteria for VCAs to be covered in OPTN policy, OPTN membership requirement for VCA transplant programs, data requirements, data collection procedures, etc.^[7].

Face transplantation is a relatively new and rapidly developing field, and experience and expertise in this field is still limited. The American Society of Anesthesiologists (ASA) has not yet developed any guidelines to manage face transplantation procedures. This article reviews the anesthetic considerations for management for face transplantation, and some of the perioperative challenges faced.

ANESTHETIC CONSIDERATIONS

Donor

The ASA Physical Status Classification System typically

classifies a declared brain dead patient whose organs are being removed for donor purposes as ASA VI. Prior to harvest of the face, a tracheostomy is usually performed because endotracheal intubation may hamper the surgical procedure^[8]. The donor operation involves removal of the facial segment which varies as per the recipient's requirements^[9]. The donor graft may contain skin, multiple vessels, nerves, muscles, and facial bones. The dissection can be very prolonged and may take 12-15 h, even up to 22 h^[9,10]. The osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss needing transfusion of blood products. Explantation is done after systemic heparinization. The vascular pedicle consisting of carotid and internal jugular vessels is also dissected and used to flush the graft with cold preservative solution such as University of Wisconsin solution^[9]. Though the total ischemia time tolerated by facial grafts is unknown, approximately 4 h should be well tolerated^[11-13]. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity^[8,14].

If the donor is a multiorgan donor, co-ordination with other solid organ teams is vital. If there is elevated blood loss and hemodynamic instability, then the solid organ team should ideally be prepared to harvest the other organs immediately. Otherwise, solid organ retrieval could be delayed till just prior to the face explantation. The solid organs should ideally be given priority over the VCAs^[8].

Recipient

The common indications for face transplantation have been devastating facial injuries which not only produce subsequent disfigurement but also compromise key facial functions, such as breathing, eating, facial expressions, vision etc.^[3]. Though face allotransplantation may not be life saving, it certainly has a significant impact on an individual whose face has been severely injured, and constitutes a major reconstructive procedure^[15,16]. It is essential for both, physical and social survival, and optimal social survival makes physical life worth living^[15].

The ASA Physical Status Classification System typically classifies patient with end organ stage disease undergoing a transplant surgery as ASA IV, i.e., a patient with systemic disease that is a constant threat to life. However, since a face transplant is not theoretically life saving, the patient may fall into category ASA III, i.e., a patient with a severe systemic disease, with substantive functional limitations. However, the patient may have several other comorbidities which may increase the ASA Grade. Reports published so far have cited damage to other organs as well, due to thermal burns, animal attacks, radiation injury, ballistic trauma, electrical burns, lye burns etc.^[1,3,16].

The airway management most commonly used in facial transplantations has been *via* a primary intubation of an existing tracheostoma with a flexometallic endotracheal tube^[17,18]. Primary orotracheal intubation may be challenging in cases of restricted mouth opening,

with facial skin contractures as commonly seen in burns, chemical trauma, etc. In such cases, fiberoptic intubation, awake or asleep, depending on the patient airway and the risks of aspiration, can be performed. Prior to commencement of surgery, a tracheostomy is done and a soft flexometallic endotracheal tube is inserted into the trachea. This is then sutured rather than tied, in order to prevent compression to venous outflow from the face by pressure exerted by the circumferential tie^[17,18].

Face transplantation surgery has a very long duration, usually averaging to approximately 19-20 h^[17,18]. One case has been reported to have a surgical time of 36 h^[19]. Venous access and hemodynamic monitoring would depend on the patient and existing comorbidities. An arterial line allows accurate monitoring of hypotension especially during massive blood loss, and also sampling for hematocrits, blood gases and coagulation profiles. Radial or femoral arterial lines can be placed, depending on accessibility.

A central line is usually preferred to administer fluids and pressors. The internal jugular and subclavian veins may be at risk of thrombosis, or maybe inaccessible. Though femoral venous access is associated with a higher degree of infection^[20], it has been used in several cases^[17,18]. Whenever feasible, a subclavian central venous line is preferable, to reduce risk of infections in this group of patients receiving immunosuppressive therapy postoperatively. A slight reverse trendelenberg position (15 degrees) can be used to facilitate venous drainage and reduce blood loss.

Patients are usually induced using an induction agent such as propofol or etomidate, an opioid such as fentanyl or sufentanil, and a muscle relaxant. Muscle relaxants are usually avoided during the course of the procedure during dissection and reconstruction phases involving neural repair. Anesthesia is usually maintained using propofol, opioid, e.g., remifentanyl and inhalationals eg. sevoflurane. No particular anesthetic technique has been proven more superior than the other in face transplantation or free flap surgery. Normothermia is usually maintained by appropriate surface warming and by warming intravenous fluids and blood products administered to the patient. A mean arterial pressure of 65 mmHg ensures adequate perfusion and oxygen deliver to the vital organs including the graft. Urine output of 0.5-1 mL/kg per hour is usually adequate. In cases of severe hypotension, apical and subcostal views in transthoracic echocardiography maybe useful in assessing cardiac function. Antibiotics, timely redosing of antibiotics and immunosuppressants are crucial to the success of this surgery.

Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase. Moreover, osteotomy sites can bleed excessively. Anesthesiologists involved in this surgery have reported that quantification of the bleeding is often difficult due to diffuse bleeding into the drapes and poor visualization of surgical site. Prior to reperfusion, often, after one sided anastomosis of the

graft, the contralateral side is allowed to bleed to get rid of the preservation solution and other additives which maybe used for allograft preservation, such as heparin or tissue plasminogen activator^[18].

A median of 20 U of packed red blood cells, 13 U of FFP, 2 platelet units, and 13 L of crystalloid administration has been reported^[18]. Though usually, massive transfusion protocols advocate 1:1:1 replacement of red blood cells, FFP and platelets^[21], the amount of plasma and platelets transfused have been on the lower side due to fear of risk of thrombosis of the facial vessels. Intraoperative product replacement should be guided by laboratory values and point of care testing for coagulation and hemostasis such as thromboelastography. Use of colloids such as dextrans^[22] are not preferred, and there is no data currently available on use of albumin for this surgery.

Many surgeons usually discourage use of pressors in microsurgical procedures, and though it is not typically a first line strategy, intraoperative use of pressors should be discussed in advance with the surgical team. It has been observed that there has been no difference in the outcomes when pressors were used or not used, and there is no reliable evidence to support contraindication of pressor use^[23]. Frequency of flap necrosis and postoperative complications and adverse events were similar with or without use of intraoperative pressors^[24]. Norepinephrine has been analysed as the most potential suitable agent for free flap transfer when compared to epinephrine, dobutamine and doxepine. This is because with norepinephrine, control of blood flow depends mostly on low frequency vasomotion or average blood pressure^[22,25]. Though vasoconstriction increased, the blood pressure increased too, resulting in overall increased flap blood flow^[26]. Dobutamine increases flap skin conductance, thereby benefiting flap blood flow^[26]. Epinephrine decreased flap blood flow^[26]. In face transplantation, bolus doses of pressors or pressor infusions have been used intraoperatively in several patients to manage hypotension^[17,18].

Post procedure, the regular flexometallic endotracheal tube maybe replaced by a regular tracheostomy tube, prior to transfer of the patient to the intensive care unit.

CONCLUSION

Face transplantation is a long procedure and involves complex planning for airway management, vascular access, fluid and pressor management. Teamwork between the surgeon, anesthesiologist and intensivist is essential for a successful outcome.

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Older candidates for kidney transplantation: Who to refer and what to expect?

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Abstract

The number of older end-stage renal disease patients being referred for kidney transplantation continues to increase. This rise is occurring alongside the continually increasing prevalence of older end-stage renal disease patients. Although older kidney transplant recipients have decreased patient and graft survival compared to younger patients, transplantation in this patient population is pursued due to the survival advantage that it confers over remaining on the deceased donor waiting list. The upper limit of age and the extent of comorbidity and frailty at which transplantation ceases to be advantageous is not known. Transplant physicians are therefore faced with the challenge of determining who among older patients are appropriate candidates for kidney transplantation. This is usually achieved by means of an organ systems-based medical evaluation with particular focus given to cardiovascular health. More recently, global measures of health such as functional status and frailty are increasingly being recognized as potential tools in risk stratifying kidney transplant candidates. For those candidates who are deemed eligible, living donor transplantation should be pursued. This may mean accepting a kidney from an older living donor. In the absence of any living donor, the choice to accept lesser quality kidneys should be made while taking into account the organ shortage and expected waiting times on the deceased donor list. Appropriate counseling of patients should be a cornerstone in the evaluation process and includes a discussion regarding expected outcomes, expected waiting times in the setting of the new Kidney Allocation System, benefits of living donor transplantation and the acceptance of lesser quality kidneys.

Key words: Kidney transplant outcomes; Frailty; Elderly; Expanded criteria donor; Quality of life

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Core tip: Transplant physicians must be well-versed in the intricacies of evaluating older kidney transplant candidates. This includes the appropriate selection of candidates which can be challenging due to the extent of comorbidity and frailty in this patient population. For patients who are deemed appropriate for transplant, physicians must be able to counsel them regarding expected outcomes and explain the expected benefit that transplantation confers over remaining on the deceased donor waiting list. Living donor kidney transplantation, even from older donors, should be encouraged. If no living donor is available, the rationale for accepting lesser quality kidneys should be discussed.

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INTRODUCTION

Although the incidence of end stage renal disease (ESRD) in the United States for patients ≥ 65 years old is declining, prevalence continues to increase due to increasing patient survival. Older patients (≥ 65 years) now constitute over 40% of the ESRD population and with an aging general population, this is likely to grow further. Among 116990 incident ESRD patients in 2013, 56977 (48.7%) were ≥ 65 years and the mean age was 62.5 years^[1]. Due to the above trends, the number of older patients referred for kidney transplantation will likely continue to increase as well. As such, transplant physicians must be well versed in the unique issues that arise in the evaluation of older candidates. In this review, we answer key questions that confront both physicians and patients during the evaluation process.

WHO IS AN APPROPRIATE CANDIDATE?

An appropriate candidate is a patient whose survival and quality of life are expected to improve with transplantation as compared to remaining on dialysis. Unfortunately, there are no clinical criteria that accurately and reliably predict this. Older age alone is not a contraindication to transplantation^[2]. Transplant centers, however, may arbitrarily set their own age cut-offs. For candidates who do not have a living donor, this may be influenced by the expected waiting time in an individual center. In choosing the appropriate candidate, it is logical to only consider patients with reasonable long-term prognosis. However, determining who these patients are can be quite complex and there may be an inherent bias to exclude older patients due to perceived poor outcomes. Grams *et al*^[3] developed a prediction model specific to older patients (≥ 65 years) using United States Renal Data System (USRDS) data of 128850 incident Medicare-

primary older adults with ESRD and United Network for Organ Sharing (UNOS) data of 6988 Medicare-primary first kidney transplant recipients aged ≥ 65 years. They identified 19 variables (15 comorbidities, age, dialysis vintage, sex and transplantation year) that predicted post-transplant outcomes. Based on the model, 11756 (9.1%) were found to be excellent kidney transplant candidates with a predicted 3-year post kidney transplant survival of 87.6% or higher. Of note, 76.3% of these patients were never placed on the waiting list or referred for living donor kidney transplantation. The authors concluded that using a simple risk prediction model may help identify suitable candidates and ultimately improve older candidates' access to transplantation. In another more recent study, Dusseuz *et al*^[4] developed a simple clinical scoring system using data from the French national prospective registry. By applying this scoring system on incident dialysis patients aged 70 or above, they identified a subgroup of patients that had a 70% probability of survival within 3 years, representing about 20% of the entire cohort. They suggested that this subgroup of patients, despite their older age, were worthy of being referred for kidney transplant evaluation.

Medical evaluation

The primary reason for graft loss in the older patient population is death with a functioning graft hence a great deal of emphasis is usually placed on the medical evaluation to determine suitability for transplant. Transplant centers may have variable selection criteria especially in older patients. Although several guidelines^[2,5,6] exist with regards to the medical evaluation of a kidney transplant candidate, these are not specific for the older population. In general, however, individual organ systems are evaluated by means of history taking, physical examination and ancillary testing. If there is end-stage or severe disease, for example multi-vessel coronary artery disease not amenable to revascularization, then this usually becomes a reason to exclude patients from transplantation. Screening for infection and malignancy is also inherent to the evaluation especially in older patients due to their heightened susceptibility for both^[7].

Particular focus is given to the cardiovascular work-up because cardiovascular causes comprise the leading cause of death among transplant recipients^[1]. Unfortunately, the optimal method of screening for cardiovascular disease, in particular coronary artery disease, is not known^[8,9]. Transplant centers may have variable approaches, usually ranging from cardiac stress testing to more invasive testing such as coronary angiography. Stress testing is relatively easy and inexpensive to perform, but has suboptimal sensitivity and specificity especially in diabetics^[10]. As such, some centers may opt to go straight to a coronary angiogram. For example, at our center patients who are older than 70 years of age are required to undergo coronary angiography and if there is a significant burden of coronary artery disease, then a patient is deemed to be "too high risk" and therefore unsuitable for kidney transplantation. As part of the

cardiovascular work-up, additional attention is also given to imaging the iliac vessels to assess for patency and calcification. The imaging modality of choice at our center is computed tomography \pm angiogram but a non-contrast magnetic resonance angiogram may also be a reasonable alternative if calcific burden is the main concern. At our center, not surprisingly, the primary reasons for excluding patients aged 60 years old or above are coronary artery disease, peripheral vascular disease (PVD), or both. It must be noted, however, that there are no studies that specifically compare the survival of these "very high risk" patients with transplantation as opposed to remaining on dialysis. Therefore, the decision to exclude these patients from transplantation remains rather subjective.

Measures of global health

Although a medical evaluation is able to closely scrutinize individual medical conditions, measures of global health and overall burden of disease may be more predictive of an older patient's prognosis post-transplant. Measures of global health that are increasingly being recognized as important predictors of outcome in kidney transplantation include comorbidity indices and measures of functional status, physical performance, and frailty.

Comorbidity refers to the presence of two or more chronic diseases or conditions. The Charlson Comorbidity Index (CCI) is the most widely used tool to quantify comorbidity. In the kidney transplant population, high CCI scores, indicating increased comorbidity, have been shown to correlate with an increased risk of death^[11,12]. However, in a study by Heldal *et al.*^[13], although increasing CCI scores predicted mortality in younger patients (ages 45-54 and 60-69 years), these were not predictive in those aged 70 years or older. Additionally, the applicability of the CCI, however, has been questioned in kidney transplant candidates^[14]. In a recently published Dutch study, Laging *et al.*^[14] developed the Rotterdam Comorbidity in Kidney Transplantation (RoCKeT) score as an alternative to the CCI. The RoCKeT score is determined by the presence of cardiovascular disease (3 points), cerebrovascular accident (2 points), PVD (2 points), diabetes mellitus (2 points), liver disease (2 points), lung disease (2 points), malignancy (2 points) and human immunodeficiency virus (1 point). Not surprisingly, comorbidity was highest in the oldest age group in that 75% of patients aged 70 to 79 had comorbidity (at least 1 point). When RoCKeT scores were categorized and analyzed for the influence on patient survival, the group with the highest scores (5-9) had a significantly lower survival than those without comorbidity (score of 0). After multivariate analysis, patients with a score of 5-9 had a 2.7 increased risk of death compared to patients with a score of 0. Despite this, 50% of patients in the highest comorbidity category survived more than 10 years. The authors concluded that patients with severe comorbidity should not be excluded from transplantation due to superior patient survival compared with published survival data of hemodialysis patients. Moreover, meticulous selection of high-risk patients for kidney

transplantation can lead to successful outcomes.

Functional status is measured by a patient's self-report of his or her ability to perform certain tasks. These tasks may include the ability to walk a certain distance, climb stairs, or perform activities of daily living. Functional status measurements are subjective and are obtained *via* questionnaires such as the short form-36 (SF-36) Physical Function (PF) scale, Vulnerable Elderly Survey-13, or Physical Activity Scale for the Elderly. A number of studies have reported an association between functional status and patient survival^[15-19]. In the largest study to date, Reese *et al.*^[19] analyzed 19242 *Fresenius* dialysis patients who had answered the SF-36 PF scale pre-transplant and had linked post-transplant data *via* the UNOS registry. Patient PF scores were divided into PF quartiles and these were correlated with time to kidney transplantation and the net survival benefit of kidney transplantation vs remaining on the waiting list. Patients in the lowest quartile were significantly older than those in the highest quartile (median age 54 years vs 46 years). In terms of survival, patients who were in the lowest PF quartile had the worst 3-year survival rates (84% compared to 92% for the highest quartile). When compared to remaining on the waiting list, patients across all PF quartiles had a survival benefit with transplantation. The lowest PF quartile had a survival benefit evident by 6 mo after transplantation. Another important finding in this study is that patients in the lowest PF quartile were more likely to be inactivated on the waiting list (adjusted hazard ratio vs highest quartile, 1.3) and less likely to be transplanted (adjusted hazard ratio vs higher quartile, 0.64). The authors concluded that functional status measures may be more useful in counseling patients regarding their probability of transplantation. It must be noted however that this study did not examine patients who were excluded from kidney transplant listing and who presumably had poorer baseline functional status, *i.e.*, the study only examined the best patients referred for transplant. Also, only 12% of the cohort were 65 years or older. Therefore, for patients referred for transplant who are older or with potentially worse baseline functional status, the applicability of this study's findings in regards to the survival benefit of transplant vs remaining on the waiting list remains to be determined.

Physical performance is the measured ability to perform tasks or exercise. Examples include measurements of gait speed or grip strength. The short physical performance battery (SBBP) is a combination of tests with a sub-score assigned. Measures of physical performance are objective and may be superior to reports of functional status in that these avoid reporting bias and overestimation of patients of their health status. Hartman *et al.*^[20] in a study of 26 patients aged ≥ 60 years and referred for kidney transplantation, found that these patients with renal failure had lower SBBP scores, gait speed and grip strength compared to patients with diastolic heart failure (71 patients), chronic obstructive pulmonary disease (176 patients) or those with high cardiovascular risk (294 patients). Interestingly, despite

their inferior physical performance, renal failure patients were less likely to report functional impairment on disability questionnaires. We are not aware of any studies to date that have measured physical performance and correlated these with outcomes in kidney transplant patients. In other solid organ transplant candidates, particularly in lung transplant, the six-minute walk test (6MWT)^[21] has been used routinely in pre-transplant evaluations and has been shown to be a predictor of morbidity and mortality^[22,23]. The 6MWT measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 min. It would certainly be interesting to see if the 6MWT can be used similarly in older kidney transplant candidates to predict outcomes.

Frailty is a state of decreased physiologic reserve and is defined by the presence of 5 features, namely unintentional weight loss, weakness as measured by decreased grip strength, slow walking speed, low physical activity and self-reported exhaustion. It has been shown by McAdams-DeMarco *et al.*^[24-26] in successive papers that frailty is associated with increased hospital readmission post-transplant, graft loss and mortality. In a prospective study of 537 kidney transplant recipients in a single center^[26], frailty was measured at time of transplantation. Those who were frail, defined as having at least 3 out of the 5 features, were found to have a hazard ratio for death post-transplant of 2.22 (1.03-4.81, $P = 0.042$) compared to patients who were non-frail. In the subgroup of patients who were 65 years or older, 1-year survival was 85.8% in the frail group as opposed to 97.4% and 97.5% in the intermediately frail and non-frail groups, respectively. The authors suggested that frail patients should be identified pre-transplant and that patient survival may improve with appropriate management and closer monitoring of these patients.

A common theme to all the global measures of health discussed above is that it is not clear as to who is "too sick", "too debilitated", "too weak" or "too frail" to undergo kidney transplantation. Although these tools may help risk stratify patients, each candidate should be assessed on an individual basis and all data considered as a whole in determining a patient's suitability for transplant.

WHAT OUTCOMES CAN BE EXPECTED POST-TRANSPLANT?

Older recipients have decreased patient and graft survival compared to younger patients^[27]. Graft loss is commonly due to patient death, the top 3 causes being cardiovascular disease, infection and malignancy^[28]. There is less acute rejection in older patients and if graft loss is censored for death, graft survival actually improves with increasing age^[7,29].

Despite inferior patient survival in older compared to younger patients, kidney transplantation is pursued due to the survival benefit that it confers when compared to remaining on the deceased donor waiting list. In a study by Rao *et al.*^[30], 5667 patients aged ≥ 70 years who

were waitlisted for kidney transplantation were analyzed based on scientific registry of transplant recipients (SRTR) data. Of these patients, 2438 ultimately underwent kidney transplantation and when compared to those who remained on the waiting list, the transplanted patients had a 41% reduction in risk of death (0.59 relative risk of death). The time to equal risk was 125 d and the time to equal survival was 1.8 years from transplant. Of note, the mortality benefit that was seen in this study extended to the subgroup of patients aged ≥ 75 years, those with diabetes and those who received an expanded criteria donor. This study confirmed the findings of an earlier study by Wolfe *et al.*^[31] wherein the subgroup of patients aged 60-74 years was found to have a 61% lower mortality (0.39 relative risk of death 18 mo after transplantation) compared to similar patients on the waiting list. This survival advantage was calculated to translate into a 4-year increase in life expectancy (from 6 to 10 years).

In addition to superior patient survival compared to remaining on the waiting list, kidney transplantation is pursued due to the improvement in quality of life (QOL) that it confers^[32,33]. Transplant patients have superior QOL compared to dialysis patients^[34], though this may not be a fair comparison given that transplanted patients are a highly selected group. Age may have an effect on post-transplant QOL^[35-37]. In a single center study by Weber *et al.*^[36], they compared the post-transplant health-related QOL of patients ≥ 65 years with younger patients and with the general population. They found that physical QOL in older patients was significantly lower compared to younger patients and the general population. However, mental QOL was better than younger patients and similar to the general population. Humar *et al.*^[37] compared QOL of patients ≥ 65 years to younger patients and with national norms for this age group. They found that older transplanted patients scored higher in their general health perception, social functioning and mental health compared to national norms and also scored higher on social functioning and mental health compared to younger transplanted patients. Both these studies, however, did not look at pre-transplant QOL data to determine if there was an actual improvement in QOL before and after transplant. In a study by Laupacis *et al.*^[38] of 166 patients, 22 of whom were ≥ 60 years, they found that mean health-related QOL scores of almost all measures improved from pre-transplant to 6 mo after transplantation.

WHICH TYPE OF KIDNEY IS BEST?

Clearly, living donor (LD) transplantation confers the best outcomes in terms of patient and graft survival^[39]. This eliminates time on the waiting list, reduces dialysis vintage and allows for preemptive transplantation, affords patients better quality kidneys, and reduces the incidence of delayed graft function and a potentially tumultuous immediate post-transplant course. Moreover, due to the

elective and scheduled nature of LD transplant surgery, recipient issues can be addressed in a controlled manner prior to surgery thereby reducing perioperative risk. This was shown in a study by Gill *et al.*^[40] of 25468 patients aged ≥ 65 years based on USRDS data who were listed for kidney transplantation, of which 11072 received a kidney transplant either from a LD, standard criteria deceased donor (SCD), or expanded criteria deceased donor (ECD). All patients were categorized based on cardiovascular (CV) risk as either being high, intermediate, or low CV risk. Among patients transplanted and across all CV risk categories, the death rate was lowest for patients who received a LD transplant and highest for recipients of an ECD kidney. Compared to patients who remained on the waiting list, a survival advantage was obtained, but importantly, times to equal risk and equal survival differed depending on the type of kidney transplanted and a patient's risk category. For patients who received a LD transplant, those who were low or intermediate CV risk had an immediately lower risk and higher survival post-transplant, and those who were high CV risk had a time to equal risk of only 43 d compared to similar patients who received an SCD (110 d) or ECD (180 d).

Despite the known advantage that living donor transplantation confers, older patients may have more limited living donor options as they may be hesitant to accept kidneys from younger donors such as their children or grandchildren. An alternative would be to pursue living donor transplantation from older donors such as their spouses or peers. Several studies have shown that recipients of kidneys from older living donors have reasonable outcomes^[41-43]. Englum *et al.*^[41] studied 250827 patients based on UNOS data who received a kidney transplant, of which 92646 were LD kidneys and 4186 from donors aged ≥ 60 years. Not surprisingly, graft and patient survivals of patients who received a kidney from an older LD were worse compared to those who received a kidney from a younger LD. However, patients who received a kidney from an older LD aged 60-64 years and 65-69 years had similar graft survivals to patients who received a SCD kidney, superior graft survivals to ECD recipients and superior patient survivals to both SCD and ECD recipients. Patients who received a kidney from a LD aged ≥ 70 years had graft survivals similar to ECD recipients but significantly better patient survival. Given the organ shortage and current waiting times for a deceased donor kidney, it would make sense for an older patient who has an available older LD to pursue transplantation from an older LD rather than wait for an SCD or ECD kidney.

For those without living donor options, patients are faced with an increasing waiting time on the deceased donor list. The median number of years to deceased donor transplant was 5.5 years in 2003 and 7.6 years in 2007^[39]. Waiting time could be shorter or longer depending on where a patient is listed and his or her sensitization status and blood type. As older patients' time on the waiting list increases, the less likely they are to be transplanted as their health deteriorates

and they are either removed from the waiting list or they die^[44]. Compared to younger patients, the risk of death while waiting for a transplant is higher for older patients^[39]. It is therefore of paramount importance for older patients to get transplanted sooner rather than later. Kidneys that are thought to be of lesser quality should be considered for older candidates as waiting times for these kidneys are usually shorter. Rao *et al.*^[30] and Merion *et al.*^[45] demonstrated that recipients of ECD kidneys had superior survival compared to similar patients who remained on the waiting list or those who received standard therapy (waiting list and non-ECD transplantation). Massie *et al.*^[46] examined the outcomes of patients who received high kidney donor profile index (KDPI) kidneys and compared these to outcomes of patients who remained on the waiting list until receipt of a KDPI $< 70\%$ kidney. The times to equal risk and equal survival post-transplant with the comparison group were 6 and 18 mo, respectively for the KDPI 81%-90% group and 7.2 and 19.8 mo, respectively for the KDPI 91%-100% group. At 4 years post-transplant, the KDPI 81%-90% group and 91%-100% group had a 17% and 10% lower mortality, respectively, than the comparison group. However, after 4 years the mortality rate was not statistically significantly different. The study found that the benefit of the high KDPI kidneys was greatest in patients ≥ 50 years who were listed at centers with a median wait time of ≥ 33 mo. In another study, Rose *et al.*^[47] found that among 5257 patients that received a kidney from a deceased donor aged ≥ 65 years (defined in this study as an ECD kidney) in the United States, 10-year mean death-censored graft survival exceeded patient survival in patients aged ≥ 60 years. Among those aged ≥ 70 years, the difference was over 20 mo. Of note, there was a 7-8 mo difference in the 10-year mean patient survival between those who received an ECD kidney and similar patients who received a kidney from a deceased donor aged < 65 with a KDPI of 60%-69%. The authors concluded that for patients aged ≥ 60 years, kidneys from older donors can provide a lifetime of allograft function and that ECD transplantation should be encouraged in this age group. In a study from Spain, Pérez-Sáez *et al.*^[48] looked at outcomes of 2040 patients waitlisted for transplant, of whom 389 (mean age 68.9 ± 5.8 years) received a kidney from a deceased donor aged ≥ 75 years. They found that there was a 56% lower risk of death in patients who received a transplant compared to those who remained on the waiting list. However, patients ≥ 70 years, diabetics and those with chronic obstructive pulmonary disease did not derive any statistically significant benefit.

HOW DOES THE NEW KIDNEY ALLOCATION SYSTEM (KAS) AFFECT OLDER PATIENTS?

In an attempt to balance equity with utility, kidney allocation in the United States was changed in December

2014^[49]. One of the goals of the new KAS is to increase unrealized graft years by matching high quality kidneys with recipients who have longer life expectancy^[50,51]. As a result, transplant rates among older candidates are expected to decrease^[51]. In an analysis of the early impact of the new KAS a year after its implementation, Stewart *et al.*^[52] noted a significant reduction in transplants where donor and recipient age differed by more than 30 years (21.1% pre-KAS vs 16.3% post-KAS). Among recipients aged 65 years or older, transplant rates significantly decreased from 22.9% of all kidney transplants pre-KAS to 18.1% post-KAS across all donor KDPI's, with the most prominent reduction in transplants from donor kidneys with a KDPI of 0-20%. This occurred despite an increase in the number of waitlisted patients aged ≥ 65 years (21.3% pre-KAS to 24.9% pre-KAS).

Another important feature of the new KAS is that kidneys from donors with a KDPI $> 85\%$ are now being allocated nationally. Whether this would lead to increased utilization of these organs and subsequent shorter waiting times remains to be seen. Broader sharing of these kidneys may lead to increased cold ischemic times and increased discard rates of marginal kidneys. In early analysis^[52], there was a significant reduction in transplant rates of kidneys from donors aged ≥ 65 years (3.1% pre-KAS vs 2.5% post-KAS, $P = 0.0085$) and a non-significant reduction in transplanted kidneys with a KDPI of 86%-100% (8.6 pre-KAS vs 7.9% post-KAS, $P = 0.0645$). The kidney discard rate 1-year post KAS was slightly higher (19.4% post-KAS vs 18.5% pre-KAS, $P = 0.05$).

With these changes in the new KAS, we believe that older recipients should be motivated further to look for living donors including older living donors. If no living donor is available, then listing for kidneys with a KDPI $> 85\%$ should be highly considered. Consenting for KDPI $> 85\%$ kidneys should include a discussion regarding expected outcomes and rationale for accepting these kidneys.

CONCLUSION

Determining who among older kidney transplant candidates is appropriate for transplantation can be challenging and complex. A thorough medical evaluation with particular focus on cardiovascular health must be employed. Additional tools such as measures of comorbidity, functional status, physical performance, and frailty may be helpful. Those older patients who ultimately undergo transplantation have decreased patient and graft survival compared to younger counterparts, but have superior patient survival compared to those who remain on the deceased donor waiting list. Living donor transplantation confers the best outcomes for older recipients with reasonable outcomes from older living donors. If no living donor is available, most older patients will likely benefit from accepting lesser quality kidneys such as those that have a KDPI $> 85\%$. In the era of the new KAS where transplant rates among older patients are expected to decrease, appropriate counseling of older recipients regarding their options is of paramount

importance.

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More than skin deep? Potential nicotinamide treatment applications in chronic kidney transplant recipients

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Abstract

Non-melanoma cutaneous carcinomas, or skin cancers, predominantly squamous cell carcinomas (SCCs), are the most common malignancies occurring in kidney transplant recipients (KTRs). Squamous cell carcinoma risk is dramatically elevated in KTRs, occurring at rates of up 45-250 times those reported in general populations. New non-melanoma skin cancers in KTRs with a prior non-melanoma skin cancer also develop at 3-times the rate reported in non-KTRs with the same clinical history. The unique aggressiveness of SCCs in KTRs increases patient morbidity, due to the high rate of new lesions requiring treatment, frequently surgical excision. Oral nicotinamide shows promise in the chemoprevention of the especially aggressive non-melanoma skin cancers which occur in KTRs. This benefit might be conferred *via* its inhibition of sirtuin enzymatic pathways. Nicotinamide's concurrent hypophosphatemic effect may also partially ameliorate the disturbed calcium-phosphorus homeostasis in these patients-a putative risk factor for mortality, and graft failure. Conceivably, a phase 3 trial of nicotinamide for the prevention of non-melanoma skin cancers in KTRs, lasting at least 12-mo, could also incorporate imaging and laboratory measures which assess nicotinamide's impact on subclinical cardiovascular and chronic kidney disease risk, and progression.

Key words: Kidney transplantation; Skin neoplasms; Nicotinamide; Phosphorus

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Core tip: Our unique review describes promising evidence

that oral nicotinamide may have dual therapeutic clinical applications in chronic kidney transplant recipients (KTRs). First, nicotinamide, *via* its inhibition of sirtuin-mediated enzymatic pathways, may reduce the rate of KTR non-melanoma skin cancers. Second, nicotinamide's hypophosphatemic effect could lower the rate of cardio-renal outcomes in KTRs. These hypothesized benefits warrant further study in randomized, placebo-controlled trials of nicotinamide treatment with both intermediate, and eventually, hard clinical outcomes.

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NON-MELANOMA SKIN CANCER IN GENERAL AND KIDNEY TRANSPLANT RECIPIENT POPULATIONS

Updated United States incidence estimates for non-melanoma cutaneous carcinoma, or skin cancer (NMSC) in the Medicare fee-for-service population indicate a pronounced rise of 100% for the entire two decade period from 1992 to 2012, and a sustained 6-year 2006 to 2012 elevation in NMSC rates of 35%^[1]. Data recorded from kidney transplant recipient (KTR) populations spanning over four decades now, consistently yield 45- to 250-fold general population standardized incident rates for squamous cell carcinomas (SCCs), accompanied by 10-fold greater rates for basal cell carcinomas (BCCs)^[2,3]. Analyzing data from 24088 United States KTRs who underwent an initial kidney transplant between 1995 and 2001, Kasiske *et al.*^[3] reported 3-year age-adjusted NMSC rate ratios of 90.0 for male KTRs, and 92.3 for female KTRs, relative to general population controls. Comparison of KTR and non-solid organ transplant recipient (SOTR) populations with a prior history of NMSC, undergoing surveillance for new NMSCs, also demonstrates a persistently greater risk for additional skin cancers in KTRs. While non-KTRs experience a 3-year cumulative 18% risk of a subsequent SCC after a first SCC^[4], 3-year rates of 52%, and 59%, for a (at least one) new SCC in KTRs after an initial SCC, have been reported^[5].

Pre-cancerous actinic keratoses (AKs), Bowen's disease (SCC *in situ*), and keratoacanthomas are commonly associated with SCCs in KTRs^[2]. Most SCCs among KTRs, as in non-SOTRs, develop, typically, on sun-exposed areas^[2]. However, KTR SCCs have an increased tendency to be multiple, and aggressive, compared to SCCs which develop in non-SOTRs not exposed to chronic immunosuppression^[2] (Figure 1). The characteristic aggressiveness of KTR SCCs exacerbates patient



Figure 1 The unique aggressiveness of squamous cell carcinoma in kidney transplant recipients. Deeply invasive, recurrent, poorly differentiated and ulcerated squamous cell carcinoma in a 73-year-old female kidney transplant recipient with background alopecia from prior radiation therapy for this squamous cell carcinoma.

morbidity, because of the disproportionate rate of new lesions requiring, cryotherapy, electro-dessication and curettage, or surgical excision^[2]. This increased morbidity, although non-fatal, also results in significant medical costs, reflecting the national United States economic burden of NMSC and AK care, tabulated, as of 2007-2011, at \$4.8 billion, annually^[6]. KTR SCCs, additionally, have a greater potential for metastasis, and death^[2,5].

Successful interventions to reduce the incidence and complications associated with all NMSCs, SCCs, in particular, as well as AKs, would represent a significant advance in the management of KTRs. Despite heroic "conversion" protocols from calcineurin inhibitor-based to mechanistic/mammalian target of rapamycin (mTOR) inhibitor-based (primarily, sirolimus) immunosuppressive regimens, KTRs with a predilection for NMSCs, especially SCCs, continue to develop new malignant skin lesions at grossly elevated rates^[7,8]. The sirolimus converted group reported by Euvrard *et al.*^[7], for example, still experienced a 2-year incidence of 22% for SCC, and 47.6% for total NMSCs (71 new lesions in 20 patients). NMSC-prone KTRs converted to sirolimus also appear to increase their relative risk for death after mTOR conversion. A recent meta-analysis of such "conversion trials" underscored the lingering therapeutic dilemma: While sirolimus use significantly lowered SCC risk, it conferred an overall mortality penalty-a 1.59-fold excess risk of death^[8].

Ultraviolet (UV) radiation, immunosuppressive therapy, and human papillomaviruses (the latter with an ostensible link to SCC, specifically), are all believed to contribute to the development of NMSC among KTRs, while their precise etiologic pathomechanisms, alone, or in concert, require elucidation^[2]. Although topical sunscreens are an effective prophylactic against sunburn, their use may not afford protection from UV radiation-induced immunosuppression of the skin^[9], and the incidence of skin carcinomas, especially SCCs, continues to climb, steeply^[1]. The ideal chemopreventive treatment, as an adjunct to barrier sun protection, would

be an oral agent that is safe, well-tolerated, inexpensive, and readily available. A potential candidate emerging to fill that therapeutic niche is oral nicotinamide (NAM).

Nicotinamide as a potential non-melanoma skin cancer chemopreventive agent

Overt, pellagrous nicotinic acid (NA; niacin) deficiency has long been recognized as a cause of severe sunlight sensitivity in exposed skin^[10]. Recent clinical trial reports from an Australian investigative group suggest that oral NAM can reduce the occurrence of both AKs and NMSCs in non-SOTRs who have a history of AKs, and/or NMSCs^[11,12]. The initial 4-mo, placebo-controlled phase 2 studies ($n = 35$, and $n = 41$ participants, respectively) reported that oral NAM lowered the rate of appearance of new AKs (*i.e.*, total AK counts) by 29% to 35%^[11]. In a subsequent 12-mo phase 3, placebo-controlled trial of 386 patients with prior NMSCs, NAM treatment ($n = 193$ active; $n = 193$ placebo) reduced the average unadjusted new NMSC rate (total lesions per patient) by 27%. NAM treatment resulted in comparable rate reductions for SCCs (-30%), and BCCs (-20%), as separate outcomes^[12]. Chen *et al.*^[13], from the same Australian investigative group which conducted these non-SOTR NAM chemoprevention studies^[11,12], recently reported consistent results, in terms of effect sizes, from a phase 2 study of 22 KTRs. Patients at least 12 mo post-transplant, with stable renal function, and a history of ≥ 2 histologically-confirmed NMSCs in the previous 12 mo, were randomized 1:1 ($n = 11$ per group) to receive NAM 500 mg, or placebo, twice daily, for 6 mo. Skin exams and AK counts were performed at 2-mo intervals. The 6-mo NMSC rate (mean lesions per patient) was non-significantly lower for the NAM group (mean = 2.7; 95%CI: 1.4 to 5.3; total = 30 cancers), compared to placebo (mean = 4.2; 95%CI: 2.2 to 7.8; total = 45 cancers), although the numeric trend was dominated by one patient in the placebo group with 20 NMSCs (8 BCC and 12 SCC). The estimated relative rate difference was 0.35 (95%CI: -0.62 to 0.74, $P = 0.36$). Baseline AK counts (reported as means of 61.6 in the placebo, and 60.1 in the NAM groups, respectively), were also non-significantly lower in KTRs receiving NAM compared to placebo by 16% at 6 mo (95%CI: 7% to 34%; $P = 0.15$)^[13]. Also of importance, there were no between groups differences for adverse clinical events, or changes in complete blood counts, liver function studies, eGFR, or urinary microalbumin/creatinine ratios^[13]. Additional, if limited, independent confirmatory data have been provided in a research letter published by Drago *et al.*^[14], in *The New England Journal of Medicine*. These Genoa, Italy investigators studied NAM given at a dose of 250 mg thrice daily, relative to matched placebo, in 24 KTRs ($n = 12$ per group), also followed for 6-mo. The following is a verbatim description of their findings^[14].

At baseline, no significant differences were observed between the sizes of light-damaged areas in patients (identified visually, by touch, and by means of polarized

light dermoscopy), in the two groups. At 6 mo, 88% of the patients who received nicotinamide had partial regression of some or all actinic keratoses and surrounding light-damaged areas; in 44% of the patients who received nicotinamide, there was complete resolution in some of these areas (no lesions were detected on biopsy). In 91% of the patients who received placebo, the size of light-damaged areas increased, new light damaged areas developed, or both.

Two plausible anti-cancer biological effects of nicotinamide have been described by the Australian investigators which provide some independent validation of their clinical trial observations: Nicotinamide has been shown to promote DNA repair after UV exposure, and lessen local UV immunosuppression, in the skin^[15]. NAM's reported effects on sirtuin enzymes and mediated pathways might also confer anti-cancer properties. Briefly, sirtuin (Sir2) enzymes, are an ancient class of nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylases which have been conserved from bacteria to humans (for example, SIRT1, *etc.*). They perform a myriad of biological functions, including transcriptional silencing, DNA recombination and repair, apoptosis, axonal protection, fat mobilization, and lifespan regulation^[16-18]. Sirtuins deacetylate various proteins, notably p53, as well as histones, acetyl-coA synthetase, alpha-tubulin, Foxo3, Ku70, and NF kappa-beta. Lysine residues are the specific targets of sirtuin deacetylation reactions, allowing for tight cellular regulation. Sirtuin-catalyzed deacetylation is linked to the cleavage of NAD⁺, producing NAM, and O-acetyl ADP-ribose (OAADPr), in conjunction with deacetylated ribose. NAM produced from this reaction is a non-competitive inhibitor of sirtuins, which provides a mechanism for modulation of these enzymes by cellular nicotinamide concentrations^[16-18]. Importantly, the inhibitory activity of NAM on sirtuin-mediated deacetylation is not conferred by nicotinic acid (NA)^[18] (Figure 2). *SIRT1* overexpression has been observed in human prostate cancer, adult T-cell leukemia, primary colon cancer, and, significantly, in skin tissue biopsies from patients with AK, Bowen's disease, SCC, or BCC^[16,17]. One mechanism by which elevated *SIRT1* concentrations are believed to enhance malignant cell growth is *via* deacetylation of "tumor suppressor" p53 protein, inhibiting p53's apoptotic activity^[16,17]. *SIRT1* interacts directly with p53 and deacetylates the protein's C-terminal (Lysine)382 residue, which prevents p53 from trans-activating apoptotic genes, and promoting apoptosis^[17]. NAM may also affect another of *SIRT1*'s downstream target proteins, the retinoblastoma tumor suppressor protein (Rb), reducing Rb phosphorylation (or "hyper"-phosphorylation)^[19]. This has already been demonstrated, for example, in a mouse model of polycystic kidney disease, where NAM treatment reduced sirtuin-enhanced cyst formation^[19]. Anti-cancer chemopreventive agents such as curcumin (a natural phenol responsible for the yellow color of turmeric), which suppresses Rb phosphorylation in prostate cancer cells, are under investigation^[20].

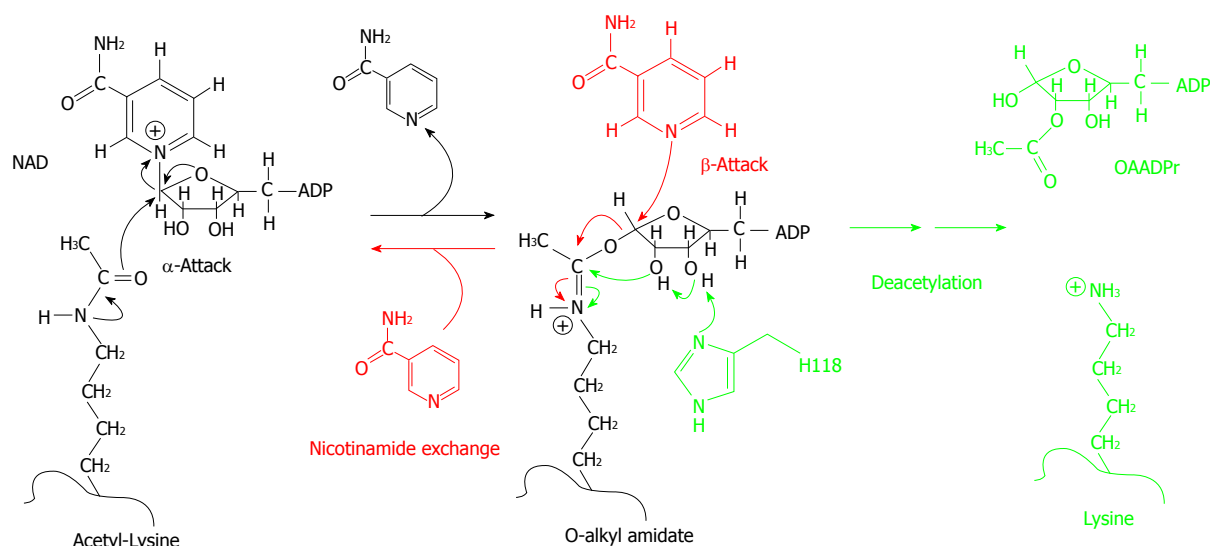


Figure 2 Nicotinamide-SIRT binding. At high concentrations, NAM can bind to SIRT when it contains the O-alkyl-amide intermediate, resulting in “NAM exchange”, reforming NAD⁺ and acetyl-lysine, and decreasing deacetylation activity - a process unique to NAM, not NA. Reproduced from^[18]. NA: Nicotinic acid; NAM: Nicotinamide.

Comparable NAM-induced p53 and Rb alterations could mediate the potential benefits of NAM treatment for the chemoprevention of AKs and NMSCs, in general, and KTR patient populations^[11-14]. NAM's putative *in vivo* role as an inhibitor of sirtuins, and sirtuin-catalyzed “pro-oncogenic” de-acetylation, and hyper-phosphorylation reactions^[16-19] warrants investigation, as another pathophysiological correlate, in NAM-treated patients.

NICOTINAMIDE AND HYPOPHOSPHATEMIA: FROM TOXICITY MONITORING TO THERAPEUTIC INTERVENTION

Extensive analyses by others^[21], and our group^[22-26] (Figure 3), demonstrate that both NAM and NA lower serum phosphorus in chronic kidney disease (CKD) patients, and indeed across the entire spectrum of renal function. Mediated *via* an elegant mechanism, *i.e.*, direct inhibition of sodium-dependent transport of phosphorus in the small intestine^[21], this consistent phosphorus-lowering effect mandates surveillance of serum phosphorus levels in patients on chronic oral NAM/NA to avoid the theoretical development of clinical hypophosphatemia^[21]. But such potential toxicity might be counterbalanced by a distinctly positive clinical phenomenon: Since baseline serum phosphorus concentrations appear to predict total and/or cardiovascular disease (CVD) mortality in CKD and KTR populations^[27-31], as well as native kidney, or kidney graft failure^[27,30-32], conceivably, NAM-induced phosphorus-lowering could reduce such hard outcomes in these patients. Moreover, as NAM does not induce prostaglandin-mediated flushing, cause hyperuricemia, or adversely affect glucose tolerance, we believe it will have a better tolerability and safety profile relative to NA, confirming

a substantive, decades old body of clinical evidence^[33,34] from non-SOTR patient populations-now updated to include those studied on oral NAM therapy in the recent phase 2 and 3 Australian AK/NMSC prevention trials^[11-13]. The sporadic occurrence of mild thrombocytopenia in end-stage renal disease (ESRD; stage 5 CKD) patients treated with NAM^[35], has not been confirmed from either the recent placebo-controlled studies conducted among patients with normal renal function^[11,12], or KTRs^[13], for NMSC or AK prevention, nor was it reported in the large multicenter ENDIT trial^[34], or a 30-year toxicity review of NAM trials which preceded ENDIT^[33]. COMBINE, an ongoing, randomized, placebo-controlled trial (NCT02258074) in patients with an estimated glomerular filtration rate (eGFR) of 20-45 mL/min per 1.73 m², which includes a NAM treatment arm (1.5 g/d, given as 0.75 g twice daily), will provide more definitive data on the incidence of this toxicity in stage 3b-4 CKD^[35,36].

OVERVIEW OF DYSREGULATED CALCIUM-PHOSPHORUS HOMEOSTASIS AND ITS CLINICO-PATHOLOGIC IMPLICATIONS IN CKD AND KIDNEY TRANSPLANTATION

Deranged calcium-phosphorus metabolism often complicates CKD^[36], worsens with the progressive development of ESRD^[21,36], and is not fully reversed after kidney transplantation^[28-30,37,38]. Moreover, notwithstanding abnormalities, particularly hyperparathyroidism (with resultant increased fractional excretion of urinary phosphorus/decreased tubular reabsorption of phosphorus)^[37], which may persist long term despite successful transplantation, and excellent kidney graft function, chronic

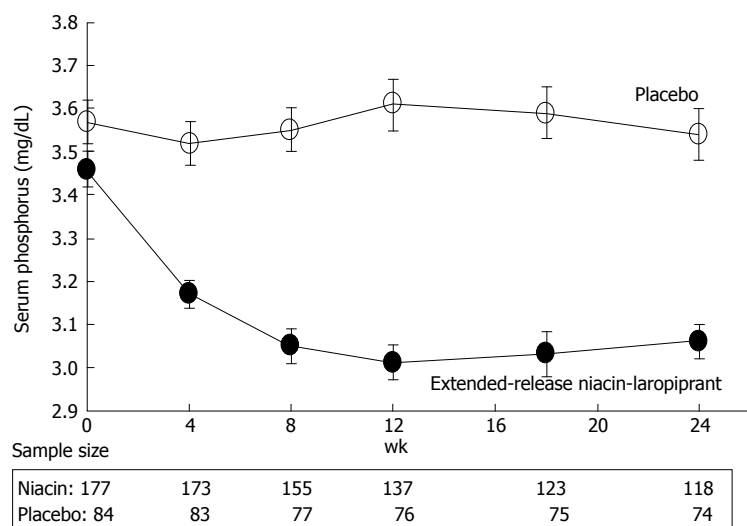


Figure 3 Phosphorus-lowering effect of nicotinic acid in chronic kidney disease. Mean serum phosphorus concentrations in dyslipidemic patients with stage 3 chronic kidney disease receiving extended release niacin-laropiprant, or placebo. Error bars are standard errors. Reproduced from Ref. [24].

KTRs whose GFR subsequently declines to stage 4 (15–29 mL/min per 1.73 m²) to 5 (< 15 mL/min per 1.73 m²), or even 3b (30–44 mL/min per 1.73 m²) CKD, are prone to the hyperphosphatemia, inadequate vitamin D status (*i.e.*, deficiencies of 25-hydroxy vitamin D3, and/or 1,25-dihydroxy vitamin D3), elevated concentrations of parathyroid hormone (PTH), and increased levels of the phosphatonin fibroblast growth factor 23 (FGF23), characteristic of stage 3b to 5 CKD in the native kidneys of non-KTRs^[28–31,37,38].

Chronic upregulation of the hormonal mechanisms evolved to maintain calcium-phosphorus homeostasis, including normal serum phosphorus concentrations, may have adverse clinical sequelae. For example, higher serum concentrations of phosphorus (*via* increased dietary intake, and/or a reduced GFR) apparently stimulate production of the bone-derived hormone FGF23, which induces compensatory renal phosphorus excretion^[30,36]. Elevated FGF23, in turn, may directly induce cardiac myocyte hypertrophy, clinically manifest as left ventricular hypertrophy (LVH)^[36], and lower endogenous production of calcitriol (1,25-OH vitamin D3), from 25-OH vitamin D3^[36]. Significant associations have been reported between FGF23 elevations LVH, CVD events, mortality, and progression to ESRD in patients with CKD stage 3–4^[36]. Accordingly, sustained elevations in FGF23 concentrations, despite being a homeostatic adaptation to maintain normal serum phosphorus, may enhance the risk for developing LVH, CVD, and ESRD^[36]. Higher phosphorus concentrations also stimulate chronic excess PTH secretion, eventually leading to clinical hyperparathyroidism^[36,39]. Inexorable CKD progression, accompanied by further nephron loss, ultimately overwhelms these compensatory hormonal mechanisms, causing a preponderance of advanced CKD patients to manifest concurrent hyperphosphatemia, increased FGF23 and PTH levels, and suppressed 1,25-OH vitamin D3 concentrations^[36,39].

The precise *in vivo* molecular mechanisms through which extracellular phosphate exerts its cytotoxic effects are not fully elucidated. However, investigations have demonstrated that extracellular phosphorus can form insoluble nanoparticles with calcium and fetuin-A,

commonly dubbed calciprotein particles (CPPs)^[39,40]. Highly bioactive ligands, CPPs can have cytotoxic effects such as causing cell death, or inducing osteogenic transformation of vascular endothelium, and renal tubular epithelium. CPPs, furthermore, are detectable in the circulation of both animal models, and humans, notably in patients with CKD, implicating their potential role in tissue injuries mediated by phosphatemia^[39,40]. Recently, a novel assay of serum calcification propensity, the transformation time (“T50”) from primary calciprotein particles to secondary calciprotein particles, has been validated, and appears to reflect the pathophysiological milieu engendered by derangement of calcium-phosphorus metabolism which may predispose to ectopic, including vascular, calcification^[29,30]. These interrelated perturbations-hyperphosphatemia, inadequate status of vitamin D, elevations in PTH and FGF23, and more recently, greater calcification propensity (reduced T50)-are of epidemiological, and potentially, clinical relevance, because they have been associated, with fatal CVD^[26,35], graft failure^[30–32,40] or rapid decline in eGFR^[38], and total mortality^[28–31,40], among KTRs.

Even after possible “over-adjustment” for these co-variable measures of disturbed calcium-phosphorus homeostasis-which may be in the causal pathway between “phosphorus toxicity”^[39], and its clinical sequelae-the relationship between serum phosphorus concentrations, and outcomes, can persist in sizable observational KTR cohort studies. Pihlström *et al.*^[31], for example, investigated the association between baseline phosphorus concentrations and major CVD events, kidney graft loss, and all-cause mortality by proportional hazard survival analyses in 1840 stable KTRs derived from the Assessment of LEscal in Renal Transplantation (ALERT) trial, a multicenter randomized, double-blind, placebo-controlled study examining the effect of fluvastatin (40–80 mg daily) on CVD (primarily coronary heart disease, CHD), and renal outcomes in 2102 KTRs. Patients were recruited a mean of 5.1 years after transplantation, and followed for 6 to 7 years. During a mean follow-up of 6.7 years, death censored graft loss was recorded in 333 patients,

277 patients experiencing a major CVD event (defined as time to cardiac death, nonfatal myocardial infarction, or undergoing a coronary revascularization procedure), and 342 died, 168 from CVD, including cerebrovascular, or other major (*e.g.*, thoraco-abdominal aortic) vascular disease. Serum phosphorus (per 1 mg/dL increase) was associated with death from all causes, hazards ratio (HR) 1.23 (CI: 1.07-1.43, $P = 0.005$), and graft loss, HR 2.61 (CI: 2.25-3.04, $P < 0.001$), in unadjusted models. The relationship between serum phosphorus and mortality lost significance, HR 1.07 (CI: 0.89-1.28, $P = 0.488$), upon multivariable modeling (with PTH > 65 pg/mL), but persisted for graft loss, HR 1.52 (CI: 1.27-1.82, $P < 0.001$)^[31]. Similarly, Wolf *et al.*^[30] studied a single center cohort of 984 chronic, stable Hungarian KTRs (median transplant vintage, 72 mo, interquartile range 40-114 mo; mean eGFR 51, SD = 21; 57% men). After a median follow-up of 37 mo (interquartile range, 35-39 mo), 87 patients died and 101 patients suffered kidney graft loss. Outcome data were analyzed in full models that adjusted for eGFR, age, gender, systolic blood pressure, body mass index, albumin, calcium, the modified Charlson Comorbidity index, and graft vintage, as well as serum phosphorus, PTH, and FGF23. These investigators reported that a 0.9 mg/dL (*i.e.*, a 1 SD) increase in phosphorus predicted the composite endpoint of death or graft failure when analyzed as a continuous variable per SD increase (0.9 mg/dL), HR 1.23 (CI: 1.08-1.40, $P = 0.002$), in the fully adjusted modeling, which included FGF23^[30].

CONCLUSION

NAM shows promise^[11-14] as an agent for the chemoprevention of the especially aggressive^[2-5,7] NMSCs which occur in KTRs. This beneficial phenomenon may be mediated *via* its inhibition of sirtuin enzymatic pathways^[16-19]. Phase 3 trials to test this specific hypothesis merit serious consideration. NAM treatment also confers a consistent reduction in serum phosphorus concentrations^[21,35,36], with the accompanying potential to correct disturbed calcium-phosphorus homeostasis—for example, lower FGF23 concentrations, as with NA^[26], and possibly improve calcification propensity^[40]. These combined ameliorative effects of NAM treatment afford unique trial design opportunities. Conceivably, a phase 3 trial of NAM for NMSC prevention in KTRs, lasting at least 12-mo^[12], could also incorporate imaging and laboratory measures which assess NAM's impact on subclinical CVD and CKD risk, and progression, a strategy being employed by the 12-mo COMBINE trial^[36] in stage 3b-4 CKD patients.

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Overview of the progress on haploidentical hematopoietic transplantation

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Abstract

Allogeneic hematopoietic stem cell transplant (HSCT) remains the only potentially curative option for variety of hematologic disorders. Lack of a suitable fully HLA-matched donor limits this option for many patients. Without a suitable related or unrelated HLA-matched donor,

umbilical cord blood and haploidentical family members provide a potential source of stem cells. Timely donor availability makes haploidentical donors an attractive alternative donor source. Initial attempts at haploidentical HSCT was associated with significantly increased mortality owing to high rates of graft rejection and severe graft-versus-host disease caused by major donor-recipient HLA-disparity. However, over the past decade, outcomes of haploidentical HSCT have improved significantly. Here, we review the advantages and challenges of haploidentical transplantation. We also discuss new developments to attempt to overcome the challenges to a successful haploidentical transplantation.

Key words: Haploidentical donor; Hematopoietic stem cell transplantation; Hematological malignancies; Transplant related mortality

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Core tip: Over the past decade, haploidentical donors have emerged as a viable alternate graft source for patients without a HLA-matched donor. Several strategies including graft manipulation, conditioning regimen optimization and better graft-versus-host disease control have significantly improved the outcomes of haploidentical hematopoietic stem cell transplant (HSCT). Here, we summarize some of the recent advances in the field of haploidentical HSCT in adults.

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INTRODUCTION

Hematopoietic stem-cell transplantation (HSCT) is

considered to be the only potentially curative therapy for several hematologic diseases. Most institutions currently consider a HLA-matched sibling as a preferred donor source, typically followed by either HLA-matched unrelated or an alternative graft source depending on the clinical scenario. The likelihood of having an HLA-matched sibling donor is approximately thirty percent after consideration of factors such as donor consent and health status. The probability of finding a suitable matched unrelated donor is strongly influenced by patient's ethnicity and can range from more than 75% for Caucasians to less than 20% for certain ethnic groups such as African Americans^[1]. In absence of related or unrelated HLA-matched donor, umbilical cord blood and haploidentical family members provide a potential source of graft. The use of haploidentical hematopoietic stem cell transplantation as an alternative graft source has been substantially increasing.

Utility of haploidentical related donors has a number of advantages including immediate donor availability for many patients facilitating a shorter interval to transplant. In addition, having a related donor makes post-transplant donor-derived cellular therapy more easily accessible. Challenges include major donor-recipient HLA-disparity which can cause delayed immune reconstitution, graft failure and severe graft vs host disease (GVHD) due to T-cell alloreactivity^[2,3]. This review highlights the major advances over the past decade to overcome the obstacles to successful haploidentical transplantation.

DONOR SELECTION

In contrast to unrelated donor transplant HSCT where finding the best HLA matched donor is the most important factor in determining transplant outcome, increasing HLA disparity in haploidentical matching does not have the same detrimental impact with dedicated techniques such as modification of post-transplant T cell reconstitution with cyclophosphamide. In 2010, Kasamon *et al*^[4] evaluated the impact of donor and recipient HLA in 185 patients who underwent un-manipulated bone marrow haploidentical transplant. Post-transplant cyclophosphamide was used as GVHD prophylaxis. In this study, the number of HLA-mismatches did not influence the rate of acute GVHD or disease free survival.

Donor characteristics that influence the outcome of haploidentical transplant were also investigated in a large study by Wang *et al*^[5] involving 1210 patients with hematologic diseases. Grafts consisted of G-CSF mobilized T-cell replete bone marrow and peripheral stem cells. Similar to the prior studies, the degree of HLA disparity did not influence the incidence of acute GVHD and treatment related mortality (TRM). Younger donor age (< 30 years) was associated with a lower incidence of acute GVHD compared to older donor age (> 30 years). Younger donor age and male gender were also associated with less TRM and better overall survival (OS). The benefit of male recipient gender was lost when maternal donors were excluded. There was a higher risk of grade II-IV acute

GVHD with maternal donors compared to paternal donors. In a male recipient, a maternal donor also correlated with a higher TRM rate and decreased OS. The impact of non-inherited maternal antigen (NIMA) disparities was evaluated in 264 patients. NIMA mismatched donors conferred a lower incidence of acute GVHD compared to non-inherited paternal antigen (NIPA) mismatched donors. Based on these results, authors concluded younger, male, NIMA-mismatched donor is a preferred donor in setting of T-cell replete haploidentical transplant. This study did not evaluate the influence of natural killer (NK) cell alloreactivity and donor CMV status. In contrast to Wang *et al*^[5], several trials demonstrated decreased risk of relapse and survival advantage with using maternal donors^[6]. A more potent anti-leukemic effect of maternal donor grafts has been attributed to the maternal immune system exposure to fetal antigens during pregnancy^[7].

Another factor influencing haploidentical transplant outcome is donor vs recipient NK cell alloreactivity. Tumor cells are able to escape T-cell adoptive immune response by down regulating cell surface MHC class I. NK cells are an important component of innate immunity and have MHC-unrestricted ability to target malignant cells. Cytotoxic activity of NK cells are mainly under the negative feedback control from inhibitory killer immunoglobulin-like receptors (KIRs) through binding to self HLA class I antigen. This phenomenon is known as "missing self"^[8-10]. KIR-KIR ligand mismatched in the donor-recipient direction lead to loss of the inhibitory feedback and activation of donor NK cells targeting recipient hematopoietic cells and leukemic cells. In contrast to allo-reactive T-lymphocytes, NK cells are thought to be capable of inducing graft vs leukemia (GVL) effect without promoting GVHD. In 2002, a study by the Perugia group demonstrated therapeutic efficacy of allo-reactive NK cells in 57 patients with acute myeloid leukemia (AML) following haploidentical transplant^[11]. Twenty out of 57 patients had KIR-ligand incompatibility in the graft vs host direction. The probability of OS at 5 years was markedly improved in patients with AML who had NK allo-reactive donors (60% vs 5%, $P = 0.0005$). Similar results were observed in the updated analysis of 112 patients with high risk AML who received T-cell depleted haploidentical transplants^[12]. Fifty one of 112 patients had NK cell allo-reactive donors. The conditioning regimen included TBI (8 Gy), fludarabine (40 mg/m² per day for 4 d), thiopeta (5 mg/kg per day for 2 d) and rabbit ATG. A significantly lower relapse rate (3% vs 47%, $P < 0.003$) and better EFS (67% vs 18%, $P = 0.02$) was observed in patients transplanted in any CR with NK allo-reactive donors compared to recipients of non-allo-reactive grafts. Although transplantation from NK allo-reactive donors improved survival in the entire cohort, subset analysis suggested that transplantation from NK allo-reactive donors did not decrease the incidence of relapse in patients transplanted at chemo-resistant relapse. There was no significant difference in incidence of acute GVHD between the two cohorts (10% vs 11%). These findings reinforced the theory that GVL activity by allo-reactive NK cells translated into prolonged OS. Subsequently, several

studies revealed a favorable impact of allo-reactive NK cells on transplant outcome in patients undergoing HLA-haploidentical transplant^[11,13-15]. An important role of donor-recipient KIR mismatch was also demonstrated after non-myeloablative T cell-replete haploidentical transplantation using post-transplant cyclophosphamide in a retrospective study involving 86 patients with high risk hematologic malignancies^[16]. On the contrary, a deleterious effect of KIR mismatches was seen in the earlier studies^[17,18]. Due to ongoing controversy, currently the KIR testing is not considered mandatory for donor selection in haploidentical transplant setting.

HAPLOIDENTICAL STEM CELL TRANSPLANT STRATEGIES

T-cell depletion

The first successful haploidentical transplants were done in the 1980s in children with severe combined immunodeficiency syndrome (SCIDS) using T-cell depleted bone marrow grafts. T-lymphocyte depletion in this setting mitigated GVHD associated with crossing a major HLA-barrier without compromising engraftment^[19]. Subsequently, this approach was implemented successfully in several studies of patients with SCIDS. In contrast to SCIDS, haploidentical transplantation was less successful in the setting of acute leukemia owing to a high rate of graft failure. Increased risk of graft failure was attributed to host derived T-lymphocytes that survived the conditioning regimen^[20-22]. A decade later, it was shown in preclinical studies (murine models) that infusion of a large number of donor hematopoietic stem cells can overcome the MHC barrier and promote engraftment^[23]. In 1993, cell dose escalation approach was tested in 36 patients with acute leukemia following myeloablative total body irradiation (TBI) based preparative regimen. Mega doses of stem cells (on average $> 10 \times 10^6$ CD34⁺ cells/kg body weight) were obtained by supplementing T cell-depleted bone marrow transplants with granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells. Using this approach, nearly 80% of patients achieved primary engraftment. The sole GVHD prophylaxis consisted of T-cell depletion of the graft. Only 18% of the patients developed grade II-IV acute GVHD^[24,25]. Subsequently, several modifications were introduced to optimize the T-cell depletion of the graft including positive immunoselection of the CD34⁺ cells using the Cephate system in 1995 and Clinimacs device in 1999^[13,26]. In addition, to reduce the toxicity associated with the myeloablative TBI based conditioning regimen, fludarabine was substituted for cyclophosphamide in 1995^[27]. After optimizing the conditioning regimen and graft processing, Aversa *et al.*^[28] investigated haploidentical transplantation in 284 patients with acute leukemia. Ninety five percent of the patients achieved engraftment with minimal GVHD. The relapse rate was 17% in acute myeloid leukemia (AML) and 27% in acute lymphoblastic leukemia (ALL) patients transplanted in any CR. Incidence of TRM was 40% mainly

due to opportunistic infections. Seventeen year DFS was 30% in ALL and 43% in AML patients transplanted in any CR. Among the long term survivors, chronic GVHD was not observed in any patients^[28].

The major disadvantages of using T-cell depleted grafts are the high rate of relapse and life-threatening infections post-transplant^[29]. Due to poor thymic function in adults, post- allogeneic transplant T-cell immune recovery depends on peripheral expansion of donor T-lymphocytes. In a T-cell depleted graft, passive transfer of T-lymphocytes is minimal leading to profound delay in immune recovery. To overcome these obstacles several strategies have evolved over the past decade including selective T-cell depletion, adoptive transfer of donor T-cells post-transplant, and T-regulatory cell (T-reg) add backs.

Selective T cell depletion

The principle behind adoptive T-cell therapy is to eliminate donor allo-reactive T cells responsible for GVHD while sparing other immune cells, which facilitate immune reconstitution. To selectively deplete allo-reactive donor T-cells, *ex vivo* T-cells are activated against host antigen presenting cells. Activated T-cells are removed using several methods including immunotoxin, immune-magnetic selection and photodynamic purging^[30-32].

Another innovative approach is to selectively remove T-cells responsible for GVHD (TCR alpha-beta) while sparing gamma-delta T-cells ($\gamma\delta$ T-cells). Gamma-delta T-cells account for 1% to 10% of peripheral T-cells. Based on *in-vitro* studies, human T lymphocytes which express $\gamma\delta$ T-cells receptor have MHC-unrestricted innate cytotoxic activity against tumor cells^[33,34]. In a recent study, Lang *et al.*^[35] retrospectively evaluated the immune recovery after TCR $\alpha\beta$ /CD19-depleted haploidentical HSCT in 41 pediatric patients with acute leukemia, myelodysplasia and nonmalignant disease. Primary engraftment was seen in 88% of the patients. The incidence of grade II and grade III-IV acute GVHD was 10% and 15% respectively. At one year follow up, the event free survival (EFS) of patients with acute leukemia or myelodysplasia transplanted in CR1-CR3 was 100%. One year EFS of patients with subsequent HSCT (CR2-CR6) or with active disease was 29% and 11%, respectively. The use of TCR $\alpha\beta$ /CD19-depleted stem cells substantially accelerated immune recovery. In comparison to CD34⁺ selected grafts (historic control), patients achieved a higher CD3⁺ at days +30 and +90, CD34⁺ at day +30 and CD56⁺ at day +14. The Italian group also reported similar results in 16 adults with high risk acute leukemia after TCR $\alpha\beta$ /CD19-depleted haploidentical HSCT.

A more recent strategy to separate GVHD and the GVL effect involves selectively depleting naïve T cells identified by CD45RA⁺ expression^[36,37]. Naïve T-cells are shown to be the most allo-reactive amongst the T-cell subsets. *Ex vivo* depletion of CD45RA⁺ T-cells and adoptive transfer of CD45RA-memory T cells hasten the immune reconstitution post-transplant, enhances the GVL effect while abrogating GVHD. This strategy was recently evaluated in a study of

17 adults with high risk hematologic malignancies (16 AML and 1 myelodysplasia) with KIR receptor-ligand mismatched haploidentical donor^[38]. The conditioning regimen included total lymphoid irradiation (8 Gy), fludarabine (150 mg/m²), cyclophosphamide (60 mg/kg), thiopeta (10 mg/kg) and melphalan (140 mg/m²). Patients received a CD34⁺ selected stem cell graft on day 0 followed by an infusion of CD45RA-depleted stem cells on day +1. NK cell infusion was given on day +6. Post-transplant GVHD prophylaxis included sirolimus and mycophenolate mofetil (MMF). All patients achieved primary engraftment. Neutrophil and platelet engraftment was rapidly achieved at median day +11 and +17 respectively. Acute GVHD was not seen in any of the patients. There was no infection related mortality. A phase II study of selective depletion of CD45RA⁺ T Cells from allogeneic peripheral blood stem cell grafts from HLA-matched related and unrelated donors for prevention of GVHD is currently under investigation^[39].

SELECTIVE T-CELL ADD BACK

Con-infusion of donor-derived regulatory T-cells (Tregs) with conventional T-cells (Tcons) is another method to manipulate the T-cell depleted graft to improve haploidentical transplant outcome. In pre-clinical studies of bone marrow transplantation, infusion of donor-type CD4⁺CD25⁺Tregs abrogated GVHD without compromising the cytotoxic ability of T-cons against tumor cells^[40,41]. A first in human study by Di Ianni *et al.*^[42] investigated infusion of Tregs, followed by Tcons in 28 patients with high risk hematologic malignancies who underwent haploidentical transplantation. After TBI containing conditioning regimens, patients received infusion of donor derived T-cons (2 × 10⁶ Tregs) on day-4. CD34⁺ stem cells were infused on day 0 followed by Tcons. Two out of five patients who received 2 × 10⁶ Tcons/kg developed acute GVHD which led to decreasing the cell dose of Tcons to 1 × 10⁶ cells/kg. Chronic GVHD was not observed in any patients. All patients achieved primary engraftment. Compared to conventional mismatched HSCT, pathogen specific CD4⁺ and CD8⁺ were detected earlier in the study cohort (as early as 2 mo vs 9-12 mo). CMV-related death, a major cause of mortality in original T-cell depleted HSCT, was not observed. At median 1 year follow up, 46% of the patients were disease free. Subsequently, Martelli *et al.*^[43] evaluated the impact of Tregs - Tcons infusion in reducing post-transplant relapse risk in 43 adults with acute leukemia. This method significantly reduced the risk of relapse and ameliorated GVHD. Grade 2 or more acute GVHD was seen in 15% of patients. At median follow up of 46 mo, only two patients relapsed resulting in an incidence of relapse that was significantly lower than historical controls. Despite promising results of T-cell depleted haploidentical transplant, this approach is costly, technically demanding and labor intensive which limits its application to highly experienced centers.

T CELL REPLETE GRAFT

Earlier attempts at using un-manipulated haploidentical

transplant were associated with an unacceptably high rate of GVHD related mortality due to donor T-cell alloreactivity. To overcome this obstacle, several strategies have evolved over the past decade including G-CSF primed graft^[44,45] and more recently post-transplant high dose cyclophosphamide.

HIGH-DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE

Cyclophosphamide is an alkylating chemotherapeutic agent which has been used for many years as a component of conditioning regimens. Preclinical trials in the early 1970s revealed short course of cyclophosphamide after bone marrow transplantation can target allo-reactive T-cells and reduce the risk of GVHD^[46-48]. In contrast to calcineurin inhibitors, cyclophosphamide is capable of inducing T-lymphocyte apoptosis^[49]. Hematopoietic stem cells are resistant to high dose cyclophosphamide due to expression of high levels of aldehyde dehydrogenase^[50]. Original clinical trials exploring cyclophosphamide efficacy as the post-transplant GVHD prophylactic agent were performed in the haploidentical transplant setting. In 2002, O'Donnell *et al.*^[51] evaluated the transplant outcome of 13 patients with high risk hematologic malignancies who received T-cell replete haploidentical transplant after a non-myeloablative conditioning regimen with TBI and fludarabine. GVHD prophylaxis included post-transplant cyclophosphamide 50 mg/kg on day +3 in combination with MMF and tacrolimus. Due to high rate of graft failure (2 out of 3 patients) the protocol was amended to add cyclophosphamide 14.5 mg/kg to the conditioning regimen. Subsequently, 8 of 10 patients obtained primary donor cell engraftment. After 99 d follow up, 6 patients (46%) developed acute GVHD. Six months incidence of DFS was 50%. This study demonstrated the feasibility and possibility of rapid engraftment in a non-myeloablative haploidentical transplant setting using post-transplant cyclophosphamide.

Subsequently, Luznik *et al.*^[52] compared safety and efficacy of administration of cyclophosphamide on day +3 and +4 rather than only on day +3 among 68 patients with hematologic malignancies after non-myeloablative haploidentical bone marrow transplant. Primary engraftment was achieved in 87% of the patients. Notably, a very low incidence of grade III acute GVHD (6%) with no grade IV acute GVHD was observed at one year follow up. The only difference between the two cohorts was a trend toward a lower incidence of chronic GVHD after two doses of post-transplant cyclophosphamide (5% vs 25%, *P* = 0.05). The 2-year OS and EFS rates were 36% and 26%, respectively. A major contributor to the low OS rate was a high incidence of relapse (58% at 2 years).

A similar outcome was observed in a large phase II study of high dose post-transplantation cyclophosphamide as GVHD prophylaxis after non-myeloablative HLA-haploidentical bone marrow transplantation in 210 patients with hematologic malignancies^[53]. Sustained donor cell

engraftment was obtained in 87% of the patients. The cumulative incidences of grades II-IV acute GVHD was 27%. At 5 year follow up, OS and EFS were 35% and 27%, respectively. As seen in the prior studies, relapse was a major cause of mortality. Five year cumulative incidence of relapse was 55%.

In parallel multicenter phase 2 trials, BMT CTN 0603 and BMT CTN 0604, patients with acute leukemia or lymphoma underwent reduced intensity bone marrow haploidentical transplantation (0603) or double cord blood transplant (0604)^[54]. The conditioning regimens contained 200 Gy TBI in addition to fludarabine and cyclophosphamide. In CTN 0603, the GVHD prophylaxis consisted of post-haploidentical transplant cyclophosphamide 50 mg/kg on day +3 and +4 followed by tacrolimus and MMF. In CTN 0604, GVHD prophylaxis included MMF and cyclosporine after double umbilical cord transplant. Among haploidentical transplant recipients, 100-d incidence of grade II-IV acute GVHD and 1-year incidence of chronic GVHD were 32% and 13%, respectively. After double cord transplant 100-d incidence of grade II-IV acute GVHD and 1-year incidence of chronic GVHD were 40% and 24%, respectively. One year cumulative incidence of relapse after haploidentical and double umbilical cord transplant were 45% and 31%, respectively. The OS and EFS rates were 62% and 48% respectively after the haploidentical transplants. Similar OS (54%) and EFS (46%) were seen after double cord transplant. The authors concluded that both RIC haploidentical and double umbilical cord HSCT are valid options in patients with hematologic malignancy. Currently a multicenter randomized phase III trial (BMT CTN 1101) is investigating the effectiveness of haploidentical and double umbilical transplant in patients with leukemia or lymphoma^[55].

Despite relatively low rates of GVHD with non-myeloablative haploidentical transplant, a high incidence of relapse has remained the main challenge in high risk hematologic malignancies. To address this obstacle, use of more intense (myeloablative) preparative regimens and peripheral blood stem cell graft was explored. In a prospective study by Solomon *et al.*^[56], 20 adults with high risk (relapsed/refractory) hematologic malignancies were treated with myeloablative conditioning followed by peripheral blood derived haploidentical transplant. The conditioning regimen consisted of fludarabine 30 mg/m² for 4 d, intravenous busulfan 130 mg/m² per day for 4 d, and Cy 14.5 mg/kg per day for 2 d. GVHD prophylaxis included high dose cyclophosphamide on day +3 and +4 followed by tacrolimus and MMF. All patients achieved primary engraftment. One year cumulative incidence of grade II-IV acute GVHD and chronic GVHD were 10% and 5%, respectively. At median follow up of 20 mo, DFS and OS were 69% and 50%, respectively. The cumulative incidence of relapse was approximately 40%. The major drawback of this trial was high incidence of hemorrhagic cystitis due to BK virus infection. This adverse event was observed in two third of the patients. This was attributed to the combination of high dose busulfan and cyclophosphamide. Association

of BK induced hemorrhagic cystitis and high dose busulfan in setting of mismatched HSCT was reported previously in several studies^[57]. To alleviate this problem, the conditioning regimen was changed to TBI-based myeloablative regimen in the subsequent study^[58]. In this phase II prospective trial, 30 patients underwent peripheral stem cell haploidentical transplant using fludarabine 25 mg/m² per day for three days and 1200 cGy TBI as the preparative regimen. All patients achieved primary engraftment. Median time to neutrophil and platelet engraftment was 16 d and 25 d, respectively. Incidence of grade II-IV acute GVHD was 23%, whereas moderate to severe chronic GVHD occurred in 22% of patients. In the entire cohort, 2-year NRM and OS were 3% and 78%, respectively. Among patients with low or intermediate risk disease NRM and OS were 0% and 100%, respectively. Relapse rate was significantly reduced in comparison to patients treated at the same center with matched related transplant. Incidence of post-transplant BK virus associated hemorrhagic cystitis was significantly reduced after TBI-based regimen compared to the busulfan-based conditioning regimen (30% vs 75%, $P = 0.005$).

Similar results were observed in several other trials of myeloablative haploidentical transplant^[59,60]. Raiola *et al.*^[59] confirmed the low rate of GVHD and encouraging rate of DFS and OS in 50 patients with high risk hematologic disease (23 patients in CR and 27 patients with active disease) after un-manipulated myeloablative haploidentical transplant^[59]. GVHD prophylaxis contained post-transplant cyclophosphamide on day +3 and +5 followed by cyclosporine and MMF. In the entire cohort, 12% of the patients developed grade II-III acute GVHD. Moderate chronic GVHD was seen in 10% of patients. The actuarial 22-mo DFS for patients transplanted in CR and patients with active disease was 68% and 37%, respectively^[61]. The overall risk of relapse after myeloablative haploidentical HSCT was approximately 40% which compares favorably with that reported for non-myeloablative haploidentical HSCT. Therefore, despite the lack of randomized trials, myeloablative haploidentical transplant may be a reasonable option in younger patients with high risk hematologic malignancy in absence of timely access to a conventional donor.

Haploidentical related donor vs matched related sibling or matched unrelated donor (Table 1)

Encouraging results of haploidentical transplant compared to matched related or matched unrelated transplant has been suggested by several non-randomized studies. In 2015, a large retrospective study compared the transplant outcome of 868 patients with acute leukemia after haploidentical transplant and 9815 patients with HLA-matched sibling donor (MRD)^[62]. However, leukemia free survival was significantly longer after matched sibling donor transplant compared to haploidentical transplant (T-cell depleted or T-cell replete grafts). Haploidentical transplant was associated with higher TRM. The probability of relapse was not significantly different between the two

Table 1 Unmanipulated haploidentical hematopoietic stem cell transplant *vs* matched related and matched unrelated hematopoietic stem cell transplant

Ref.	Disease	Conditioning regimen (n)	Graft type (n)	GVHD prophylaxis	Neutrophil engraftment	Grade II-IV acute GVHD	Chronic GVHD	Relapse rate	DFS	OS
Bashey <i>et al</i> ^[69] 2013 n = 271	Acute leukemia/ CML/ myeloma/ lymphoma/ MDS	RIC (102) MA (169)	MRD (117) MUD (101) Haplo (53)	CNI based CNI based CNI + MMF + PT-Cy	NR	6 mo 27% 39% 30% (P = NS)	2 yr 54% 54% 38% (P < 0.05)	2 yr 34% 34% 33% (P = NS)	2 yr 53% 52% 60% (P = NS)	2 yr 76% 67% 64% (P = NS)
Di Stasi <i>et al</i> ^[70] 2014 n = 227	AML/MDS	RIC (227)	MRD (81) MUD (108) Haplo (32)	CNI + MTX CNI + MTX + ATG CNI + MMF + PT-Cy	30 d 99% 96% 97% (P = 0.44)	100 d 24% 19% 26% (P = 0.68)	3 yr 46% 42% 24% (P = 0.52)	1 yr 28% 23% 33% (P = 0.75)	3 yr 36% 27% 30% (P = 0.12)	NR
Luo <i>et al</i> ^[71] 2014 n = 305	Acute leukemia/ lymphoma/ MDS	MA + ATG (305)	MRD (90) MUD (116) Haplo (99)	CNI + MMF + MTX CNI + MMF + MTX CNI + MMF + MTX	15 d 97% 97% 78% (P < 0.001)	3 mo 15.60% 39% 42% (P < 0.0001)	2 yr 24% 41% 41% (P = NS)	5 yr 34% 21% 14% Haplo <i>vs</i> MRD P = 0.008 Haplo <i>vs</i> MUD P = 0.17	5 yr 63% 58% 58% (P = 0.57)	5 yr 77% 63% 60.80% Haplo <i>vs</i> MRD P = 0.026 Haplo <i>vs</i> MUD P = 0.38
Ciurea <i>et al</i> ^[63] 2015 n = 2174	AML	RIC (825) MA (1349)	MUD (737) Haplo (88) MUD (1245) Haplo (104)	CNI + MMF or MTX CNI + MMF + PT-Cy CNI + MMF or MTX CNI + MMF + PT-Cy	30 d 93% 96% (P = 0.25) 90% 96% (P = 0.02)	3 mo 19% 28% (P = 0.05) 16% 33% (P = 0.001)	3 yr 34% 52% (P = 0.002) 30% 53% (P < 0.0001)	3 yr 58% 42% (P = 0.006) 44% 39% (P = 0.37) (P = 0.14)	3 yr 9% 23% (P = 0.0001) 14% 20% (P = 0.14)	3 yr 46% 44% (P = 0.71) 45% 50% (P = 0.38)
Wang <i>et al</i> ^[64] 2015 n = 450	AML in CR1	MA (ATG in haplo cohort)	MRD (219) Haplo (231)	CNI + MMF + MTX CNI + MMF + MTX	NE engraftment 2 d longer after MRD P = 0.004	100 d 36% 13% (P < 0.001)	1 yr 42% 15% (P < 0.001)	3 yr 15% 15% (P = 0.98)	3 yr 74% 78% (P = 0.34)	3 yr 79% 82% (P = 0.36)
Ghosh <i>et al</i> ^[67] 2016 n = 987	Lymphoma	RIC (987)	MRD (807) Haplo (180)	CNI based PT-Cy ± CNI	28 d 95% 97% (P = 0.31)	100 d 25% 27% -0.84 (P < 0.001)	1 yr 45% 12% (P < 0.001)	3 yr 37% 40% (P = 0.51)	3 yr 48% 48% (P = 0.98)	3 yr 62% 61% (P = 0.82)
Kanate <i>et al</i> ^[72] 2016 n = 917	Lymphoma	RIC (917)	MUD + ATG (241) MUD (491) Haplo (185)	CNI based CNI based PT-Cy based	28 d 97% 97% 94% (P = 0.32)	100 d 17% 12% 8% (P = 0.44)	1 yr 33% 51% 13% (P < 0.001)	3 yr 36% 28% 36% (P = 0.07)	3 yr 38% 49% 47% (P = 0.02)	3 yr 50% 62% 60% (P = 0.2)

AML: Acute myeloid leukemia; ATG: Anti-thymocyte globulin; CR: Complete remission; CNI: Calcineurin inhibitor; DFS: Disease free survival; GVHD: Graft *vs* host disease; Haplo: Haploidentical; MMF: Mycophenolate mofetil; MTX: Methotrexate; RIC: Reduced intensity conditioning; MA: Myeloablative; MDS: Myelodysplasia; MUD: Matched unrelated donor; MRD: Matched related donor; NE: Neutrophil; NR: Not reported; NS: Not significant; OS: Overall survival.

cohorts. Therefore, the authors concluded haploidentical GVL effect is similar to MRD.

Ciurea *et al*^[63] also retrospectively compared the transplant outcome of patients with AML after haploidentical transplant ($n = 192$) using post-transplant cyclophosphamide and MUD ($n = 1982$). In the haploidentical cohort, 104 patients received MA and 88 had reduced intensity conditioning. In MUD cohort, 1245 patients (63%) received MA and 737 (37%) received RIC regimens. Compared to MUD, thirty day neutrophil engraftment was lower after haploidentical transplant in

MA setting (97% *vs* 90%, $P = 0.02$). In RIC setting, day 30 neutrophil engraftment rate was similar between the two cohorts (96% and 93%, $P = 0.25$). Acute and chronic GVHD was notably lower after haploidentical transplant. In the MA setting, three month incidence of acute GVHD (16% *vs* 33%, $P < 0.0001$) and 3-year incidence of chronic GVHD (30% *vs* 53%, $P < 0.0001$) were significantly lower with haploidentical in comparison to MUD transplant. Similar results were obtained in RIC setting. A lower rate of GVHD with haploidentical transplant was attributed to the use of bone marrow as a graft source and the use

of post-transplant cyclophosphamide. Among patients receiving myeloablative and RIC regimens, three-year DFS and OS were comparable in haploidentical and MUD transplant.

Transplant results of matched sibling donor (MSD) transplant and T-cell replete haploidentical transplant was also evaluated by Wang *et al.*^[64]. In this prospective, multicenter, nonrandomized trial, 450 patients with acute leukemia in CR1 underwent MSD ($n = 219$) or haploidentical ($n = 231$) transplant. Cyclosporine, MMF, and low dose methotrexate was used as GVHD prophylaxis regimen in both groups. All individuals in both cohorts achieved donor-cell engraftment. The median time to achieve neutrophil engraftment was 2 d longer after MSD transplant. The 100-d cumulative incidence of grade II-IV acute GVHD after haploidentical and MSD transplant was 36% and 13% ($P = 0.001$), respectively. The incidence of chronic GVHD was significantly higher after haploidentical transplant compared to MSD (42% vs 15%, $P < 0.001$). However, the rate of GVHD related death was similar in both groups. Among haploidentical and MSD recipients, the 3 year probability of DFS (74% vs 78%, $P = 0.34$) and OS (79% vs 82%, $P = 0.36$) were comparable. There was no difference in 3-year cumulative incidence of relapse between the two cohorts (15% vs 15%, $P = 0.98$). Lower incidence of GVHD after MSD was attributed to combination of cyclosporine, methotrexate and MMF for GVHD prophylaxis. Prior studies also reported significantly lower rate of GVHD using this combination in recipients of MSD transplant^[65,66].

More recently Ghosh *et al.*^[67] performed a registry analysis comparing outcomes of 987 patients with lymphoma following reduced intensity haploidentical HSCT ($n = 180$) with MSD HSCT ($n = 807$). GVHD prophylaxis for the haploidentical group consisted of post-transplant cyclophosphamide with or without calcineurin inhibitor and MMF. GVHD prophylaxis for the MSD group contained calcineurin inhibitor based approaches. The cumulative incidence of grade II-IV acute GVHD was similar between the two cohorts (27% in haploidentical cohort vs 25% in MSD cohort, $P = 0.84$). Cumulative incidence of chronic GVHD was significantly lower with haploidentical HSCT (12% vs 45%, $P < 0.001$). Chronic GVHD was the main cause of death in 5 patients in MSD cohort. There was no significant difference in the three-year cumulative incidence of relapse (37% in haploidentical vs 40% in MSD, $P = 0.51$), DFS (48% vs 48%, $P = 0.96$) and OS (61% vs 62%, $P = 0.82$). Therefore, based on this retrospective registry study in patients with lymphoma, RIC haploidentical HSCT using post-transplant cyclophosphamide provides comparable survival outcome to MSD HSCT with significantly lower risk of chronic GVHD.

CONCLUSION

HSCT is the only curative option for a large number of hematologic diseases. A minority of patients (30%) have a suitable HLA-identical sibling donor. For patients who lack MSD, MUD HSCT is frequently the preferred graft source.

However, the presence of a suitably matched unrelated donor depends on factors such as the ethnicity of the patient, with a likelihood of finding an acceptably matched unrelated donor less than 20% in certain minorities compared to approximately 80% in Caucasians. A major disadvantage of MUD transplant is the prolonged time from patient referral to donor identification and collection of stem cells. Delay in the process of unrelated donor search due to logistical issues may increase the risk of disease progression or relapse^[68]. Immediate availability of a haploidentical donor makes this approach an attractive treatment option for patients who lack an HLA-identical MSD or those for whom a MUD cannot be found in a timely manner. The field of haploidentical HSCT has matured significantly over the past two decades. In earlier studies of haploidentical HSCT, HLA-incompatibility barrier resulted in unacceptably high rate of GVHD and graft rejection leading to inferior OS. While effective T-cell depletion followed by infusion of mega doses of highly purified stem cells permitted high engraftment rates and reduced incidence of GVHD, higher risk of relapse and delay in immune reconstitution remained a significant obstacle. Newer methods of graft manipulation including adoptive T-cell immunotherapy and selective T-cell depletion have been shown to hasten immune recovery and reduce the risk of relapse. Despite the promising results, these approaches are costly and labor intensive, hence may not be globally available. In recent years, use of post-transplantation cyclophosphamide for GVHD prophylaxis after T-cell replete haploidentical HSCT has yielded encouraging results in adults. In several non-randomized studies, survival outcomes following haploidentical HSCT with post-transplant cyclophosphamide have been comparable to MSD or MUD transplant. Ultimately, a prospective randomized controlled trial such as BMT CTN 1101 is needed to determine the optimal approach to haploidentical transplant.

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Review of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in solid tumors excluding breast cancer

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Abstract

Solid tumors in adults constitute a heterogeneous group of malignancy originating from various organ systems. Solid tumors are not completely curable by chemotherapy, even though some subgroups are very chemo-sensitive. Recently, oncologists have focused on the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity conditioning (RIC) for the treatment of some refractory solid tumors. After the demonstration of allogeneic graft-versus-leukemia effect in patients with hematological malignancies who received allo-HSCT, investigators evaluated this effect in patients with refractory metastatic solid tumors. According to data from experimental animal models and preliminary clinical trials, a graft-versus-tumor (GvT) effect may also be observed in the treatment of some solid tumors (*e.g.*, renal cell cancer, colorectal cancer, *etc.*) after allo-HSCT with RIC. The use of RIC regimens offers an opportunity of achieving full-donor engraftment with GvT effect, as well as, a reduced transplant-related mortality. Current literature suggests that allo-HSCT with RIC might become a choice for elderly and medically fragile patients with refractory metastatic solid tumors.

Key words: Renal cell carcinoma; Allogeneic hematopoietic stem cell transplantation; Colorectal cancer; Ovarian cancer; Sarcoma

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Core tip: Some refractory metastatic solid tumors including renal, ovarian and even colon cancers may respond well to allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity

conditioning (RIC). Their lower toxicity profiles and lower non-relapse mortality rates constitute the advantages of RIC. The use of allo-HSCT with RIC or non-myeloablative regimens can be a feasible option among fragile patients, such as geriatric patients and patients with comorbidities. Future studies are needed for a clear-cut understanding of the mechanisms of graft-versus-leukemia and graft-versus-tumor effects of donor T-cells and their subsets in order to optimize the efficacy of such treatment modalities in patients with refractory solid tumors.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is primarily used in patients with relapsed or high-risk hematologic malignancies and the efficacy of this treatment has been substantially demonstrated. The first allo-HSCT in the literature in a patient with a solid tumor was published in late 90s^[1]. The principles of allo-HSCT consist of maximal tumor cytoreduction with high-dose chemoradiotherapy and adequate immunosuppression in order to provide engraftment of donor stem cells, as well as graft-versus-tumor (GvT) effect^[2]. Studies investigating high dose chemotherapy with autologous stem cell rescue in patients with solid tumors yielded controversial and disappointing results^[3-7]. This has led to the development of novel approaches, including allo-HSCT with reduced intensity conditioning (RIC) regimens, which aim to create and take advantage of a GvT effect in order to induce more durable responses^[1,2,8-10]. Today, types of conditioning regimens that are used prior to allo-HSCT include myeloablative (MA), RIC and non-myeloablative (NMA) regimens. MA regimens lead to irreversible cytopenia and therefore, stem cell support is needed. In contrast, NMA regimens cause minimal cytopenia and can be given without stem cell support. RIC regimens do not completely fit in the criteria for MA and NMA regimens. The marrow aplasia is reversible; however, stem cell support is mandatory.

NMA/RIC regimens for allo-HSCT have introduced a new era for treating elderly and those with comorbidities^[11-13]. The RIC regimens are currently being used for as much as 40% of all allo-HSCTs and becoming increasingly popular. The growing knowledge on the immune system and T-cell biology has made allo-HSCT a promising approach for the treatment of some solid tumors. Several phase I and II studies, which were conducted by the European Society for Blood and Marrow

Transplantation Solid Tumors Working Party (EMBT-STWP) documented the presence of a GvT effect in patients with various solid tumors, such as renal, ovarian and colon cancers and soft tissue sarcomas^[2].

This novel strategy provides a switch from a chemotherapy-based to an immunotherapy-based approach^[14]. Replacing conventional MA regimens with NMA/RIC regimens prior to allo-HSCT has two main goals: (1) to diminish the high transplant-related morbidity and mortality^[15-19]; and (2) to induce allo-reactivity against the metastatic solid tumor via a GvT effect^[1,12].

The successful engraftment rates together with a lower transplant related mortality (TRM) and the presence of GvT effect made allo-HSCT with RIC an attractive option for the treatment of several solid tumors within the last decade^[20-24]. The lower toxicity obtained by the reduction of chemoradiotherapy dose also enables allo-HSCT with RIC to become a choice for the elderly and medically fragile patients with metastatic solid tumors^[1,12]. This review briefly describes the background, rationale, and clinical results of allo-HSCT with RIC as an immune-based strategy via GvT effect for the treatment of some metastatic solid tumors, including renal cell carcinoma (RCC), metastatic colorectal cancer (mCRC) and ovarian cancer.

CYTOTOXIC ADOPTIVE T-CELL THERAPY

Advances in systemic therapy for metastatic cancer have focused on important cellular pathways with critical roles in cancer development and progression^[25]. Although a dramatic success is obtained in the minority of patients, this approach provides a relatively short-term benefit in the majority and exposes them to chronic toxicities, including cardiac and dermal toxicities and thus, is not cost-effective^[26].

The mechanisms during the evasion of adoptive immune system by tumor cells have been described as growth, angiogenesis and tissue remodeling. During this process, the tumor cells also exploit the innate inflammatory response. Besides these mechanisms, the role of tumor microenvironment is also regarded as a new target for therapy^[27]. Advances in understanding of cancer immunology and especially the role of the adoptive immune system, have identified new targets for the treatment of solid tumors^[27].

The term, adoptive T-cell therapy (ATCT), involves the expansion of cytotoxic immune effector cells. It may be either specific or non-specific^[25]. The GvT effect and tumor response after allo-HSCT with RIC may be regarded as a non-specific ATCT, as it involves leukocyte-activated killer cells (LAKs) and cytokine-induced killer cells (CIKs), which are described and discussed in this paper. ATCT is not yet considered as a standard treatment modality in the medical oncology practice. However, it is considered as the most potent immunotherapeutic approach according to the results of some early phase trials^[27].

GVT EFFECT

The effect of immune system in inducing tumor regression is well-described. Graft-versus-host disease (GvHD) that occurs after allo-HSCT contributes to and maintains an anti-leukemic effect^[28]. Thus, it is referred as graft-versus-leukemia (GvL) effect. This effect was first demonstrated with the eradication of leukemia in mice receiving non-syngeneic allogeneic transplant after irradiation^[29]. Since then, several direct and indirect evidences of GvL effect after allo-HSCT have been reported. The GvL effect is generally associated with GvHD^[30]. A stronger GvL effect is observed in chronic GvHD than in acute GvHD^[31]. The probability of being in remission is also higher in patients with GvHD when compared to patients without GvHD^[32]. Other strong evidences for the presence of an immune-mediated GvL effect are the significantly increased relapse risk in patients receiving T-cell depleted transplants and the lower risk of relapse observed in patients undergoing allo-HSCT rather than autologous HSCT^[2,33-36]. The direct evidence of GvL effect comes from the studies reporting that donor lymphocyte infusions (DLI) given after transplant might augment the GvL effect of allo-HSCT and DLI infusion without cytotoxic therapy might induce and maintain remission in patients who relapse after allo-HSCT^[37-40].

The GvL effect, which eradicates malignant cells *via* fas-dependent killing and perforin degranulation, is mediated by donor T cells (CD4⁺, CD8⁺ and natural killer - NK-cells)^[41,42]. The major cytokines that potentiate the GvL effect include interleukin-2 (IL-2), interferon- γ and tumor necrosis factor- α ^[43]. Post-transplant adoptive therapy with cytotoxic T-lymphocytes (CTLs) against human cancer-associated antigens, minor histocompatibility antigens (e.g., HA-1, HA-3, etc.) or T-cell receptor genes may be used to induce anti-tumor effects^[44]. The development of acute and chronic GvHD has been linked to a better response to therapy in solid tumors^[2]. Identification of antigen targets of GvT and development of targeted therapies may further improve the immune effect of allo-HSCT for solid tumors and reduce the treatment toxicity^[2].

Allo-HSCT is an immuno-modulatory therapy aiming at exploiting a GvT effect. However, it has to be emphasized that a delicate balance between effective immuno-suppression, GvHD and relapse should still be considered.

Allo-HSCT with RIC in renal cell carcinoma

RCC is a common malignancy diagnosed in patients older than 50 years of age and almost one third of cases are metastatic at the time of diagnosis^[45]. Despite various treatment strategies including hormonal therapy, chemotherapy and immunotherapy, the prognosis of metastatic RCC is extremely poor with a median survival of 10 mo and a 5-year survival of less than 5%^[46,47]. RCC is sensitive to immunotherapy. Interferon- α with or without IL-2 (especially at high doses) have been widely used. However, the rates of response (10%-20%) and long-term progression-free survival (4%-15%) are still

unsatisfactory^[48-50]. Allo-HSCT with RIC is considered as a promising option in this setting^[11,13].

A response rate of 53% has been reported in the first series of allo-HSCT with NMA conditioning for cytokine-refractory RCC^[11]. Another trial included 75 metastatic RCC patients and reported a sustained engraftment in 74 out of 75 patients after allo-HSCT with NMA conditioning^[51]. In this study, chronic GvHD was observed in 50% and was associated with a significant tumor response.

The largest series of allo-HSCT with NMA conditioning in RCC patients was published by the EBMT-STWP, in which a fludarabine-based conditioning regimen was administered to all 124 patients prior to peripheral blood allo-HSCT^[52]. Engraftment failure was observed in 2.4%. TRM at the end of first year was 16% and associated mostly with acute GvHD. A response rate of 22.5% was achieved including complete response in 4 patients at a median of 150 (42-600) dpost-transplant.

Nowadays, patient selection for allo-HSCT has become an important issue, since disease progression after transplantation is more frequent among patients with rapidly progressive tumors. In order to determine which patients benefit most from allo-HSCT, 70 patients who underwent allo-HSCT were evaluated according to pre-transplant characteristics, such as performance status, C-reactive protein and lactate dehydrogenase levels in a study conducted by EBMT. This study suggested that these parameters could be used to stratify patients with advanced RCC who are candidates for allo-HSCT and to assist clinicians in decision-making and selection of an appropriate treatment program. As a result the patients with good prognostic criteria had a longer median survival than those with poor prognostic criteria, 23 mo vs 3.5 mo, respectively^[45]. Another study reported a higher response rate in the presence of an early transplantation, HLA-mismatched donors, higher Karnofsky score, lower number of metastatic sites and limited chronic GvHD^[52]. Currently, some other scoring systems are also developed for predicting survival in previously treated RCC patients^[46].

In conclusion, NMA conditioning followed by allo-HSCT in patients with RCC is feasible and it might prolong survival, especially in patients with favorable prognostic characteristics.

Allo-HSCT with RIC in colorectal cancer

Inoperable metastatic colorectal cancer (mCRC) is an incurable disease. Despite advances in therapy, median survival with fluorouracil-leucovorin, irinotecan, and oxaliplatin as first-line therapy is 18 to 22 mo and in case of resistance to these agents, the median survival declines 9 to 12 mo with second-line chemotherapy^[53,54]. Combination of chemotherapy with monoclonal antibodies such as cetuximab or bevacizumab improves remission rates and survival; however, long-lasting remission usually cannot be achieved, especially in the presence of resistant disease^[55,56].

Allo-HSCT following RIC has emerged as a novel

immunotherapy-based therapeutic strategy for the management of mCRC^[15,57,58]. In a study including six advanced mCRC patients, one complete response and one mixed response, including regression of lung and lymph-node metastasis and progression of liver metastasis were obtained^[59]. In a multicenter EBMT trial, among 39 patients with progressive mCRC overall disease control was achieved in 18 (46%) and 1 complete (2%), 7 partial (18%), and 10 stable disease responses (26%) were reported after allo-HSCT^[60]. Allo-HSCT with RIC might be an alternative to conventional strategies, especially in young patients with refractory mCRC.

Allo-HSCT with RIC in ovarian cancer

Ovarian cancer (OC) is the most fatal gynecologic malignancy and the fifth-leading cause of death among women in the developed countries^[61]. Despite extensive surgery and use of new generation drugs such as taxanes (mostly in combination with carboplatin), relapse rates may reach up to 50%. Although sensitive to high-dose chemotherapy (especially based on carboplatin combinations), the median overall survival is about 2 years for relapsing disease^[62,63]. The only benefit of high-dose chemotherapy does appear to be delayed relapse^[64,65].

In a study, including five refractory OC patients who underwent allo-HSCT with RIC, tumor regression were observed in four patients during acute or chronic GvHD and relapse occurred in one patient treated with methylprednisolone for chronic GvHD^[66]. A retrospective study from the EBMT-STWP database included 17 heavily pre-treated OC patients and mortality was reported in 11 patients, 8 of which were due to tumor progression at a median follow-up of 296 d (5-1599)^[67]. Grade 2-4 acute GvHD was reported in eight patients, seven (41%) of which had a partial response. Tumor regression was achieved in one out of three patients who received DLI. This data supports the existence of a graft-versus-ovarian cancer effect in correlation with GvHD. In another retrospective multicenter study with 30 allografted OC patients, objective response was observed in 50% and TRM was 20% at the end of first year^[68]. The median overall survival was 10.4 mo with a median follow-up of 74.5 mo (16-148). Overall survival was significantly higher among patients with chronic GvHD (17.6 mo vs 6.5 mo, $P < 0.05$).

Allo-HSCT with RIC for OC could be a feasible treatment option. However, supporting data are limited.

Allo-HSCT with RIC in soft tissue sarcomas

Soft tissue sarcomas (STS) constitute a rare and heterogeneous group of malignant tumors, which include less than 1 percent of all adult malignancies. Prognosis of STS is poor with a median survival of about 1 year with conventional treatments^[69].

In experimental animal models of allogeneic transplantation, immune-mediated effect against sarcoma has been shown^[70,71]. However, reports on STS treated with allo-HSCT mostly consist of single case reports and small series of patients from HLA-matched sibling donors.

Although some authors have reported the evidence of a graft-vs-sarcoma effect, no evidence of cancer regression following allo-HSCT with RIC regimens were reported among patients with various histologic subtypes^[72-74]. In a retrospective study, 14 adult patients from EBMT database with advanced STS received allo-HSCT with RIC for chemo-refractory disease, excluding rhabdomyosarcoma (most frequently a pediatric disease with an extremely different natural history) and they were assessed regarding whether a GvT effect could be generated in this setting. TRM was reported in two patients and progressive disease was observed in eight patients. Four patients experienced long-lasting disease stabilization following allo-HSCT. Authors concluded that an immune-mediated effect cannot be excluded in some STS^[75].

In conclusion, allo-HSCT with RIC may give rise to some degree of significant responses in some refractory metastatic solid tumors, such as renal, ovarian and even colon cancers. The advantages of RIC regimens are their lower toxicity profiles and lower non-relapse mortality rates. Allo-HSCT with RIC or NMA can be a feasible option for geriatric patients and patients with comorbidities. Future studies are needed for a clear-cut understanding of the mechanisms of GvL and GvT effects of donor T-cells and their subsets in order to optimize the efficacy of such treatment modalities in patients with refractory solid tumors.

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"Contrast nephropathy" in renal transplantation: Is it real?

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Abstract

The risk of contrast-induced nephropathy (CIN) in renal transplant recipients is increased in diabetics, patients with impaired basal kidney function, patients in shock, patients presenting with acute emergency and in old age recipients. Approximately one-third of all hospitalized patients with acute kidney injury is attributed to CIN. In the United States, it is the third leading cause of hospital-acquired renal failure. Therefore, efforts should be directed to minimize CIN-related morbidity and mortality as well as to shorten hospital stay. While the role of peri-procedural prophylactic hydration with saline is unequivocal; the use of acetyl cysteine is not based on robust evidence. The utility of theophylline, aminophylline, calcium channel blockers, natriuretic peptide, and diuretics does not have proven role in attenuating CIN incidence. We aim to analyze the evidence for using various protocols in published literature to limit CIN-associated morbidity and mortality, particularly during surveillance of the renal allograft survival.

Key words: Contrast; Renal; Transplantation; Nephropathy

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Core tip: The renal transplant is usually a solitary kidney with diverse hemodynamic changes and exposed to the immunosuppressive agents for a long period. Any superadded stress such as contrast-induced nephropathy (CIN), will definitely affect allograft function. We provide in this article a comprehensive review of the current evidence on the true incidence, the mechanism of damage induced by CIN and available preventive measures to counteract the possible effect induced by CIN.

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INTRODUCTION

Perioperative transplant complications are reported to involve about 15%-20% of the kidney transplant recipients. Diagnostic ultrasound (US) is the most common and first line imaging modality^[1], since it is safe, noninvasive, gives a rapid diagnosis and also a portable tool for many surgical emergencies requiring bedside imaging^[2-5]. The utility of ultrasonography in management of hydronephrosis, renal masses, renal artery stenosis (RAS) and pyelonephritis in renal allograft is well documented^[1].

Computed tomography (CT) scanning and CT-guided interventions play a vital role in investigating post-operative complications. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) can be used safely where there is renal dysfunction, since the "Gadolinium-based" contrast media can be safely used with minimal nephrotoxic effects. Post-transplant complications such as vessel thrombosis can also be assessed using these modalities. Allograft "morphology and function" can be effectively assessed by using intravenous Gadopentetate Dimeglumine (DTPA) to the MRA technique^[6].

However, the patients with pacemakers, aneurysmal clips, or evident claustrophobia cannot be safely exposed to MRI studies. Gadolinium-based media have been linked to the development of nephrogenic systemic fibrosis (NSF). Another drawback of MRI, is the "layering" of the excreted gadolinium in the urinary bladder causing multiple image artifacts. Alternatively, CT is better for the evaluation of the kidney and urinary bladder for renal stones and ureter and bladder abnormalities. To summarize, MRI is usually dedicated to the evaluation of transplant recipient, whereas CT and CT angiogram are reserved for potential donors^[6].

MECHANISM OF CONTRAST-INDUCED NEPHROPATHY

Vasoconstriction induced by the contrast media (CM) can be explained by the direct action of contrast media on vascular smooth muscle and from metabolites such as adenosine and endothelin. Moreover, the osmotic criteria of contrast media, especially in the tubular lumen, affects water reabsorption, leading to a magnifying interstitial pressure. This will be augmented by the increased salt and water load to the distal tubules, will decrease GFR and lead to local compression of the vasa recta. All these factors will aggravate medullary hypoxemia and renal vasoconstriction in an already volume depleted patient.

Finally, contrast media could increase resistance to blood flow by increasing its viscosity and by deranging red blood cells (RBCs) deformability. These manifest as local ischemia leading to activation of reactive oxygen species that result in damage to renal tubules^[7].

Up till now, we are sure why renal failure patients are sensitive to contrast utilization. Whether their primary disease is a contributing factor or not, this has to be elucidated by additional future research.

CONTRAST NEPHROPATHY IN RENAL TRANSPLANTATION

Intravenous contrast (Table 1)

Only a relatively handful of studies have looked into the contrast-induced nephropathy (CIN) in the renal transplant recipients. Light *et al*^[8], 1975 studied thirty-four renal transplant recipients received drip infusion urograms post-transplantation. Twenty-two patients exhibited a change in renal function within 1-4 d of the urogram that was indistinguishable from allograft rejection that is a tender swollen kidney, a rise in serum creatinine, oliguria, diminished urinary sodium, weight gain and hypertension. Two patients developed acute tubular necrosis (ATN) and required hemodialysis, but renal function in the remaining 20 patients improved after therapy for "graft rejection" with intravenous methylprednisolone sodium succinate. Kidneys from older-age donors that were functioning sub-optimally at transplant and kidneys, which exhibited subsequent clinical allograft rejection, were more at risk for CIN. These suggested occult vascular lesions might have been present in the allograft, which was exacerbated when exposed to the irritant vascular effects of contrast media, producing a mild, reversible toxic nephritis. However, several renal grafts with normal function and also those, which never exhibited rejection activity, were also adversely affected by exposure to contrast media. Therefore these agents should be used cautiously, if at all, in the early post-transplant period^[8]. CIN was more common and more severe in those with impaired kidney function. This study also found that kidneys from older donors were at higher risk for CIN. In this study, contrast was used before stable creatinine was achieved, these kidney transplant recipients were not on a CNI, and there is no mention of use of any prophylaxis to prevent CIN. More than half of these patients were thought to have acute rejection and were treated as such without consistent biopsy documentation^[8].

The incidence of acute kidney injury (AKI) induced by CIN resulting from direct exposure to contrast media in kidney transplants recipients still controversial. The main insult is the ensuing vasoconstriction of the afferent glomerular arterioles and reduction in renal blood flow and glomerular filtration rate. Renal vasoconstriction, as well as direct tubular epithelial toxicity, is the two major mechanisms by which contrast causes AKI as explained by Haider *et al*^[9] in 2015. Immediately after contrast

Table 1 Trials concerned with contrast nephropathy

No.	Trial	Year	No. of KTRs	Need for HDX	CIN	Comments
1	Light <i>et al</i> ^[8]	1975	34	Two	22	20 patients improved after therapy for "graft rejection"
2	Moreau <i>et al</i> ^[12]	1975	231	None	Nil	No increase in risk of CIN in KTRs if contrast studies were performed with normal renal function
3	Peters <i>et al</i> ^[11]	1983	93	None	Very high (84.3%)	No increased risk was found > 120 d post-transplant
4	Ahuja <i>et al</i> ^[10]	2000	35	None	> 21%	Patients received high osmolality contrast, and 94% were on CyA therapy
5	Charnow <i>et al</i> ^[16]	2015	76	None	> 13.2%	CIN did not affect allograft function and survival, according to the researchers
6	Haider <i>et al</i> ^[9]	2015	124	None	5.60%	The largest retrospective study evaluating incidence of CIN in KTRs. CNIs were being used in 95% patients at the time of contrast administration
7	Bostock <i>et al</i> ^[15]	2016	40	One	12.50%	Renal dysfunction is 3 times more frequent in KTR treated with EVAR, though overall survival did not differ between groups. Decreased pre-operative eGFR and higher iodine/eGFR ratio are associated with post-operative renal dysfunction
8	Fananapazir <i>et al</i> ^[14]	2016	104	None	7% and 3%	Incidence of CNI = 7% (7/104) based on a rise of ≥ 0.3 mg/dL and 3% (3/104) based on a rise of ≥ 0.5 mg/dL. With a strict definition (≥ 0.5 mg/dL) had a pre-CT eGFR < 60 mL/min per 1.73 m ² . No patients required DX or had allograft loss 30 d after contrast use

CIN: Contrast-induced nephropathy; HDX: Hemodialysis; KTRs: Kidney transplant recipients; CNIs: Calcineurin inhibitors; EVAR: Endovascular aortic aneurysm repair; eGFR: Estimated glomerular filtration rate.

use, there is a transient increase in renal blood flow followed by a prolonged reduction in flow resulting in renal ischemia. So, there is "clustering" of two risk factors here, as both calcineurin inhibitors and IV contrast cause renal ischemia by the dual mechanism: (1) by increasing the release of the vasoconstrictors such as endothelin; and (2) by blocking the release of vasodilators including prostaglandins and nitric oxide^[10].

Ahuja *et al*^[10] (2000) also studied 35 kidney transplantation recipients (KTRs) as regard the effect of "volume expansion" as well as the effect of cyclosporine therapy; which documented the presence of CIN in a percentage exceeding 21%, with incidence of CIN was about 15% in patients received volume expansion and exceeds 42% in those who did not. None of these patients had AKI requiring dialysis. In this study, two main insults were reported, first: They received "high osmolality" contrast, and second: 94% were on cyclosporine therapy. The baseline serum creatinine in patients with and without CIN was 1.54 ± 0.17 mg/dL and 1.97 ± 0.20 mg/dL, respectively, $P = 0.15$, but the volume of contrast was not reported here. Another study- demonstrated by Peters *et al*^[11] in 1983-reported a very high incidence of CIN (84.3%) in the early post-transplant period, but no increased risk was found > 120 d post-transplant.

Moreau *et al*^[12] (1975) demonstrated clear evidence that there was no increase in the risk of CIN in kidney transplant recipients if contrast studies were performed against a background of normal renal function. Data observed from these studies showed that older donor kidney, early post-transplant period, impaired baseline kidney function, and lack of prophylactic volume expansion, appear to be important risk factors for increasing the incidence of CIN in kidney transplant recipients (Figure 1). In fact, a direct comparison between these studies regarding the incidence

of CIN among is challenging, as the definition of AKI used was not uniform. There were differences in baseline serum creatinine; use of hyper-, hypo-, or iso-osmolar contrast; volume of contrast given; and the proportion of patients with known risk factors for CIN, including: Diabetes Mellitus, congestive heart failure, and concomitant use of CNI, in these studies which make it difficult to conclude the actual incidence of CIN in kidney transplant recipients.

To date, Haider *et al*^[9] (2015) study is considered the largest retrospective study evaluating the incidence of CIN in kidney transplantation. The incidence of CIN in this study was low (5.6%), much lower than reported by Ahuja *et al*^[10] (2000). Two fundamental factors were implicated in this low incidence of CIN in this landmark study first: the relatively elevated baseline eGFR (> 70 mL/min per 1.73 m²) and second: The use of "hypo-osmolar" contrast applied in this procedure^[9].

Another possible explanation for the low incidence of CIN in this study is that Diabetes Mellitus and hypertension in these patients may not have damaged the renal allograft to the extent to potentiate CIN. Another important factor is the age of the kidney rather than the age of recipient may affect the susceptibility for CIN. Furthermore, among all procedures utilizing iodine-based contrast, coronary angiography with the percutaneous intervention was responsible for 49% of cases of CIN^[13]. However in Haider *et al*^[9] (2015) work, only 4.8% of patients have had cardiac catheterization (none of them had CIN), and this might also have leading to low incidence of CIN-AKI in this group of patients. Their inability to identify association with known risk factors for CIN may be explained by the very small number of patients complicated by CIN events.

On the other hand, Fananapazir *et al*^[14], 2016, declared in the most recent trial that CIN incidence was very low,

Incidence of CIN after kidney transplant

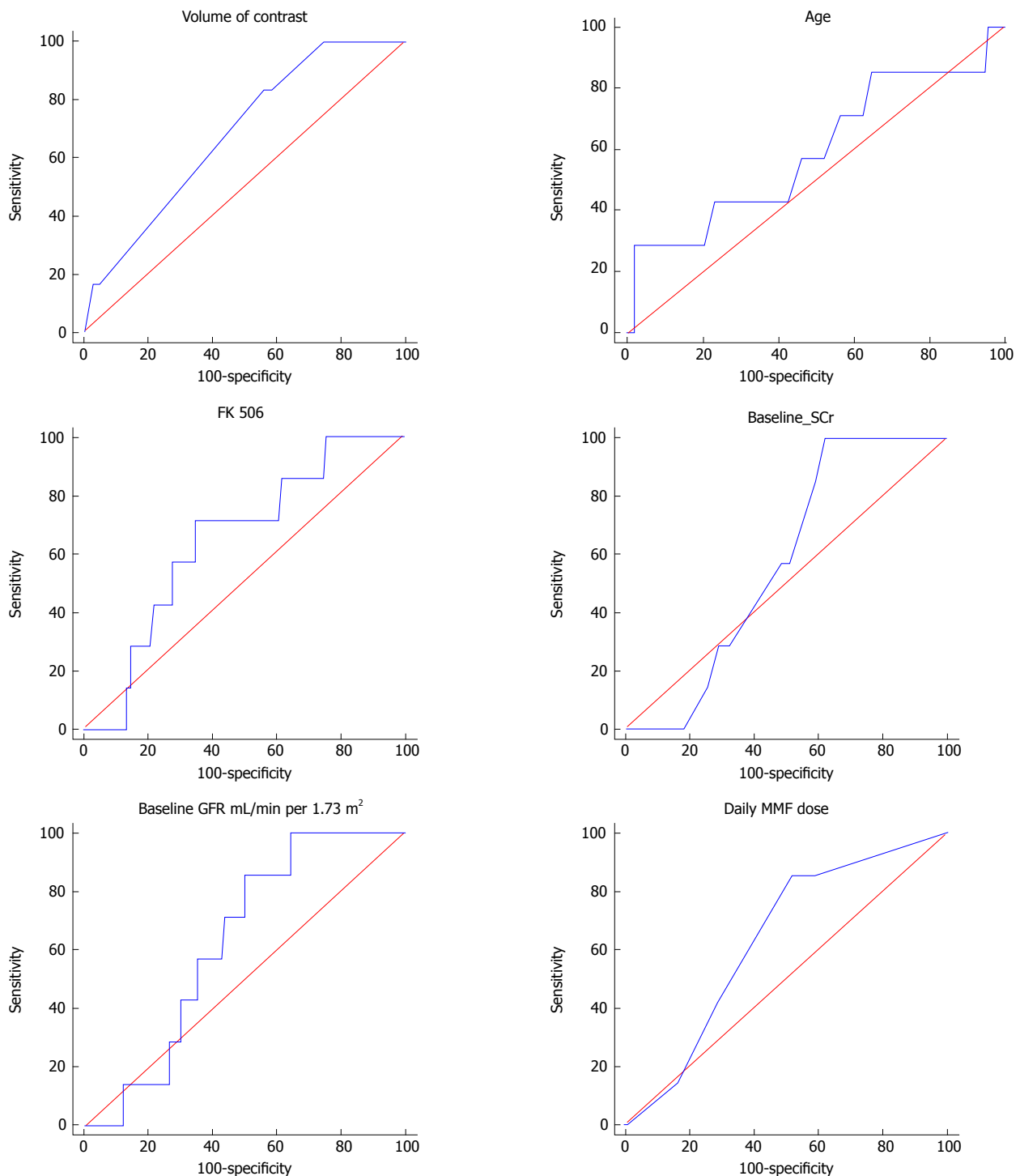


Figure 1 Receiver operating characteristics curves for age, FK506 levels, daily Cellcept dose, baseline Cr., eGFR, and volume of IV contrast. Area under the curve (AUC) for age, FK506 levels, daily Cellcept dose, baseline creatinine, eGFR, and volume of IV contrast were 0.60, 0.64, 0.63, 0.57, 0.63, and 0.68, respectively^[9]. Adapted from Haider *et al.*^[9], Incidence of Contrast-induced Nephropathy in Kidney Transplant Recipients. *Transplantation Proceedings* 2015; **47**: 2379-2383 (with permission). GFR: Glomerular filtration rate.

i.e., 7% and 3% according to an elevation of SCr of > 0.3 and 0.5 respectively, after a low osmolality contrast administration. There was with no need for emergent dialysis or an allograft loss 30 d post-operative^[14].

Moreover, Bostock *et al.*^[15] in 2016, also demonstrated that CIN following endovascular aortic aneurysm repair

(EVAR) in kidney transplant recipients could have de-ranging sequelae. The Vascular Quality Initiative (VQI) database was interrogated to select all kidney transplant recipients who underwent EVAR between 01/2003 and 12/2014. Their primary outcome was renal dysfunction, defined as AKI (rise in serum creatinine concentration

> 0.5 mg/dL above the baseline or new post-operative hemodialysis requirement). Within the EVAR VQI dataset, 40 subjects were kidney transplant recipients (40/17, 213, 0.2%). Renal dysfunction occurred in 5/40 patients in the kidney transplant recipients group in comparison to 779/17173 patients in the non-transplanted group (12.5% vs 4.5%, $P < 0.01$). Emergency EVAR was indicated in 2 (5%) patients who required hemodialysis after surgery and died later. One-year survival after EVAR was similar in both groups (92.9% vs 93.1%, $P = 0.73$). Kidney transplant recipients who developed renal dysfunction had significantly lower pre-operative eGFR's (29.5 vs 54.7, $P = 0.007$) and a significantly higher iodine/eGFR ratio (0.78 vs 0.39, $P = 0.02$) despite receiving a similar volume of contrast (70.0 vs 68.8, $P = 0.97$). Renal impairment was three times more frequent in kidney transplant recipients treated with EVAR, despite the overall survival did not differ between groups. Diminished pre-operative eGFR and a higher iodine/eGFR ratio were associated with post-operative renal dysfunction^[15]. Charnow *et al*^[16] 2015, showed an incidence about 13% of CIN in allograft recipients undergoing CT or cardiac catheterization with contrast media. CIN was relatively common in kidney transplant recipients undergoing (CT) or cardiac catheterization with contrast media. Charnow *et al*^[16] (2015) at the University of Cincinnati in Ohio studied 76 contrast exposures (45 CT scans and 31 catheterizations) in 50 kidney transplant recipients (50% male) with a mean age of 53.3 years and means. Cr level of 1.46 mg/dL. The investigators reported CIN - defined as a rise in s. Cr by > 0.3 mg/dL or 25% from baseline within 4 d. after the procedure - in 10 of 76 procedures (13.2%). Results demonstrated: 6 (13.3%) of the 45 CT scans and 4 (12.9%) of the 31 catheterizations resulted in CIN^[16].

Abu Jawdeh's group (2015)^[16] also examined the risk factors for CIN. In a multivariate model, exposure to N-acetylcysteine (NAC) and a lower hemoglobin level was significantly associated with an increased risk of CIN, but not with CNI use. They assumed that NAC might have been used in high-risk subjects for CIN, a bias that could explain the increased risk of CIN associated with NAC use. At the last follow-up, CIN did not affect allograft function and survival, according to the investigator^[16].

CIN is accompanied by a significant rise in mortality and morbidity; Abu Jawdeh suggested that extrapolation of knowledge about CIN affecting the native kidneys and applying this to allografts might not necessarily reflect the best practice. Allografts are solitary kidneys that exposed to significant hemodynamic alterations and also under the effect of lifelong immuno-suppressive agents. Both these factors might affect susceptibility to contrast-induced renal injury. They also suggested that CIN is potentially modifiable if risk factors are well identified and the proper preventive precautions are performed. The 13.3% incidence of CIN identified in this study is consistent with previous studies looking at native kidneys^[16]. Due to the retrospective nature of

this study and the small sample size, this study should be interpreted with caution.

Finally, it appears that the strict "definition of CIN" in various studies was not universal. While Charnow *et al*^[16] defined CIN as a rise in s. Cr by > 0.3 mg/dL or 25% rise from baseline within four days of contrast exposure, Bostock *et al*^[15] defined CIN as an AKI with elevation of SCr > 0.5 mg/dL from baseline, or new post-operative hemodialysis (HD) requirement%. Haider *et al*^[9] (2015) defined CIN as either an absolute rise in serum creatinine of ≥ 0.5 mg/dL or a $\geq 25\%$ drop in estimated glomerular filtration rate (eGFR) after contrast administration. On the other hand Fananapazir *et al*^[14] (2016) applied two definitions for CIN in the most recent study, they found CIN in 7% based on a rise of ≥ 0.3 mg/dL and 3% based on a rise of ≥ 0.5 mg/dL. Patients with the more strict definition (≥ 0.5 mg/dL) had a pre-contrast eGFR < 60 mL/min per 1.73 m².

"Ultrasound with contrast": Contrast enhanced ultrasound (CEUS) is a promising radiological technique with increased popularity. It has a superiority over the color Doppler ultrasound in evaluation of kidney microvasculature studies. A wide variety of diagnoses can be applied including differentiation of cystic from solid lesion, solid mass assessment, pseudotumor and RAS. Moreover, CEUS can help in elucidating the hemodynamic changes associated with chronic allograft nephropathy (CAN)^[17].

US contrasts are gas microbubbles of nearly the same size of RBCs, which enclosed in a protein, lipid or polymer shell^[18]. They last intravascular only for few minutes (time of CEUS examination), after that, the gas exhaled through the lungs and the shell metabolized by the liver^[19], so renal excretion is not a possibility. As these contrast agents is not excreted through the kidney, allograft integrity cannot be deranged. So, their use in KTRs with impaired renal function is completely safe. Furthermore, CEUS is the sole available technique for dynamic evaluation of kidney perfusion, particularly so, when the use of contrast media is mandatory in CT and MR studies in patients with renal dysfunction. CEUS has a wide safety margin in comparison with other radiological modalities^[20,21].

Prevention of CIN-induced AKI in the renal transplant recipient: There are no specific measures dedicated to prevent CIN-induced AKI in the renal allograft, but rather universal recommendations. The optimal recommendations for CIN prevention are still uncertain.

The following precautions are suggested with increased risk of CIN (S. creatinine ≥ 1.5 mg/dL (132 micromols/L) or an eGFR < 60 mL/1.73 m²), especially in diabetics: (1) Avoid volume depletion and NSAID^[22,23]; (2) Avoid use of high osmolar agents (1400-1800 mosmol/kg)^[24,25]; (3) Try to use US and MRI without gadolinium contrast, or CT scanning without contrast media when possible; (4) Choose iodixanol or nonionic low-osmolar

agents, *e.g.*, iopamidol or ioversol rather than iohexol^[25]; (5) Apply lower doses of contrast and avoid repetitive, closely spaced studies (< 48 h apart)^[12,13,15,16,25]; (6) In an absence of contraindications to volume expansion, start isotonic intravenous fluids before and continued several hours after contrast use. Optimal type and timing are not well documented. "Isotonic bicarbonate" is preferred to isotonic saline as a "volume expander"^[23,26-29]. "Isotonic bicarbonate" regimen: A bolus of 3 mL/kg for one hour prior to the procedure, and continued at a rate of 1 mL/kg per hour for "6" h after the procedure^[23,26-29]. Suggested regimen for isotonic saline: Isotonic saline (1 mL/kg per hour), starting at least 2-6 h before, and continued for 6-12 h after the procedure. Duration of intravenous fluid should be directly proportional to the degree of renal dysfunction (*i.e.*, longer duration for severe renal impairment); (7) Based upon potential benefit, low toxicity, and cost, Acetylcysteine (AC) can be given: 1200 mg orally twice/day, the day before and the day of the procedure. Intravenous AC is NOT recommended due to lack of evidence of benefit and potential risk of anaphylactoid reactions^[30,31]; and (8) Prophylactic use of "mannitol" or other diuretics is NOT recommended^[32,33]. Prophylactic HF/HDX after contrast exposure is NOT advised on stage 3 and 4 CKD^[34].

Oral contrasts

Two documented contrast media are already in use for oral imaging procedures: First: Barium sulphate, a commonly used oral contrast agent (for GI studies); Second: Gastrografin, which is a substitute agent for the barium in special situations. Generally, barium, as well as gastrografin, is safe, passing through the gastrointestinal tract easily like food and drink^[35].

Barium sulphate is by far the most common contrast material used orally. It can also be utilized rectally. Multiple forms are available, including powder, liquid, paste and tablets. They are generally safe. Only mild unpleasant taste can be observed. If given by enema, abdominal fullness, change in bowel habits and whitish discoloration may be observed for only a few days^[36].

Nephrostogram

A nephrostogram is a radiological tool performed to check the nephrostomy catheter and to rule out any abnormalities in the kidney and ureters, for example, obstructive uropathy. It is performed by disconnecting the catheter from its drainage bag and injecting the iodinated contrast through its lumen, monitored with fluoroscopy and static X-ray imaging. Nephrostogram is a very safe technique with few documented complications. Only mild pain with the possibility of the introduction of infection can occur. Unfortunately, this procedure has no known alternative technique^[37].

CONCLUSION

The risk of CIN affecting renal allograft function is

significant especially in diabetics, old age and in volume depleted subjects. This risk can be greatly mitigated through optimizing the hydration status in peri-procedure period, by avoiding nephrotoxic medications, by careful use of safe and widely spaced contrast media with the possible minimal amount of contrast media and possibly by prophylactic peri-procedural administration of isotonic bicarbonate. Some of the questions remain unanswered that require randomized controlled trials involving larger number of renal transplant recipients in order to maximize safety of the renal allograft.

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Retrospective Cohort Study

Impact of preformed donor-specific antibodies against HLA class I on kidney graft outcomes: Comparative analysis of exclusively anti-Cw vs anti-A and/or -B antibodies

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Abstract

AIM

To analyze the clinical impact of preformed antiHLA-Cw vs antiHLA-A and/or -B donor-specific antibodies (DSA) in kidney transplantation.

METHODS

Retrospective study, comparing 12 patients transplanted with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B.

RESULTS

One year after transplantation there were no differences

in terms of acute rejection between the two groups (3 and 6 cases, respectively in the DSA-Cw and the DSA-A-B groups; $P = 1$). At one year, eGFR was not significantly different between groups (median 59 mL/min in DSA-Cw group, compared to median 51 mL/min in DSA-A-B group, $P = 0.192$). Moreover, kidney graft survival was similar between groups at 5-years (100% in DSA-Cw group vs 91% in DSA-A-B group, $P = 0.528$). The sole independent predictor of antibody mediated rejection (AMR) incidence was DSA strength (HR = 1.07 per 1000 increase in MFI, $P = 0.034$). AMR was associated with shortened graft survival at 5-years, with 75% and 100% grafts surviving in patients with or without AMR, respectively (Log-rank $P = 0.005$).

CONCLUSION

Our data indicate that DSA-Cw are associated with an identical risk of AMR and impact on graft function in comparison with "classical" class I DSA.

Key words: Donor-specific antibodies; Antibody-mediated rejection; Anti human leukocyte antigen class I ; AntiHLA-Cw antibodies; Graft survival; Solid-phase immunoassays

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Core tip: The clinical importance of preformed antiHLA-Cw donor-specific antibodies (DSA) in kidney transplant patients remains controversial, so we performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival frequency, respectively, at one and at five years of follow-up, were comparable between groups. Our data support a similar deleterious impact considering DSA-Cw or DSA-A/-B in terms of risk of AMR and impact on graft function.

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INTRODUCTION

In kidney transplantation the presence of preexisting anti human leukocyte antigen (HLA) donor-specific antibodies (DSA) has impact on graft outcomes. Their presence is associated with an augmented risk of antibody-mediated rejection (AMR)^[1] and worst graft survival^[2].

Classically, antibodies against major HLA Class I (A and B) and Class II (DR and DQ) antigens are considered to be responsible for most cases of AMR. AntiHLA-Cw are considered less immunogenic when are paralleled

to other class I antiHLA antibodies, mainly due to minor HLA-Cw antigen expression on cell surface^[3]. Indeed, some studies found that the incidence of antiHLA-Cw antibodies in sensitized patients was lesser than that for HLA-A or HLA-B antibodies^[4-6].

However, the progress of additional sensitive assays that identify HLA antibodies, namely solid-phase immunoassays, demonstrated that HLA-C locus may induce an antibody reaction comparable to the other usually tested loci^[4,5,7,8]. In 2012, Ling *et al*^[5] showed that kidney transplantation in patients with isolated antiHLA-Cw antibodies was effective (no rejections occurred) when using induction treatment with anti-thymocyte globulin (ATG) and IVIG. Another study evaluated 22 patients with pretransplant DSA antiHLA-Cw in comparison with 88 patients allosensitized but with no detectable preformed DSA and concluded that they seem to be at superior risk for AMR occurrence^[9]. Recently, Bachelet *et al*^[10] in their retrospective and multicenter study showed that antiHLA-Cw DSA have the same effect on graft outcome as DSA against "classical" HLA loci (A, B, DR, DQ), suggesting that antiHLA-Cw should also be considered in transplant allocation procedures and in immunologic risk stratification of patients.

As this subject remains controversial, we decided to conduct a retrospective study in kidney transplant patients to investigate the clinical impact of preformed antiHLA-Cw DSA comparing them to DSA against the other HLA class I loci, namely antiHLA-A and/or B.

MATERIALS AND METHODS

Patients

From the database of our Histocompatibility Center 35 adults who received a kidney transplant since 2007 were identified as having pretransplant donor specific antibodies (DSA) exclusively antiHLA-A and/or -B or exclusively antiHLA-Cw. Twenty-three patients had DSA antiHLA-A and/or antiHLA-B: 6 with DSA antiHLA-A only; 11 with DSA antiHLA-B only and 6 with DSA antiHLA-A and -B. This group was designated DSA-A-B. Twelve patients had DSA exclusively antiHLA-Cw, and this group was designated DSA-Cw. The patients were all transplanted with a negative T- and B-cell cytotoxic crossmatch (standard NIH technique). The Institutional Review Board at Hospital Santo António, CHP approved this study.

AntiHLA antibody testing

Patients in the waiting list were examined for antiHLA IgG by multiplex microsphere based on Luminex X-map® Technology (LABScreen® Mixed kit, OneLambda, Canoga Park, CA, United States). The cut-off for positive samples was the Normalized Background (NBG) ratio advocated by the manufacturer and executed by the HLA fusion® software (One Lambda Inc.). To determinate the specificity of the HLA antibodies, single-antigen bead (SAB) assays (LabScreen Single Antigen Beads®, OneLambda, Canoga Park, CA) were executed in patients with a positive

screening, using the same pretransplant sera. The mean fluorescence intensity (MFI) was measured using LABScan™ 100 flow analyzer (Luminex®, Austin, TX, United States). The analysis was performed using HLA fusion® software (One Lambda Inc.) and a cut-off for a positive reaction were set in MFI value of ≥ 1000 .

Donor typing and crossmatch

Samples of all deceased donors were routinely typed before recipient selection in loci HLA-A*, B*, Cw* and DRB1* using polymerase chain reaction (PCR) amplification with specific sequence primers (SSP; Olerup SSP® low resolution HLA typing kits, Stockholm, Sweden). After donor HLA typing, using that information, a virtual crossmatch (virtual XM) was executed. The strength of each single DSA was based on the MFI of one SAB. In the case of several DSA against different HLA-antigens, we considered the cumulative strength of all DSA by adding the individual MFI values.

Immunosuppression

Thirty-three of the total of 35 patients (94.3%) received induction therapy: Ten patients with a monoclonal antibody anti-IL-2 receptor (Basiliximab Novartis®, 20 mg twice at day 0 and 4), and 23 patients with polyclonal ATG Fresenius® (3 mg/kg for 5–7 d). All patients had an equivalent maintenance immunosuppression using three oral drugs: A calcineurin inhibitor [tacrolimus (FK-506) in the majority of patients (32/35 patients) or cyclosporine (CsA) in 3 patients], mycophenolate mofetil (MMF) and a corticosteroid. FK-506 was started at a dose of 0.1–0.15 mg/kg per day, and was adjusted to maintain levels between 8 and 12 ng/mL during the first month post-transplant, between 7 and 10 ng/mL the next 2–3 mo and between 5 and 8 ng/mL thereafter. MMF was started at a dose of 2000 mg/d, and decreased based on white blood cells count. Methylprednisolone was administered intravenously at doses of 500, 250 and 125 mg/d on the day of transplantation, days 1–2 and days 3–4 after the operation, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg, being then tapered to 5–10 mg/d within 2–3 mo after transplant. Living donor recipients ($n = 3$) were prescribed FK-506 and MMF 7 d before transplant.

Eight patients underwent a desensitization protocol. Five patients received intravenous immunoglobulin (IvIg) 2 g/kg at transplant (0.5 g/kg immediately before transplant, and at day 1, 2 and 3) and 1-mo after transplant (1 g/kg in 2 consecutive days). One patient received a similar dose of IvIg and underwent plasmapheresis every other day (first session immediately before transplant, for a total of 6–9 sessions) and two other patients received additionally a dose of Rituximab (375 mg/m²) on day 3 post-transplant.

Patients' data and outcomes

The data concerning patients' characteristics and transplantation variables was collected retrospectively. Estimated glomerular filtration rate (eGFR) was assessed using the 2006 Modification of Diet in Renal

Disease (MDRD) equation and dialysis requirement in the first week post-transplant was defined as delayed graft function. Patients were followed until graft failure, death or end of follow-up (five years after transplant or December 31, 2015, which came first). Graft survival was evaluated considering graft failure censored for death with a functioning graft.

Follow-up

Graft biopsies were performed "for cause" only. Allograft rejection was classified according Banff classification (updated in 2013) and defined by biopsy where specimens were evaluated by light microscopy and immunofluorescence (with C4d staining). Mild acute cellular rejection (ACR Banff grade I) was treated with 500 mg methylprednisolone for 3 d and increased maintenance immunosuppression. All other ACR were treated with ATG. AMR patients were treated with plasmapheresis every other day (the number of plasmapheresis sessions was 4 per protocol) and IvIg 100 mg/kg after each session. After the last plasmapheresis session, they received a high-dose IvIg (2 g/kg) divided in four daily doses and the same dose was repeated 1 mo later. If not used at transplant, patients received, additionally, one dose of rituximab (375 mg/m²).

Statistical analysis

Categorical data were expressed as numbers (frequencies) and continuous data were described using median (inter-quartile range). Categorical data (demographic and medical characteristics) were compared using Pearson χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared with Mann-Whitney *U* test. Predictors of AMR were explored by univariate and multivariable (using a backward elimination method, with a *P*-value < 0.05 necessary for retention in the model) Cox regression. For graft survival curves was used the Kaplan-Meier method, and the comparison between groups was done by log-rank test.

RESULTS

Baseline characteristics

Baseline characteristics of DSA-Cw and DSA-A-B groups are given in Table 1. DSA-Cw patients tended to be younger compared to patients in DSA-A-B group (respectively, 39 years vs 48 years), ($P = 0.061$). There was no significant difference between groups concerning gender, history of previous transplant or previous pregnancies. However DSA-Cw patients had significantly higher prevalence of previous blood transfusions (75% vs 39%, $P = 0.044$).

Concerning donor characteristics and pretransplant immunological data, namely donor age, donor gender, type of donor transplant (living vs deceased), peak PRA, and DSA number, none of these characteristics significantly differed between groups. Although DSA strength median was higher in DSA-A-B (MFI 7583) in comparison with DSA-Cw group (MFI 2939), this difference was not

Table 1 Baseline characteristics of donor-specific antibodies-Cw and donor-specific antibodies-A-B groups

	DSA-A-B <i>n</i> = 23	DSA-Cw <i>n</i> = 12	<i>P</i>
Recipient			
Age (yr), median (IQR)	48 (39-55)	39 (33-49)	0.061
Female gender, <i>n</i> (%)	13 (57)	6 (50)	0.713
Retransplant, <i>n</i> (%)	11 (48)	5 (42)	0.728
Previous blood transfusions, <i>n</i> (%)	9 (39)	9 (75)	0.044
Previous pregnancies, <i>n</i> (%)	8 (35)	8 (33)	1
Kidney-pancreas transplantation, <i>n</i> (%)	1 (4)	1 (8)	1
Donor			
Age (yr), median (IQR)	45 (36-56)	45 (32-54)	0.542
Female gender, <i>n</i> (%)	8 (35)	8 (33)	1
Living donor, <i>n</i> (%)	1 (4)	2 (17)	0.266
Pretransplant immunological data			
Peak PRA, median (IQR)	4 (0-80)	8 (0-52)	0.472
DSA number, median (range)	1 (1-3)	1 (1-2)	0.056
DSAsum MFI, median (IQR)	7583 (2320-12395)	2939 (2529-3650)	0.11
Transplant			
ABDR HLA mismatches, mean ± SD	3.22 ± 1.28	4.08 ± 1.16	0.056
FCXM-T + (<i>n</i> = 29), <i>n</i> (%)	1 (6)	3 (27)	0.139
FCXM-B + (<i>n</i> = 29), <i>n</i> (%)	2 (11)	0	0.512
ATG induction, <i>n</i> (%)	14 (61)	9 (75)	0.476
Tacrolimus (<i>vs</i> CsA), <i>n</i> (%)	20 (87)	12 (100)	0.536
Desensitized, <i>n</i> (%)	5 (22)	3 (25)	1
IvIg only, <i>n</i>	2	3	
IvIg + PP, <i>n</i>	1	0	
IvIg + Rtx + PP, <i>n</i>	2	0	

DSA: Donor-specific antibodies; MFI: Mean fluorescence intensity; IQR: Interquartile range; SD: Standard deviation; CKD: Chronic kidney disease; PRA: Panel reactive antibodies; HCV: Hepatitis C virus; CMV: Cytomegalovirus; HLA: Human leukocyte antigen; ATG: Anti-thymocyte globulin; CsA: Cyclosporin; IvIg: Intravenous immunoglobulin; PP: Plasmapheresis; Rtx: Rituximab.

significant ($P = 0.110$).

Flow cytometry crossmatch (FCXM) was performed for 29 of 35 patients. Positive T- and/or B- cell FCXM was similarly uncommon between groups. Three (27%) patients had a positive T-cell FCXM in the DSA-Cw group and only one (6%) in the DSA-A-B group ($P = 0.139$). Only two patients had a positive B-cell FCXM and both belonged to the DSA-A-B group.

Immunosuppression and induction treatment were similar between groups. ATG induction was used in 14 (61%) and 9 (75%) patients from the DSA-A-B and DSA-Cw groups, respectively ($P = 0.476$). Additionally, 5 patients in the DSA-A-B group were desensitized: 2 of them using only IVIG, 1 with IVIG and plasmapheresis and another 2 combining IVIG, plasmapheresis and rituximab. In DSA-Cw group 3 patients were treated with IVIG.

Clinical outcomes

Transplant outcomes are detailed in Table 2. There was no difference in terms of acute rejection at one year between the two groups (6 and 3 cases, respectively in the DSA-A-B and the DSA-Cw groups; $P = 1$). All cases of acute

Table 2 Clinical outcomes and follow-up

	DSA-A-B <i>n</i> = 23	DSA-Cw <i>n</i> = 12	<i>P</i>
Delayed graft function, <i>n</i> (%)	7 (30)	1 (8)	0.216
Acute rejection at 1-yr, <i>n</i> (%)	6 (26)	3 (25)	1
AMR at 1-yr, <i>n</i> (%)	6 (26)	2 (17)	0.685
ACR-only at 1-yr, <i>n</i> (%)	0	1 (8)	0.343
1 yr-eGFR (mL/min), median (IQR)	51 (46-60)	59 (47-64)	0.192
1 yr-ProtU, median (IQR)	0 (0-0.1)	0.1 (0-0.2)	0.163
Censored graft failure, <i>n</i> (%)	2 (9)	0	0.536
Follow-up time (mo), median (IQR) [range]	60 (45-60) [28-60]	18 (11-50) [3-60]	0.001

DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; ACR: Acute cellular rejection; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; ProtU: Proteinuria.

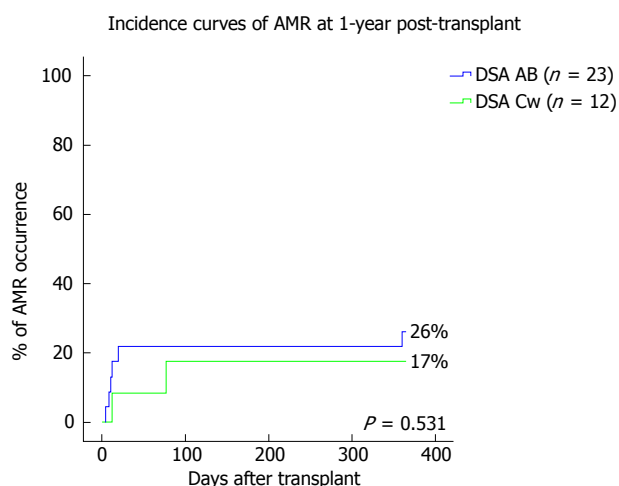


Figure 1 Incidence curves of antibody-mediated rejection at 1-year post-transplant. AMR: Antibody-mediated rejection; DSA: Donor-specific antibodies.

rejection were diagnosed as AMR in the DSA-A-B group, while in the DSA-Cw group there were 2 cases of AMR and 1 of ACR. Figure 1 shows the incidence of AMR at one-year post-transplant, between DSA-A-B and DSA-Cw patients groups, (respectively, 26% and 17%, Log-rank $P = 0.531$) with no significant difference being detected. At one year, eGFR tended to be higher in DSA-Cw group (median 59 mL/min) compared to DSA-A-B group (median 51 mL/min), ($P = 0.192$) (Figure 2). Importantly, follow-up was significantly longer for the DSA-A-B group (median 60 mo) than in the DSA-Cw group (median 18 mo) ($P < 0.001$). Kidney graft survival at 5-years was also similar between groups (Figure 3, 91% for the DSA-A-B group vs 100% for the DSA-Cw group, $P = 0.528$).

Antibody-mediated rejection: Incidence, predictors and clinical impact

AMR occurred in 8 patients (23%) of the overall cohort. Possible associations between clinical and immunological data and AMR incidence through a Cox regression analysis is shown in Table 3. The sole independent predictor of AMR incidence was the DSA strength, both in uni- and multi-

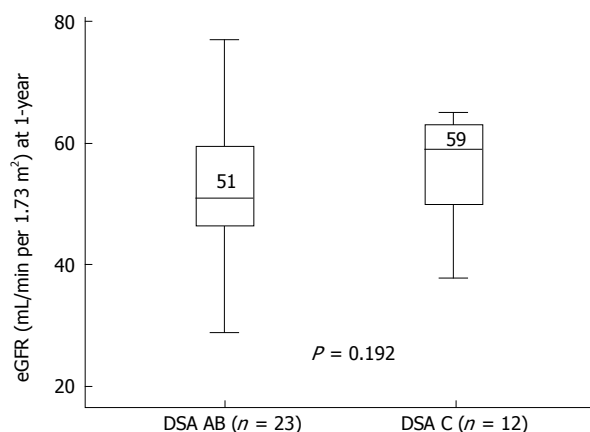


Figure 2 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to donor-specific antibodies human leukocyte antigen loci. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. DSA: Donor-specific antibodies; eGFR: Estimated glomerular filtration rate.

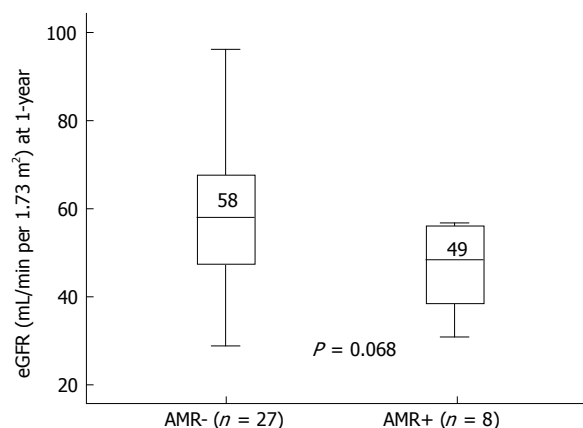


Figure 4 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to antibody-mediated rejection occurrence. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. AMR: Antibody-mediated rejection; eGFR: Estimated glomerular filtration rate.

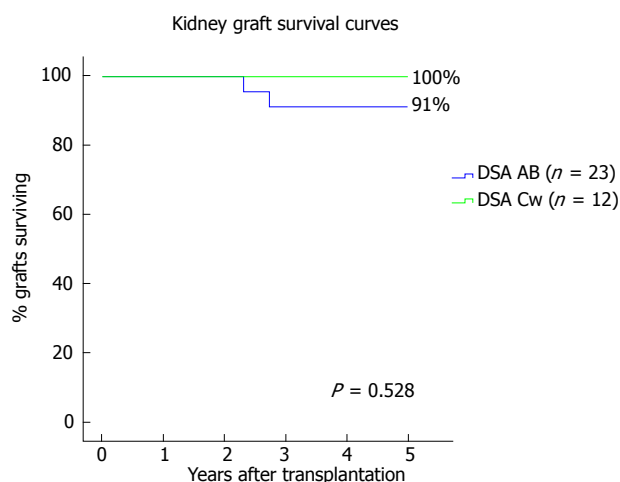


Figure 3 Kidney graft survival curves according with donor-specific antibodies human leukocyte antigen loci. DSA: Donor-specific antibodies.

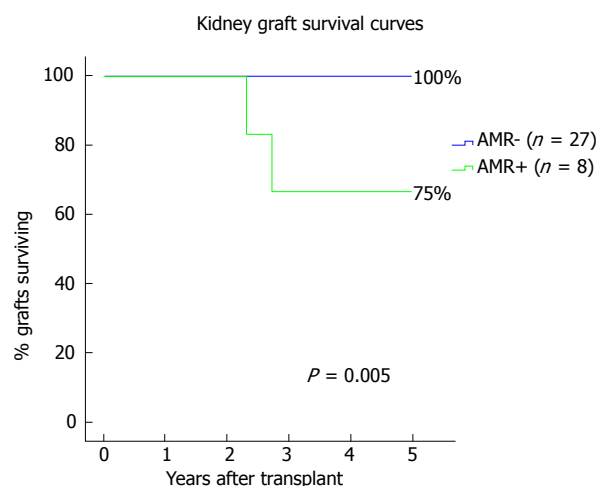


Figure 5 Kidney graft survival curves according with antibody-mediated rejection occurrence. AMR: Antibody-mediated rejection.

variable analysis (HR = 1.07 per 1000 increase in MFI, $P = 0.034$). At 1-year, eGFR was lower in AMR⁺ (median 49 mL/min) in comparison with AMR⁻ patients (median 58 mL/min) ($P = 0.068$), as shown in Figure 4. At the end of follow-up, kidney graft survival (Figure 5) was 75% in patients that experienced AMR and 100% in those who did not (Log-rank $P = 0.005$).

DISCUSSION

This retrospective study demonstrates that patients with preformed DSA solely antiHLA-Cw had a similar impact on post-transplant outcomes comparing to those patients with preformed antiHLA-A/-B DSA. Both groups had a relative high incidence of AMR at one year, 26% in the DSA-A-B group and 25% in DSA-Cw group. Also, the impact on graft outcomes measured by eGFR at one-year and graft survival at the end of follow-up was comparable

between groups.

HLA-Cw molecules are scantily expressed at the cell surface compared with HLA-A and HLA-B locus products, but intracellular HLA-A, HLA-B and HLA-Cw alleles are expressed in similar quantities^[3,11]. One reason pointed for this low amount at the cell surface is the fact that HLA-Cw alleles interact in a very stable way with the transporter associated with antigen processing (TAP) and they are kept in the endoplasmic reticulum, where they are degraded^[11]. Another justification for finding low HLA-Cw at cell proposed by McCutcheon *et al*^[3] is that HLA-Cw heavy chain mRNA is unstable and rapidly degraded, resulting in a lower rate of protein. This fact, associated with the modest sensitivity of the lymphocytotoxicity-based assays used in the past for identification of HLA-Cw antigens, probably explains why for many years they were considered less immunogenic and neglected in the matching systems of most kidney allocation procedures.

Table 3 Analysis of possible predictors of acute antibody-mediated rejection occurrence by univariable Cox regression

	HR for AMR	95%CI	P
Recipient			
Age (yr), per year	0.96	0.89-1.03	0.269
Female (<i>vs</i> male) gender	0.26	0.05-1.26	0.094
Retransplant	2.18	0.52-9.13	0.287
Previous blood transfusions	0.5	0.12-2.10	0.345
Previous pregnancies	0.24	0.03-1.99	0.187
Donor			
Age (yr), per year	1.01	0.96-1.06	0.684
Living donor	1.79	0.22-14.76	0.588
Pretransplant immunological data			
Peak PRA, per unit	1.01	1.00-1.03	0.149
DSA Cw (<i>vs</i> AB)	0.6	0.12-2.99	0.537
DSAsum MFI, per 1000 ¹	1.07	1.01-1.15	0.034
Transplant			
ABDR HLA mismatches, per unit	0.84	0.50-1.41	0.512
ATG (<i>vs</i> basiliximab) induction	1.68	0.34-8.34	0.527
FCXM + (<i>n</i> = 29)	0.75	0.09-6.21	0.787
Desensitized	1.2	0.24-5.97	0.825
Delayed graft function	2.55	0.61-10.68	0.201

¹Only independent predictor identified by multivariable Cox regression model (all variables included) using backward elimination (*P*-value < 0.050 needed for retention in the model). DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; MFI: Mean fluorescence intensity; ATG: Anti-thymocyte globulin; FCXM: Flow cytometry crossmatch.

Recent studies confirm their lower frequency. Bryan *et al.*^[6] in 2010 described in their sensitized transplant patients a 42% positivity to HLA-Cw, which was significantly lesser than sensitization to HLA-A (80%) and HLA-B (83%). In 2012, Ling *et al.*^[5], obtained similar results and showed that the frequency of antiHLA-Cw antibodies in sensitized patients was about 56%, lower than HLA-A (79%) and B (86%) antibodies. Our group evaluated 453 sensitized kidney transplantation candidates to determine the presence of antiHLA class I and class II antibodies, comparing how different sensitization events, such as pregnancy, transfusion or previous organ transplantation, affected the degree of HLA alloimmunization^[12]. For antiHLA antibodies against class I, if the sensitization event was previous transplant only, the antiHLA antibodies prevalence was 21.2% for -A, 28.8% for -B and 21.1% for -Cw; if the single sensitization event was previous transfusion, the antiHLA antibodies prevalence was 3.9% for -A, 5.5% for -B and 1.6% for -Cw. At last, if the sensitization event was pregnancy only, the antiHLA antibodies prevalence was 13.6% for -A, 11.1% for -B and 6.2% for -Cw.

In spite of their lower frequency, some reports have been published concerning their association with AMR and impact on graft function and survival^[8,13,14]. Besides, the recent development of the solid-phase immunoassays, in particular the single-antigen flow bead (SAFB) assays, allowed us to detect and properly identify anti-HLA-Cw antibodies. Tambur *et al.*^[15] compared virtual flow-cytometry cross-match to actual cross-match and described that 40% of the cases with a positive actual flow-cytometry cross-match and negative virtual cross-

match were explained by the presence of antiHLA-Cw antibodies. Gilbert *et al.*^[7] compared two groups of sensitized recipients, one group with only classical HLA-A, -B, -DR, -DQ antibodies (*n* = 176) and the other group with classical plus HLA-C and/or -DP antibodies (*n* = 27). They concluded that there was a significant increase in the number of AMR among the group with pre-transplant anti-Cw and -DP antibodies. However, they did not distinguish between pre-transplant anti-DP or anti-Cw antibodies, and they speculated that anti-DP antibodies seemed to be involved more often in poorer graft outcomes. Ling *et al.*^[5] investigated the clinical outcomes in kidney transplant patients with isolated Cw-DSA. They identified eight patients with pre-transplant DSA antiHLA-Cw, exclusively. During a median 6 mo of follow-up (range 3-24 mo), patient and graft survival was 100% without any acute rejection occurring. In this group, all the patients had induction therapy with thymoglobulin or basiliximab and additionally all patients received intravenous immunoglobulin, similar to patients with positive FCXM and/or cPRA > 50%. Even so, the median time of follow up was relatively short and may have underestimated the incidence of rejection. Aubert *et al.*^[9] evaluated retrospectively 22 renal transplant recipients with isolated antiHLA-Cw DSA at day 0 of renal transplant, comparing them with 88 allosensitized patients with no preformed DSA (control group), and followed for a period of 1 year. Acute AMR was diagnosed in six patients (27.3%) in patients with DSA-Cw vs 9% in those without DSA. In this study, the patients with DSA antiHLA-Cw received less-intensive immunosuppression than the control group of sensitized patients, including ATG induction (only 59.1%), and this may probably be a plausible explanation for this high rate of AMR. However they alert for the necessity of screening pre-transplant DSA HLA-Cw and subsequent modulation of immunosuppression in cases of positivity. More recently, Bachelet *et al.*^[10] investigated the clinical effect of DSA antiHLA-Cw and/or -DP, comparing 48 patients transplanted with isolated preformed DSA antiHLA-Cw and/or -DP with a group of HLA-sensitized recipients with no DSA (104 patients) and 47 kidney transplant recipients with preformed DSA antiHLA-A, -B, -DR, and/or -DQ. Two years after transplantation, the groups with DSA (both -Cw/-DP or -A/-B/-DR/-DQ) had similar incidence of AMR and graft survival (and worse than the group with no DSA), showing that preformed DSA anti-HLA-Cw and/or -DP were as deleterious as DSA anti-HLA -A/-B/-DR/-DQ.

Our data reached similar results of these previous studies, confirming that DSA-Cw is associated with a similar incidence of AMR and impact on graft survival in comparison with "classical" DSA against class I^[9,10].

We have also shown that patients that experienced AMR had a significant lower kidney graft survival in comparison to patients who did not (respectively, 75% vs 100%, Log-rank *P* = 0.005), with the sole independent predictor of AMR incidence being DSA strength. The negative impact of DSA for AMR occurrence and adverse results on kidney graft

survival has been previously established^[2]. Lefaucheur *et al.*^[16] stated that it is the occurrence of AMR associated with DSA that has impact on graft survival, since graft survival of DSA-positive patients, in the absence of AMR, is the same as DSA-negative patients. Furthermore, DSA characteristics as number, class or strength may have a negative impact on graft outcomes^[1,17-19]. Malheiro *et al.*^[20] showed that DSA strength (MFI) had a reasonable ability to predict AMR occurrence, with no cases of AMR occurring below a MFI < 3000. However when the MFI values increased from this value, also did the risk of AMR. Again, Aubert *et al.*^[9] in their retrospective study with 22 renal transplant recipients with preformed isolated antiHLA-Cw DSA, showed that the level of DSA at day 0 was predictive for AMR: Measurement of MFI was 4966 (978-17941) in the AMR group and 981 (530-8012) in the group of patients without AMR ($P = 0.017$).

This study has limitations. First, the small number of patients in the cohort limits our ability to generalize the results. Second, follow-up time difference may have limited the comparative analysis of graft survival according with DSA HLA loci. Contrarily, AMR incidence was not influenced by it, since it was analyzed at 1-year post-transplant. Third, there was no protocol biopsies performed in our patients and it is an important tool for HLA incompatible kidney transplantation^[21,22]. Lastly, the limitations of SAB assay are well established and their reported MFI values should be considered for analyzing our results^[23].

In summary, our data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class I locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection. Therefore, HLA-C typing and respective antibody identification will benefit sensitized patients during organ allocation.

COMMENTS

Background

Classically, antibodies to major human leukocyte antigen (HLA) Class I (A and B) and Class II (DR and DQ) antigens are considered to be responsible for most cases of AMR. Compared to other class I antiHLA antibodies, antiHLA-Cw are considered less immunogenic.

Research frontiers

Preformed antiHLA-Cw donor-specific antibodies (DSA) seem to have the same impact on graft outcome as DSA against "classical" HLA loci (-A, -B, -DR and -DQ), suggesting that it should also be considered in transplant allocation systems and in immunologic risk stratification algorithms.

Innovations and breakthroughs

The clinical relevance of preformed antiHLA-Cw DSA in kidney transplant patients remains controversial, so the authors performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival rates, respectively, at 1 and at 5-years of follow-up, were comparable between groups.

Applications

The data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class I locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection.

Terminology

HLA: Human leukocyte antigen; DSA: Donor-specific antibodies; AMR: Antibody-mediated rejection.

Peer-review

The topic is very interesting. The authors investigated the possible role of preformed donor-specific antibodies against HLA antigens, specially anti-Cw antibodies compared to standard anti A/B antibodies. The importance of Cw antibodies is still under investigation and this study is valuable about this topic. This article is worthwhile for publication.

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Retrospective Study

Tacrolimus confers lower acute rejection rates and better renal allograft survival compared to cyclosporine

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Abstract

AIM

To compare the impact of tacrolimus (FK) and cyclosporine (CYA) on acute rejection and graft survival and to assess the predominant causes of graft loss between patients receiving these two calcineurin inhibitors (CNIs).

METHODS

Retrospective review of 1835 patients who received a kidney transplant (KTX) between 1999-2012. Patients were grouped based on initial CNI utilized: 1195 in FK group, 640 in CYA group. Data on baseline characteristics, clinical outcomes, and causes of graft loss in both groups were analyzed.

RESULTS

Cumulative acute rejection rates were 14% in the FK vs 24% in the CYA group. Despite more marginal donor characteristics in the FK group, these patients had better graft survival rates compared to the CYA group. Three and five year graft survival rates were 88% and 84% respectively in the FK group compared to 79% and 70% respectively in the CYA group ($P < 0.001$). After multivariate analysis, which controlled for confounders, FK use was a strong predictor for lower acute rejection

rates [odds ratio (OR) 0.60, 95%CI: 0.45-0.79] and better renal allograft survival (OR 0.740, 95%CI: 0.58-0.94). Death with a functioning graft was the most common cause of graft loss in both groups. Common causes of death included cardiovascular disease, infections, and malignancies. Chronic allograft nephropathy was also found to be an important cause of graft loss, being more prevalent in the CYA group.

CONCLUSION

The use of FK-based maintenance immunosuppression therapy is associated with a significantly lower rate of acute rejection and better graft survival compared to CYA-based regimen. Individualizing immunosuppression through risk-stratified CNI choice may lead to improved outcomes across all spectra of KTX patients.

Key words: Tacrolimus; Cyclosporine; Renal allograft survival

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Core tip: Tacrolimus (FK) has surpassed cyclosporine (CYA) as the calcineurin inhibitor (CNI) of choice for the vast majority of kidney transplant (KTX) programs. Yet, CYA continues to be an important alternative for patients intolerant to FK. FK is associated with significantly lower rate of acute rejection and better graft survival compared to CYA. Individualizing immunosuppression through risk-stratified CNI choice may lead to improved outcomes across all spectra of KTX patients.

Kamel M, Kadian M, Srinivas T, Taber D, Posadas Salas MA. Tacrolimus confers lower acute rejection rates and better renal allograft survival compared to cyclosporine. *World J Transplant* 2016; 6(4): 697-702 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/697.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.697>

INTRODUCTION

Calcineurin inhibitors (CNIs) are the main immunosuppressive agents utilized in kidney transplantation^[1]. Cyclosporine (CYA) and tacrolimus (FK) are currently the most widely used maintenance immunosuppressants for prevention of acute rejection in kidney transplant recipients. CYA-based regimen was more common in the era of 1990 until 2002, after which FK-based regimen became more commonly used in most transplant programs. In our transplant center, FK became the primary CNI of choice in 2005. FK and CYA show variable side effect profiles. Hypertension, hyperlipidemia, gum hypertrophy, and hirsutism occur more frequently with CYA use. On the other hand, a higher incidence of post-transplant diabetes mellitus is observed with FK therapy. Prolonged use of CNI may result in nephrotoxicity.

FK use is associated with less acute rejection

compared to CYA, as documented in different studies^[2,3]. Mayer *et al*^[2] found that among 448 renal transplant recipients who were on triple therapy (FK or CYA + Azathioprine + Prednisone), patients who were in the FK group had a significant reduction in the frequency of acute rejection at 12 mo (FK 25.9% vs CYA 45.7%; $P < 0.001$). Ekberg *et al*^[3] also found that at 12 mo post-transplant, the use of FK-based regimen is associated with less biopsy-proven acute rejection compared to CYA use (12.3% vs 25.8%, $P < 0.01$).

FK is frequently preferred in patients with high immunologic risk (highly sensitized, ABO-incompatible organ recipients), delayed graft function, and African American race. Data regarding graft survival based on the use of FK vs CYA is controversial with most studies showing similar graft survival rates with the use of either agent^[4]. Vincenti *et al*^[5] showed comparable patient (79.1% vs 81.4%; $P = 0.472$) and graft (64.3% vs 61.6%; $P = 0.558$) survival between treatment arms at 5 years of follow-up among FK and CYA-treated patients. However, after accounting for patients initially on CYA who crossed over to FK, the authors found significantly reduced graft failure in the FK group^[5]. Gonwa *et al*^[6] showed that among 223 kidney transplant recipients who experienced delayed graft function, patients who used FK-based therapy had a better 3-year graft survival compared to CYA use (84.1% vs 49.9%, $P = 0.02$). Given these conflicting findings, this study aims to compare rates of acute rejection and graft loss among patients who receive FK and CYA.

MATERIALS AND METHODS

Patients

This was a retrospective cohort study of 1835 patients who received a KTX between 1999-2012 at a single center. Patients were grouped based on the type of CNI they were prescribed: 1195 patients utilized FK-based immunosuppression whereas 640 patients were on a CYA-based regimen. All patients received an antimetabolite and prednisone in combination with CNI. The initial CYA dose was 4-5 mg/kg PO BID. Target CYA levels were 350-400 ng/mL for weeks 1-4, 250-350 ng/mL for weeks 5-12, 200-300 ng/mL within the first year post-transplant, and 100-200 ng/mL thereafter. Initial FK doses were given at 0.025-0.05 mg/kg PO BID. Target FK levels were kept between 8-12 ng/mL within the first four weeks post-transplant, then 6-10 ng/mL within the first year post-transplant, and 4-6 ng/mL subsequently. Characteristics of recipients (age, race, sex, BMI, etiology of kidney disease, history of heart disease, diabetes, hypertension, years on dialysis, panel reactive antibody, preemptive transplant, living donor transplant), and donors [age, race, kidney donor risk index (KDRI)] were compared between groups. Characteristics of the kidney transplant (cold ischemia time, induction agent) as well as clinical outcomes (cumulative acute rejection rate, delayed graft function, three, and five year graft survival) were also analyzed. The Banff '97 criteria were used to define the

Table 1 Characteristics of patients in tacrolimus and cyclosporine group *n* (%)

Parameter	Cyclosporine <i>n</i> = 640	Tacrolimus <i>n</i> = 1195	<i>P</i> -value
Mean recipient age (yr)	49 ± 12	50 ± 13	0.059
Race			0.96
Non-African American	281 (44)	526 (44)	
African-American	359 (56)	669 (56)	
Sex			0.78
Male	371 (58)	693 (58)	
Female	269 (42)	502 (42)	
BMI	26 ± 7	28 ± 5	0.462
Etiology of kidney disease			
DM	172 (26.9)	375 (31.4)	0.044
HTN	317 (49.5)	559 (46.8)	0.26
FSGS	36 (5.6)	78 (7.3)	0.177
IgA nephropathy	24 (3.8)	34 (2.8)	0.291
Polycystic kidney	63 (9.8)	89 (7.4)	0.076
History of DM	186 (29)	394 (33)	0.092
History of HTN	595 (93)	1135 (95)	0.122
History of heart disease	134 (21)	227 (19)	0.38
Years on dialysis	3 ± 2.4	3 ± 2.9	0.01
PRA	5%	17%	< 0.010
Preemptive transplant	122 (19)	239 (20)	0.49
Living donor transplant	122 (19)	179 (15)	0.27
CIT (h)	13 ± 9	16 ± 9	0.621
Mean donor age (yr)	31 ± 18	36 ± 16	< 0.010
KDRI	0.9 ± 0.6	1.3 ± 0.4	< 0.010
African-American donor	122 (19)	203 (17)	0.27
Induction therapy			< 0.010
Cytolytic agents	70 (11)	550 (46)	
IL-2 receptor antagonist	570 (89)	645 (54)	

DM: Diabetes mellitus; HTN: Hypertension; FSGS: Focal segmental glomerulosclerosis; PRA: Panel reactive antibody; CIT: Cold ischemia time; KDRI: Kidney donor risk index.

different grades of rejection. Based on center protocol, Banff 1A and 1B rejection episodes were treated with Methylprednisolone IV. Rejection episodes with Banff 2A grade or higher were treated with anti-thymocyte globulin. Subset analysis was conducted on subjects who had graft loss to retrospectively investigate the factors leading to graft loss. For patients who died, causes of death were presented as overall prevalence of infections (encompassing sepsis, bacterial, fungal, CMV, and other viral infections), malignancies (encompassing solid organ tumors, hematologic malignancies, and post-transplant lymphoproliferative disorder), and cardiovascular diseases (encompassing acute myocardial infarction and cerebrovascular accident). Cause of death classified under "other" includes accidents, unknown, or undocumented. Non-adherence was defined as documentation in the medical record by a provider that a patient was not taking their immunosuppressive regimen as prescribed. Under immunosuppression was defined as evidence of kidney transplant injury related to rejection that led or contributed to graft loss.

Statistical analysis

The statistical review was performed by a clinician with advanced biostatistical training and experience.

Two-sided independent student's *t*-test was used to compare continuous data while the χ^2 test was used to compare categorical data. A two-sided *P*-value of less

than 0.05 was considered statistically significant.

Multivariate survival analysis, using both logistic and Cox regression, was used to assess the association between CNI choice and acute rejection (logistic), graft survival (Cox), and patient mortality (Cox), while controlling for additional transplant variables known to influence outcomes or those that differed across CNI choice. In a subset analysis of patients who had graft loss, causes of graft loss, and causes of death were compared between the two groups using standard univariate comparative statistics. All analyses were conducted using SPSS version 21.0 (IBM Corp, Armonk, NY).

RESULTS

Patient characteristics

Table 1 displays demographic characteristics of the two groups. Mean recipient age, race, BMI, etiology of kidney disease, comorbidities, and dialysis vintage, were similar between the two groups. Patients on FK had higher PRA compared to patients on CYA group (17% vs 5%, *P* < 0.01). Rates of living donor transplants were similar between the two groups. Among patients who received a deceased donor transplant, KDRI was higher in those who received FK. More patients in the FK group received induction agent with depleting antibodies (46% vs 11%, *P* < 0.01).

Table 2 Clinical outcomes

Parameter	Tacrolimus <i>n</i> = 1195	Cyclosporine <i>n</i> = 640	<i>P</i> -value
Mean glomerular filtration rate	56 ± 19	46 ± 17	0.09
Delayed graft function <i>n</i> (%)	179 (15)	115 (18)	0.049
Acute rejection (biopsy proven) <i>n</i> (%)	167 (14)	154 (24)	< 0.010
Three years graft survival	88%	79%	< 0.010
Five years graft survival	84%	70%	< 0.010

Table 3 Multivariate analysis of factors associated with acute rejection

Variable	Hazard ratio	95%CI	<i>P</i> -value
CNI tacrolimus	0.6	0.45-0.79	< 0.001
Retransplant	1.43	0.91-2.24	0.123
PRA	1	0.99-1.00	0.529
Cytolytic induction	0.5	0.36-0.69	< 0.001

CNI: Calcineurin inhibitor; PRA: Panel reactive antibody.

Table 4 Multivariate analysis of factors associated with graft loss

Variable	Hazard ratio	95%CI	<i>P</i> -value
CNI tacrolimus	0.74	0.58-0.94	0.012
History of DM	1.41	1.13-1.76	0.002
History of HTN	0.56	0.34-0.94	0.029
Delayed graft function	2.1	1.66-2.66	< 0.001
Acute rejection	1.59	1.26-2.01	< 0.001

CNI: Calcineurin inhibitor; DM: Diabetes mellitus; HTN: Hypertension.

Clinical outcomes

Patients in the FK group had better clinical outcomes in terms of delayed graft function (DGF) rate (15% vs 18%, *P* = 0.049), cumulative biopsy proven acute rejection rates for Banff 1A and higher, as well as antibody-mediated rejection (14% vs 24%, *P* < 0.01), three year graft survival (88% vs 79%, *P* < 0.010), and five year graft survival (84% vs 70%, *P* < 0.01) (Table 2). FK was a strong predictor of lower acute rejection rates. After multivariate analysis, which accounted for recipient immunologic risks (age, gender, re-transplant, PRA, HLA mismatches, cold ischemic time, induction), donor characteristics (deceased status, ECD, age, race) and delayed graft function, FK continued to be strongly associated with lower acute rejection rates, as compared to CYA (OR = 0.60, 95%CI: 0.45-0.79; *P* < 0.001) (Table 3). Further analysis showed that even after controlling for all other variables, including delayed graft function and acute rejection, FK remained a strong and statistically significant predictor of graft survival (OR = 0.740, 95%CI: 0.58-0.94; *P* = 0.012) (Table 4) (Figure 1).

Graft loss

During the study period, there were 106 patients in the FK group and 123 in the CYA group who had graft loss. Death with a functioning graft was the cause of graft loss

in the majority of these patients. The leading causes of death among the patients include cardiovascular disease, infections, and malignancies (Table 5). The contribution of non-adherence and underimmunosuppression in patients who had graft loss was not significantly different between the FK and CYA groups.

DISCUSSION

The utilization of potent immunosuppressive medications such as CYA and FK has led to progressive improvement in renal allograft survival. Two large studies on kidney transplant recipients showed that the incidence of acute rejection is much lower with FK-based immunosuppression compared to CYA-based regimen^[2,3]. Our study demonstrated similar findings of lower acute rejection rates in patients using FK compared to those on CYA. Acute rejection rate was significantly lower in the FK group despite the relatively higher degree of sensitization, as evidenced by higher PRA, in this group. Multivariate analysis showed that FK was a strong predictor for lower acute rejection rates while controlling for recipient, donor, and transplant characteristics.

The shortage of deceased donor kidneys and the growing number of patients on the waiting list has driven the increased utilization of organs with relatively marginal donor characteristics. Donor factors affect initial graft function and survival^[7]. Donor factors that may influence graft survival include age, gender, hypertension, and cardiovascular disease^[8]. The KDRI is a comprehensive metric that was recently developed to assess the relative risk of graft failure associated with various combinations of donor characteristics. Kidneys with the highest KDRI quintile are associated with lower graft survival^[9]. Although many trials have shown similar graft survival outcomes with FK when compared with CYA-based regimen^[4], some studies showed better survival and outcomes with FK-based immunosuppression^[6]. Our

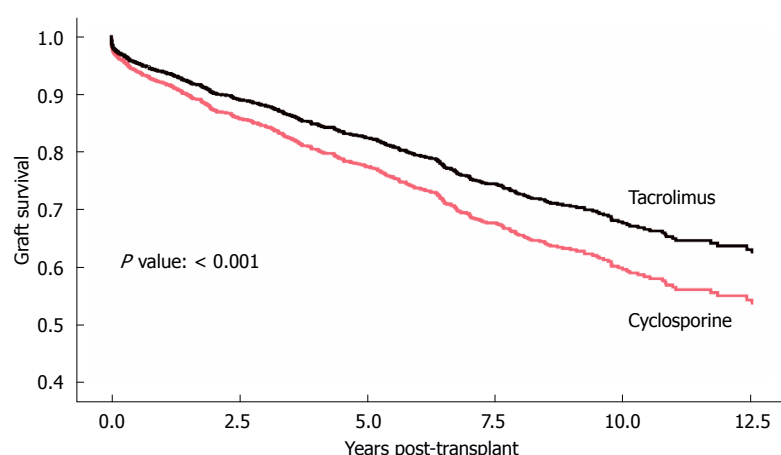


Figure 1 Kaplan Meier curve showing effect of tacrolimus vs cyclosporine on graft survival.

Table 5 Graft loss *n* (%)

Parameter	Tacrolimus <i>n</i> = 106	Cyclosporine <i>n</i> = 123	<i>P</i> -value
Death with functioning graft	61 (58)	66 (54)	0.55
Cause of death			0.85
Cardiovascular disease	19 (18)	19 (15)	
Infections	10 (9)	9 (7)	
Malignancy	10 (9)	9 (7)	
Others	33 (31)	41 (33)	
Causes of graft loss			0.44
Chronic allograft nephropathy	18 (17)	29 (24)	
Acute rejection	14 (13)	11 (9)	
Acute on chronic rejection	8 (8)	13 (11)	
Recurrent disease	1 (1)	1 (1)	
Death	63 (59)	68 (55)	
Component of non-adherence	15 (14)	20 (16)	0.65
Component of underimmunosuppression	21 (20)	25 (20)	0.92

study showed that although patients in the FK group received kidneys from more marginal donors (higher KDRI), the three year and five year graft survival was still more superior in this group compared to the CYA group (Figure 1).

The risk of infections after kidney transplant depends on the net state of immunosuppression. As FK was shown to be associated with less acute rejection compared to CYA^[10], it may concurrently cause more intense immunosuppressive effects compared to CYA. Thus, risk of infections after kidney transplant may be higher with FK compared to CYA. This may be exemplified by the higher incidence of polyomavirus (BK) viremia in patients on FK-based regimen compared to CYA^[11]. Progression of BK viremia may lead to BK nephropathy, which can then eventually cause premature renal allograft failure^[11]. However, in our subjects who had graft loss, we did not observe a significant difference in the prevalence of infections (including BK) in the FK and CYA groups.

The use of maintenance immunosuppressive medications among transplant recipients increases the long-term risk of malignancy, compared with that of the general population. The overall level of immunosuppression appears to be the principal factor that increases the risk of post-transplant malignancy. Both FK and CYA are associated

with an increased risk of malignancy following kidney transplant^[12,13]. No direct comparison between these two agents has been reported regarding the incidence of malignancy following kidney transplant. However, FK was found to have higher incidence of *de novo* malignancy after liver transplant compared to CYA^[14]. In our study, we did not find a significant difference in the prevalence of malignancies between the two groups.

Cardiovascular disease is a leading cause of mortality among kidney transplant recipients. Death from cardiovascular disease is the most common cause of renal allograft loss^[15]. CNIs potentially contribute to increased risk of cardiovascular events indirectly by the development of new-onset diabetes mellitus, hypertension, and hyperlipidemia. Clinical trials have shown a higher incidence of post-transplant diabetes mellitus with FK. However, the risk of hypertension and hyperlipidemia is slightly higher with CYA than FK. No direct comparison has been done between FK and CYA regarding the incidence of cardiovascular disease. In our study, we found that FK was associated with a slightly higher prevalence of cardiovascular disease compared to CYA, although the difference was not statistically significant.

In conclusion, FK is associated with lower prevalence of acute rejection compared to CYA. It confers better three and five year graft survival even with the use of

organs with marginal deceased donor characteristics. An individualized approach to the choice of CNI needs to be employed in order to achieve the best possible outcome while minimizing adverse effects. The use of either FK or CYA should be individualized according to the patient's comorbid conditions and immunological risk.

COMMENTS

Background

Calcineurin inhibitors (CNIs) [cyclosporine (CYA) and tacrolimus (FK)] are currently the most widely used maintenance immunosuppressants for prevention of acute rejection following kidney transplantation. However, data on the impact of these CNIs on acute rejection rate and graft survival have remained equivocal.

Research frontiers

The choice of immunosuppressive regimen that will achieve the best renal allograft outcomes remains an important focus in the care of kidney transplant recipients.

Innovations and breakthroughs

The data showed lower acute rejection rates and better graft survival in patients on FK compared to those on CYA.

Applications

The use of either FK or CYA should be individualized based on patient's comorbidities and immunological risk.

Terminology

FK: Tacrolimus; CYA: Cyclosporine; CNI: Calcineurin inhibitor; DGF: Delayed graft function; DM: Diabetes mellitus; HTN: Hypertension; FSGS: Focal segmental glomerulosclerosis; PRA: Panel reactive antibody; CIT: Cold ischemia time; KDRI: Kidney donor risk index; GFR: Glomerular filtration rate.

Peer-review

The authors are presenting their experience in the use of CNIs in the immunosuppression post renal transplantation.

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Retrospective Study

Quality of life 10 years after liver transplantation: The impact of graft histology

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Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Abstract

AIM

To evaluate the relationship between the state of transplanted liver graft and the recipient quality of life (QOL) of histologically proven lesions in a 10-year post liver transplantation (LT) cohort of patients.

METHODS

Seventy-two recipients with a functional first graft at 10 years post-LT underwent liver biopsy and completed a QOL questionnaire. Logistic regression analysis was used to explore associations between histological, clinical and

QOL criteria.

RESULTS

Ten years after LT, fibrosis was detected in 53% of patients, and affected the general health perception, while ductopenia, present in 36%, affected the well-being ($P = 0.05$). Hepatic steatosis (HS) was present in 33% of patients and was associated with the worst QOL score on multiple domains. When compared to patients without HS, patients with HS had significantly higher incidence of fibrosis ($P = 0.03$), hepatitis C virus (HCV) infection ($P = 0.007$), and more patients had retired from their job ($P = 0.03$). Recurrent or *de novo* HCV-associated fibrosis and patient retirement as objective variables, and abdominal pain or discomfort and joint aches or pains as subjective variables, emerged as independent determinants of HS.

CONCLUSION

Long-term liver graft lesions, mainly HS presumably as a surrogate marker of HCV infection, may have a substantial impact on QOL 10 years after LT.

Key words: Liver transplantation; Quality of life; Liver biopsy; Hepatic steatosis; Liver fibrosis

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Core tip: Objective and subjective parameters are helpful in the accurate assessment of long-term outcome in liver transplantation recipients. The main finding of this study was that histological lesions in the transplanted liver 10 years after liver transplantation can affect the recipient quality of life. Hepatic steatosis had the most significant impact on quality of life and this was independent of alcohol consumption, fibrosis, diabetes and body mass index. The strongest determinants of a worse quality of life in patients with hepatic steatosis were hepatitis C virus infection and retirement from job irrespective of patient-age.

Karam V, Sebah M, Rifai K, Yilmaz F, Bhangu P, Danet C, Saliba F, Samuel D, Castaing D, Adam R, Feray C. Quality of life 10 years after liver transplantation: The impact of graft histology. *World J Transplant* 2016; 6(4): 703-711 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/703.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.703>

INTRODUCTION

The goal of liver transplantation (LT) is to ameliorate not only survival, but also quality of life (QOL) while minimizing the effects of disease and costs of care. Analysis of data from the European Liver Transplant Registry (ELTR) shows that 38% of the patients transplanted in 1991 were still alive with their first graft at least 10 years post LT^[1]. The increasing proportion of recipients alive at long-term follow up has incited transplant professionals to focus on long

term morbidity-free survival and an acceptable QOL.

The QOL is increasingly recognized as an important measure of outcome after solid organ transplantation^[2-4]. We showed in a previous study that the challenge of maintaining long-term well-being is achieved to a greater extent in liver transplant recipients than in other solid-organ transplant recipients^[4]. However, short term and long term QOL in liver transplant recipients is still inferior to that of the general population^[3,4].

Studies of long-term survivors have been mainly based on clinical data and histological follow up at long term, or with respect to indication for LT, immunosuppressive regimen or recipient and donor criteria^[5-10]. In previous studies, we justified the use of biopsies in the follow-up protocol to adjust treatments, not only in HCV-infected patients (in whom fibrosis progression was rapid and non-linear), but in all recipients^[11,12].

No study has been published assessing the relationship between the state of transplanted liver graft and the recipient QOL of histologically proven lesions in a 10-year post LT cohort of patients.

MATERIALS AND METHODS

Patients

Between September 1989 and December 1992, 485 LT were performed in 432 patients at Paul Brousse Hospital (Villejuif, France). During the 10th year post LT, among the 145 patients who were alive with a first functional graft, 126 accepted to complete the QOL questionnaire, and among these 72 accepted to have a liver biopsy done. For the purpose of this study, only the 72 subjects who underwent both a 10-year post LT liver biopsy and completed the QOL auto-questionnaire were included.

Questionnaire

QOL data was obtained using the NIDDK questionnaire^[2]. The questionnaire includes 21 disease-specific items assessing symptoms related to chronic liver disease. We used a validated French version of the questionnaire developed previously using the back-translation method

Five domains of QOL; physical distress (PHD), psychological distress (PD), personal function (PF), social/role function (SRF), and general health perception (GHP) are well represented in the questionnaire. Each symptom is numerically graded according to severity and then a composite overall score is calculated from all domains^[3,4].

Histological evaluation

Prospectively obtained reperfusion biopsies and ten-year post LT liver biopsies were reviewed by the same experienced pathologist (MS) who was blinded to clinical information.

Portal tracts, hepatic veins and parenchyma were systematically analysed according to a preformed format. Fibrosis was staged on a five-point scale: 0, none; 1, portal fibrosis without septa; 2, few septa; 3, numerous septa without cirrhosis; 4, cirrhosis. Ductopenia evaluated on

liver biopsy was analysed according to the Banff criteria^[13], and ductopenia was considered as significant when the percentage of bile duct lost exceeded 20%. Steatosis was scored according to the percentage of biopsy tissue involved. Patients were considered in Hepatic Steatosis (HS) group when the percentage of steatosis exceeded 10%. Minimal changes were defined as the absence of all the above cited lesions or the existence of only one of the following criteria: steatosis < 10%, sinusoidal fibrosis, or minimal bile duct or lobular inflammation. The final diagnosis was established by joint review of records; biochemical, virological, and immuno-histochemical data.

Statistical analysis

Continuous variables are given as mean \pm SD. Comparisons of continuous variables were performed with the Mann-Whitney test and those of nominal variables with χ^2 contingency test or Fisher's exact test when appropriate. Logistic regression was conducted to examine determinants of HS. We conducted two separate regressions: (1) with the objective (clinical) variables; and (2) with the subjective (QOL) variables. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Characteristics of patients who were not included in the study

Only 72 patients accepted to complete the QOL questionnaire and undergo liver biopsy amongst the 145 patients who survived for a minimum of 10 years with a first functional graft. Since this could create a selection bias in our study, we compared the selected and unselected patients with respect to characteristics at the time of LT and at 10-years post-transplant. Clinico-demographic characteristics like age at transplantation, sex, donor age, ABO group, CMV and reperfusion biopsy status, indication for LT, liver enzyme tests at the time of 10 year control of included patients were not statistically different from the non-included patients. Moreover, comparison of all domains of QOL has not shown any statistically significant difference between the selected and unselected patients (data not shown).

Patient characteristics at the time of LT

The mean age at time of transplantation was 35 ± 19 years and proportion of female patients was 52%. Mean donor age was 27 ± 11 years. The main indications for LT were PBC (25%), acute liver failure (24%) and viral cirrhosis (20%) [mostly hepatitis C virus (HCV) related (12%)]. The reperfusion biopsies showed steatosis ($\geq 10\%$) in 18% of patients and reperfusion injury related lesions in 86% of reperfusion biopsies. Twenty-six percent of these lesions were classified as mild while 60% were of moderate to severe-degree (Table 1).

Patient status and histological findings 10 years after transplantation

As regards co-morbidities present in the recipients

at follow-up, thirty eight (53%) patients had arterial hypertension and 7 (10%) suffered from diabetes mellitus (mostly type II). According to the body mass index (BMI), 9 (13%) patients were underweight, 50 (69%) patients were within normal limits, 10 (14%) were overweight and 3 (4%) were obese. Fifteen (21%) patients consumed alcohol with 1.1 ± 0.3 drinks/day (one drink = 1 bottle of beer or 1 glass of wine or 1 mixed drink, the equivalent of 1.25 grams of alcohol) and 12 (17%) were tobacco smokers with 1.9 ± 0.8 cigarettes/day (Table 2).

Forty one patients (57%) had HCV infection, amongst them 35 (49%) had *de novo* infection whereas 6 (8%) had recurrent HCV infection. Most patients had been transplanted before the screening of blood and organ of donors for HCV serology began (pre HCV era). The predominant HCV genotype in our study cohort was genotype 1 (60%), mostly 1b subtype (51%). The proportion of other genotypes was; genotype 2 (12%), genotype 3 (9%) and genotype 4 (6%). In 13% of cases HCV-infection was established by RNA revelation. At the time of biopsy and QOL evaluation, none of the patients was being treated with interferon.

The immunosuppression was mainly using Cyclosporine-based (96%) in the study population.

The main histological findings were as follows: (1) fibrosis F1-F4 ($n = 38$, 53%), with F1 ($n = 16$, 22%), F2 ($n = 13$, 18%), F3 ($n = 4$, 6%). Cirrhosis (F4) was found in 7% ($n = 5$) of cases; (2) ductopenia ($n = 26$, 36%) with a mean percentage of bile duct loss of $40\% \pm 20\%$; and (3) steatosis ($n = 24$, 33%) with a mean percentage of $19 \pm 17\%$, which was mostly macrovacuolar ($n = 23$, 32%). Combined fibrosis and steatosis was found in 24% ($n = 17$) of patients. Only 23% ($n = 16$) of biopsies contained minimal-change lesions (as defined above).

Relation between QOL and histological lesions

Overall-QOL was not affected by fibrosis or ductopenia (Figure 1A and B). Nevertheless, GHP score was lower in patients with fibrosis ($P = 0.02$) and well-being score was lower in patients with ductopenia ($P = 0.05$). The overall-QOL score was the lowest in HS patients ($P = 0.007$) (Figure 1C). HS impaired particularly the PHD ($P = 0.002$), PD ($P = 0.01$) and GHP ($P = 0.05$). According to these results, we focused our study on the group of patients with HS.

Profile of patients with hepatic steatosis

As the worst QOL score on multiple domains was associated strikingly with HS we made a detailed analysis to compare the group with steatosis on 10 year liver biopsy, with those without. There were no statistically significant differences between the groups with respect to data at the time of LT except for recipient age (32 ± 21 years vs 42 ± 12 years; $P = 0.04$) (Table 1). At 10 year post LT follow-up, the BMI (22.6 ± 3.4 vs 22.3 ± 3.9), rate of diabetes (13% vs 9%), rate of arterial hypertension (54% vs 54%) and immunosuppressive dosage were not statistically higher in HS group. No difference was found

Table 1 Relationship between various parameters at the time of liver transplantation and the incidence of hepatic steatosis on 10-year post-liver transplantation biopsy

	All subjects <i>n</i> = 72	No HS <i>n</i> = 48	HS <i>n</i> = 24	<i>P</i> ¹
Age (yr)	35 ± 19	32 ± 21	42 ± 12	0.04
Gender (female)	52%	60%	71%	NS
Disease				
Acute hepatic failure	24%	27%	17%	NS
Primary biliary cirrhosis	25%	25%	25%	NS
HBV-related cirrhosis	8%	6%	12%	NS
Autoimmune cirrhosis	7%	6%	8%	NS
Biliary atresia	5%	8%	0%	NS
HCV-related cirrhosis	12%	6%	25%	NS
Metabolic disease (Wilson disease)	1%	2%	0%	NS
Alcohol related cirrhosis	1%	2%	0%	NS
Primary sclerosing cholangitis	4%	4%	4%	NS
Cryptogenic cirrhosis	2%	4%	0%	NS
Hepatocellular carcinoma	8%	8%	8%	NS
ABO compatible	97%	96%	100%	NS
Donor age (years)	27 ± 11	27 ± 12	27 ± 10	NS
Donor gender (female)	41%	42%	39%	NS
Urgency	25%	27%	21%	NS
Cold ischemic time (min)	410 ± 212	406 ± 215	429 ± 214	NS
Reperfusion biopsy ²				
Steatosis (≥ 10%)	18%	15%	22%	NS
% of steatosis	24 ± 15	31 ± 16	16 ± 8	NS
Reperfusion lesions				
Mild	26%	31%	17%	NS
Moderate to severe	60%	50%	79%	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. ¹Comparison between HS and No HS; ²Reperfusion biopsy not done in 10 cases (9 in Non HS group and 1 in HS group). HS: Hepatic steatosis; NS: Not significant; HCV: Hepatitis C virus.

in liver function tests (Table 2).

For the 24 patients with HS, three of the studied objective variables were statistically significant when compared to patients without HS at 10 years post LT: Fibrosis (71% vs 44%, $P = 0.03$), HCV infection (79% vs 46%, $P < 0.007$) (Table 2), and patient retirement (50% vs 21%, $P = 0.03$) (Table 3). Fibrosis was present in 17 (71%) patients and was mainly related to HCV infection. The HCV genotype 1 was predominant and represented 63%, mostly 1b subtype (42%). Despite the equally distributed mean age and the percentage of more than 60 years old patients in the two groups (29% vs 27%, $P = \text{ns}$), retired recipients were more prevalent in the HS group (46% vs 21%, $P = 0.03$).

Regarding the subjective QOL variables, a detailed analysis showed that the HS has an impact on 17 symptoms belonging to each one of the 5 domains of QOL (Table 4). The most affected physical symptoms were: Abdominal pain or discomfort ($P < 0.0001$), joint aches or pains ($P < 0.001$) and change in facial appearance ($P < 0.001$). Nervousness/anxiety was the most affected psychological symptom followed by a feeling of being depressed, sad or blue ($P < 0.01$). As regards PF, the health of HS patients currently limits their ability to perform vigorous activities such as running, heavy lifting or sport ($P < 0.001$). The SRF was affected by the patients' decreased interest in sex ($P = 0.003$). Finally, bodily pain during the last month represented the worst symptom of GHP ($P < 0.01$).

In multivariate regression analysis, two objective

variables emerged as independent determinants of HS: HCV infection ($P < 0.01$) and patient retirement ($P = 0.04$). So also, two subjective variables were significantly associated with HS: Abdominal pain or discomfort ($P < 0.01$) and joint aches or pains ($P = 0.04$) (Table 5).

DISCUSSION

The developments in surgical techniques, immuno-suppressive treatment modalities and better patient care have led to an increasing number of long-term survivors after LT, yet the QOL of transplant recipients does not always return to normal. The constant need for drug ingestion and monitoring the high incidence of recurrent or intervening diseases after LT, all seem to impair QOL^[14,15]. Nevertheless, reported data shows that most of the QOL parameters are better after transplantation than before^[2,3,16]. This study is an attempt to identify those factors which prevent long term liver transplant survivors from returning to a near normal lifestyle, with a specific focus on the relationship of QOL with graft histological status. One can recognize that a key challenge specific to this study could be its face validity, *i.e.*, comparison of histologic changes to QOL, which in the absence of advanced histologic changes is not intuitively related. In order to attenuate the relative fluctuations liver biopsies were reviewed by the same experienced pathologist (MS) who was blinded to clinical information. Moreover, we used the NIDDK questionnaire considered as one of the most appropriate and validated instruments for QOL

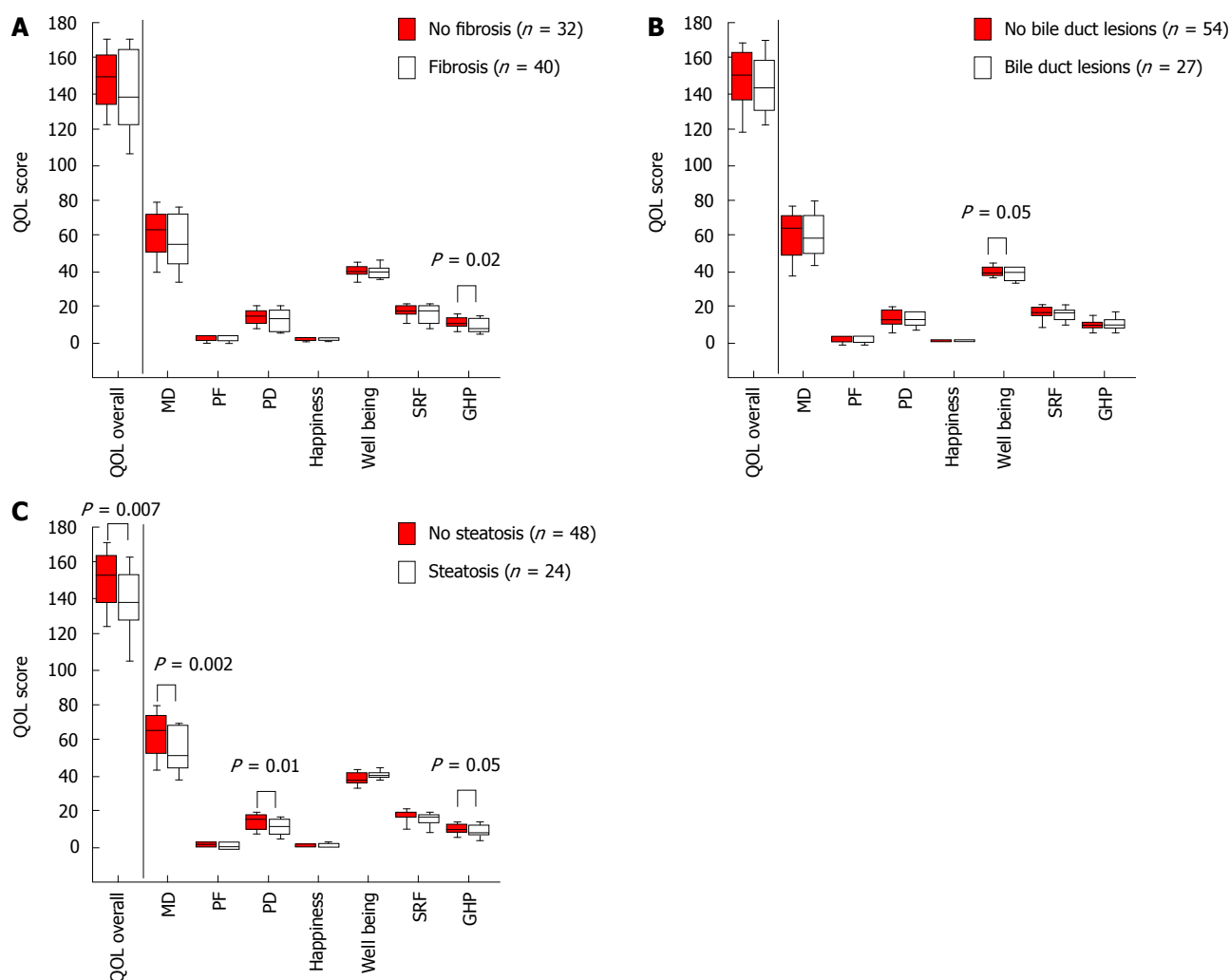


Figure 1 Relationship between quality of life domains and histological findings on 10 year post-liver transplantation liver biopsy. A: Fibrosis; B: Bile duct lesions; C: Steatosis. QOL: Quality of life; PF: Personal function; SRF: Social/role function; GHP: General health perception.

evaluation in transplant recipients^[17].

The main finding of this study was that histological lesions (especially HS) in the transplanted liver 10 years after LT can affect the recipients' QOL. Overall-QOL was not affected by fibrosis or ductopenia, but there was a significant decrease in GHP score in patients with fibrosis and in well-being score in patients with ductopenia. HS had the most significant impact on overall-QOL score and this was independent of alcohol consumption, fibrosis, diabetes and BMI.

Post-LT development of HS in recipients has only been analyzed in few studies so far^[18]. Post-transplant metabolic syndrome and graft NAFLD are being increasingly recognized as long term problems in LT recipients^[19,20]. Patients with post-LT NAFLD develop at the least an increased risk of cardiovascular events, rejection and infection^[21]. Recently, a retrospective study reported that the reasons for long term steatosis in liver allografts may be related to seven factors either present alone or in combination, such as graft steatosis at the time of transplantation, HCV infection, recurrence of NAFLD or alcoholic liver disease, metabolic syndrome, diabetes

mellitus and *de novo* NAFLD^[22]. Most of these factors are known risk factors for NAFLD in the non-transplant setting also.

For the determination of such potential underlying factors, we compared the groups with and without HS in our series. We could not find any significant difference between the groups, neither with respect to known metabolic risk factors not related to LT (such as incidence of diabetes, hypertension or recipient BMI) nor with respect to transplant-related factors (such as donor liver steatosis, reperfusion injury, alcohol abuse and immunosuppressive dosage). Only three of the objective variables were significantly different; HCV infection, fibrosis and patient retirement irrespective of age.

The post-transplant setting is a good background for the development of one or several components of the metabolic syndrome^[18], *de novo* NAFLD seems to be one of the most probable reasons for HS. For instance, high incidence of hypertension and hyperlipidemia in patients on Cyclosporine-based immunosuppressive regimen and diabetes mellitus in patients on Tacrolimus-based

Table 2 Relationship between various parameters at 10-year post-liver transplantation and the incidence of hepatic steatosis on 10-year post-liver transplantation biopsy

	All subjects <i>n</i> = 72	No HS <i>n</i> = 48	HS <i>n</i> = 24	<i>P</i> ¹
Age at the time of survey	49 ± 15	47 ± 15	53 ± 12	NS
≥ 60 yr aged patients	28%	27%	29%	NS
Histological lesions				
Steatosis	33%	-	100%	
Macrovacuolar	28%	-	82%	
Microvacuolar	1%	-	4%	
Combined Mac-Mic	4%	-	14%	
Initial and 10-yr maintained steatosis	8% (5 pat.)	0%	22%	0.002
Fibrosis (F1-F4)	53%	44%	71%	0.03
F1-F2	40%	35%	50%	NS
F3-F4	13%	8%	21%	NS
Combined fibrosis-steatosis	24%	0%	71%	< 0.0001
HCV(+) Fibrosis	44%	31%	71%	< 0.001
Bile duct lesions	36%	42%	25%	NS
Minimal change	23%	27%	17%	NS
Other potential steatosis factors				
BMI (kg/m ²)	22.4 ± 3.8	22.3 ± 3.9	22.6 ± 3.4	NS
Underweight (BMI ≤ 18.5)	13%	17%	4%	
Normal weight (BMI = 18.5-24.9)	69%	65%	79%	NS
Overweight (BMI = 25-29.9)	14%	14%	13%	
Obesity (BMI ≥ 30)	4%	4%	4%	
HCV infection (<i>de novo</i> or recurrence)	57%	46%	79%	0.007
Arterial hypertension	53%	52%	54%	NS
Glycemia (mmol/L)	5.4 ± 2.0	5.1 ± 0.9	6.2 ± 3.2	NS
Diabetes mellitus	10%	8%	13%	NS
Maintenance immunosuppression				
Cyclosporine A	96%	96%	96%	NS
Dosage (mg)	129.8 ± 58.1	135.0 ± 61.5	119.5 ± 50.4	NS
Prednisolone	93%	96%	88%	NS
Dosage (mg)	6.8 ± 3.1	6.9 ± 3.2	6.7 ± 2.9	NS
Azathioprine	43%	40%	50%	NS
Dosage (mg)	48.4 ± 15.7	51.3 ± 15.5	43.8 ± 15.5	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. ¹Comparison between HS and No HS. HS: Hepatic steatosis; NS: Not significant; BMI: Body mass index.

Table 3 Social life factors and hepatic steatosis at 10-year biopsy

	All subjects <i>n</i> = 72	No HS <i>n</i> = 48	HS <i>n</i> = 24	<i>P</i> ¹
Work				
Employed	33%	39%	23%	NS
Homemaker	13%	17%	4%	NS
Student full/part-time	3%	4%	0%	NS
Unemployed	20%	19%	23%	NS
Retired	30%	21%	50%	0.03
No. of years worked	17.9 ± 12.7	16.4 ± 12.6	20.9 ± 12.5	NS
Alcohol and smoking				
Alcohol consumption	21%	17%	30%	NS
No. of drinks ² /d in drinkers	1.1 ± 0.3	1.0 ± 0.0	1.2 ± 0.4	NS
Tobacco smokers	17%	15%	21%	NS
Cigarettes/d in smokers	1.9 ± 0.7	2.1 ± 0.7	1.6 ± 0.9	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. ¹Comparison between HS and No HS; ²One drink = 1 bottle of beer or 1 glass of wine or 1 mixed drink. HS: Hepatic steatosis; NS: Not significant.

regimen are well-known side effects^[23]. We acknowledge that at the time of this study almost all patients (96%) were on Cyclosporine and our results may not be applicable to patients who are on Tacrolimus.

Interestingly, in our series HS in the 10 year allograft

biopsies were related to HCV infection, rather than NAFLD or other causes. HCV infection is well known to highly influence the rate of not only liver fibrosis but also HS. In the non-transplant setting steatosis is a very common lesion in chronic HCV infection^[24], and

Table 4 Univariate analysis of subjective variables associated with hepatic steatosis at 10-year biopsy

QOL criteria	Univariate <i>P</i>
Physical distress	
Muscle weakness	0.04
Abdominal pains or discomfort	< 0.0001
Abdominal swelling or bloating	0.04
Joint aches or pains	< 0.001
Headaches	0.03
Poor or blurred vision	0.03
Change in facial appearance	< 0.001
Fluid retention or swelling of ankles	0.02
Psychological distress	
Sleeplessness or insomnia	0.03
Nervousness, anxiety	0.009
Feeling depressed, sad or blue	< 0.01
Low satisfaction with life as a whole	0.02
Personal function	
Health currently limits the kind of vigorous activities such as running, heavy lifting or sport	< 0.001
Social and role function	
Decreased interest in sex	0.003
Problem with sex life	0.04
General health perception	
Bodily pain during the last month	< 0.01

the pathogenesis of steatosis may differ according to the genotype of HCV. Strong clinical and experimental evidence suggests that steatosis in patients infected with genotype 3 is partly related to a direct cytopathic effect, whereas in genotype 1, steatosis is mainly related to an associated metabolic syndrome and insulin resistance^[25]. Because the predominant HCV genotype in our patients with HS was the genotype 1 (63%), mostly 1b subtype, and genotype 3 represented 16% (all in HS group), we can consider that both mechanisms were involved.

HCV seems to dominate other risk factors in our study. One explanation for this findings may be that a type II error occurred because of the relatively small sample size ($n = 72$ and only 24 in HS group). Otherwise, HS is presumably a surrogate for chronic hepatitis C, which is more directly affecting the QOL from chronic viral infection than the presence of histologic steatosis.

Unfortunately, HCV recurs in nearly all liver transplant recipients, and the reduction in long-term survival observed in these patients is the result of progressive fibrosis and evolution into cirrhosis^[26-30]. Among the recipients with HCV infection, those who achieved 10 year post-transplant survival in our series can probably be categorized as "slow fibrosers". Fibrosis was present in 71% of our patients with HS and was mainly related to HCV infection.

Other symptoms like changes in facial appearance, fluid retention or swelling of ankles, and headaches affected the QOL of long term survivors. These symptoms are probably associated to the long term medication that patients require after LT. Moreover, in addition to muscle weakness, these physical affections presumably had a repercussion on PD; predominantly nervousness, anxiety, sadness or depression associated with sleeplessness

Table 5 Multivariate analysis of independent factors of hepatic steatosis at 10-year biopsy

Factors	Multivariate <i>P</i>
Objective factors	
Retirement	0.04
Hepatitis C virus infection (<i>de novo</i> or recurrence)	< 0.01
Subjective factors	
Abdominal pains or discomfort	< 0.01
Joint aches or pains	0.04

or insomnia. As a consequence, PD, SRF, and GHP also worsened in patients with HS.

The impact of HS on QOL has been already reported in non-transplanted patients. Recent studies demonstrated the negative impact of NAFLD on the physical and psychological function^[31-33]. Newton *et al.*^[31] refuted the misconception that symptoms associated with NAFLD are entirely related to excessive weight, a concept that supported by our data. It is well recognized that the major risk factor for HS is excessive consumption of food, alcohol, or both. However, many people who over-consume do not have fatty livers, and steatosis can develop in those who do not engage in these behaviors. Thus, genetic or environmental factors or both could influence one's susceptibility to hepatic triglyceride accumulation^[34-36].

Future perspectives in the transplant setting must inevitably imply the host and the graft. At the present time, the gold standard for diagnosis remains liver biopsy but its costs and risks limit its practice in the non-transplant setting. Some demographic factors, blood tests, and imaging studies can be used to predict a higher risk of steato-hepatitis or advanced fibrosis, but are of limited sensitivity and specificity. More accurate predictors and scoring systems would allow identification of those who would benefit most from liver biopsy and monitor disease progression and response to therapy^[19].

In conclusion, we could demonstrate that in patients with long-term follow-up after LT, HS is the most important histological finding that has an impact on the patients' quality of life. Interventions are needed to restore and optimize QOL in patients with *de novo* or recurrent HS during long-term follow-up. Future research should focus on identifying factors that lead to the development of HS after LT.

COMMENTS

Background

The goal of liver transplantation is to ameliorate not only survival, but also quality of life (QOL) while minimizing the effects of disease and costs of care. The increasing proportion of recipients alive at long-term follow up has incited transplant professionals to focus on long term morbidity-free survival and an acceptable QOL. In this study the authors evaluated the relationship between the state of transplanted liver graft and the recipient QOL of histologically proven lesions in a 10-year post liver transplantation (LT) cohort of patients.

Research frontiers

Studies of long-term survivors have been mainly based on clinical data and follow up at long term with respect to indication of LT, immunosuppressive

regimen or recipient and donor criteria. Few studies assessed the graft histology by long-term graft biopsy protocol and, to our knowledge, no report assessing the relationship between the histological state of long-term transplanted liver graft and the recipient QOL has been published.

Innovations and breakthroughs

The results of this study showed a potential impact of graft's steatosis on the QOL of transplant patients 10 years after surgery.

Applications

These results are encouraging and may represent the beginning of further studies in the area and, consequently the establishment of a specific care of these patients.

Peer-review

An interesting experience on the histological explore the outcome of 10-year liver transplantation. Manuscript is well written.

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Retrospective Study

Stabilization of estimated glomerular filtration rate in kidney transplantation from deceased donors with acute kidney injuries

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Abstract

AIM

To evaluate and compare the outcomes of kidney transplant (KT) from deceased donors among standard criteria, acute kidney injury (AKI) and expanded criteria donors (ECDs).

METHODS

This retrospective study included 111 deceased donor kidney transplant recipients (DDKT). Deceased donors were classified as standard criteria donor (SCD), AKI donor and ECD. AKI was diagnosed and classified based on change of serum Cr by acute kidney injury network (AKIN) criteria. Primary outcome was one-year estimated glomerular filtration rate (eGFR) calculated from Cr by CKD-EPI. Multivariate regression analysis was done by adjusting factors such as type of DDKT, %Panel-reactive antibodies, cold ischemic time, the presence of delayed graft function and the use of induction therapy. Significant

factors that can affect the primary outcomes were then identified.

RESULTS

ECD group had a significantly lower eGFR at one year (33.9 ± 17.3 mL/min) when compared with AKI group (56.6 ± 23.9) and SCD group (63.6 ± 19.9) ($P < 0.001$). For AKI group, one-year eGFR was also indifferent among AKIN stage 1, 2 or 3. Patients with AKIN stage 3 had progressive increase of eGFR from 49.6 ± 27.2 at discharge to 61.9 ± 29.0 mL/min at one year. From Kaplan-Meier analysis, AKI donor showed better two-year graft survival than ECD (100% vs 88.5%, $P = 0.006$). Interestingly, AKI group had a stable eGFR at one and two year. The two-year eGFR of AKI group was not significantly different from SCD group (56.6 ± 24.5 mL/min vs 58.6 ± 23.2 mL/min, $P = 0.65$).

CONCLUSION

Kidney transplantations from deceased donors with variable stage of acute kidney injuries were associated with favorable two-year allograft function. The outcomes were comparable with KT from SCD. This information supports the option that deceased donors with AKI are an important source of organ for kidney transplantation even in the presence of stage 3 AKI.

Key words: Acute kidney injury donor; Rising of terminal serum creatinine; Acute kidney injury network stage; Deceased donor; Estimated glomerular filtration rate stabilization; Stabilize allograft function

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Core tip: Many concerns about problems from using kidneys donated from donors who had acute kidney injury (AKI) before organ procurement lead to underutilization of such kidneys. Several kidneys have unnecessary been discarded in recent year. Here, we describe the comparable allograft and patient outcomes between using kidney from standard criteria donor and donor with AKI. Kidney transplantations from deceased donors with variable stages of acute kidney injuries were associated with favorable allograft function. This information supports the option that deceased donors with AKI are an important source of organ for kidney transplantation and can remedy the problem of organ shortage.

Wiwattanathum P, Ingsathit A, Kantachuesiri S, Arpornsujaaritkun N, Tirapanich W, Sumethkul V. Stabilization of estimated glomerular filtration rate in kidney transplantation from deceased donors with acute kidney injuries. *World J Transplant* 2016; 6(4): 712-718 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/712.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.712>

INTRODUCTION

An increasing tendency to perform kidney transplant

(KT) from deceased donors other than standard criteria donor (SCD) is the result of disparity between the number of patient being in the waiting list for transplantation and utilized donor pool^[1]. Types of non-ideal deceased donor include donors with acute kidney injury (AKI) and expanded criteria donor (ECD) are being used for expanding donor pool^[2]. However, there are concerns about worse allograft outcomes when using kidneys from AKI donors. Therefore, a significant number of kidneys from AKI donors with high terminal serum creatinine level have been discarded. Hence, the plan to solve problem of organ shortage cannot be accomplished.

Increased incidence of delayed graft function (DGF)^[3,4] is a significant disadvantage of using kidneys from AKI donors. This can lead to increased hospital stay and cost of treatment or even worse allograft function^[5] when compare with KT from SCD. In addition, it is uncertain whether KT from AKI donor is associated with increased risk of acute rejection or allograft loss when compare with KT from using kidney from standard deceased donor^[3,4]. Since AKI can occur from different causes and have different severities, the outcomes of KT from donors with AKI may be varied. Theoretically, KT from donors with mild degree of AKI may have favorable outcomes than KT from severe AKI. However, it is not universally agreed to use kidneys from donors with AKI. There are studies reporting association of discarding kidney in the presence of AKI of deceased donor^[6]. We conducted a study aimed to determine outcomes of kidney transplantation from deceased donors with variable degrees of acute kidney injuries.

MATERIALS AND METHODS

Patients

A retrospective cohort of 243 KT recipients from our single center hospital during 1st January 2012 to 31st December 2013 was reviewed. Inclusion criteria were (1) deceased donor kidney transplant (DDKT) recipient; (2) Age ≥ 15 years old; (3) Negative lymphocytotoxic cross match result at the time of transplantation; and (4) Panel-reactive antibodies (PRA) luminex $< 20\%$. Exclusion criteria were: (1) recipients who had combined solid organ transplantation; and (2) donor whose terminal serum creatinine increased ≥ 0.3 mg/dL but not ≥ 1.5 -fold from baseline. From these inclusion and exclusion criteria (excluded 115 cases due to living related kidney transplantation, 8 cases due to age < 15 years, 1 case due to combined solid organ transplant and 8 cases due to terminal serum creatinine increased ≥ 0.3 mg/dL but not ≥ 1.5 -fold from baseline), total 111 KT recipients who received DDKT were enrolled in the study. This study was approved by the study center Institutional Review Board/Ethics Committee.

Study procedure

Baseline transplantation data and the clinical outcomes at two-year were collected from all patients then compared

outcomes by statistical analysis. Study populations were stratified into 3 groups according to the donor status: (1) Standard criteria deceased donor (SCD); (2) Deceased donor with AKI donor; and (3) Expanded criteria deceased donor (ECD). AKI donor was recognized by a rising of serum creatinine more than 0.3 mg/dL and defined by AKI Network criteria (AKIN criteria^[7]) based on baseline to terminal serum creatinine (Cr) as follows: Stage 1, increase in Cr ≥ 1.5 to < 2 -fold increase; stage 2, 2 to < 3 fold increase and stage 3, ≥ 3 -fold increase. However, we did not included AKI donors who have terminal serum creatinine less than 1.5 fold from baseline to ensure that degree of AKI was significant enough to have impacts on transplantation outcomes. ECD was defined by any donor over the age of 60, or a donor over the age of 50 with two of the following: A history of high blood pressure, a creatinine greater than or equal to 1.5 mg/dL, or death resulting from a stroke. All other donors were classified as SCD.

Outcomes

Primary outcome was estimated glomerular filtration rate (eGFR) at one year as calculated from Cr by CKD-EPI equation. Secondary outcomes were eGFR at discharge and two year, rate of DGF (defined as requirement of dialysis within 7 d after transplantation), two-year allograft and patient survival.

Statistical analysis

Continuous variables were described as mean values (SD) and median values (range) for data with normal distribution and non-normal distribution respectively. Categorical variables were described as frequency and percentage. Student *t* test (or Mann-Whitney *U* test) was used to compare the difference between groups for continuous data. A χ^2 test (or Fisher's exact test) was used to compare the difference between groups for categorical data. Multivariate regression analysis was used to determine independently significant factors (type of DDKT, %PRA, cold ischemic time, the presence of DGF and the use of induction therapy) that may affect one-year eGFR. Allograft survival and patient survival were presented by Kaplan Meier analysis. All analyses were performed using Stata statistical software, version 13.0 (Stata Corp., Collage Station, TX). $P < 0.5$ was considered significant. The statistical review of the study was performed by a biomedical statistician.

RESULTS

A total of 119 DDKT recipients were enrolled. Eight recipients receiving kidney from AKI donors whose terminal serum creatinine increased ≥ 0.3 mg/dL but not ≥ 1.5 -fold from baseline and were excluded. One hundred and eleven patients were included in the analysis. There were 32 recipients in SCD group, 51 in AKI group and 28 in ECD group. Recipient and donor characteristics are shown in Table 1. All recipient baseline characteristics

were similar among 3 groups. Donor age was older in ECD group than the other groups. Most donors were male and the proportion was highest in AKI group. Basiliximab (Simulect[®]) was commonly used for induction in both SCD (34.4%) and AKI group (47.1%). Antithymocyte globulin (ATG) was frequently used in ECD group (39.9%). However, the different in prescribing induction therapy was not statistically significant ($P = 0.19$). Maintenance immunosuppressive regimens were shown in Table 1. The combination of cyclosporine and everolimus was more commonly used in AKI and ECD donor when compared with standard criteria deceased donor ($P = 0.05$).

eGFR at discharge was 64.1 ± 22.1 , 52.5 ± 22.9 and 35.5 ± 17.9 mL/min for SCD, AKI and ECD group. eGFR at one year was 63.6 ± 19.9 , 56.6 ± 23.9 and 33.9 ± 17.3 mL/min for SCD, AKI and ECD group. eGFR at two year was 58.6 ± 23.2 , 56.6 ± 24.5 and 29.9 ± 19.2 mL/min in SCD, AKI and ECD group respectively (Table 2). Two-year eGFR was significant lower in ECD group ($P < 0.001$) when compared with the other groups but was not different between SCD group and AKI group ($P = 0.65$). For AKI group, two-year eGFR was also indifferent among degree of AKI as classified by AKIN stage 1, 2 or 3 (Table 3). Two-year eGFR for AKI group with AKIN stage 1, 2 and 3 was 53.4 ± 24.3 , 54.0 ± 21.4 and 64.0 ± 29.4 mL/min ($P = 0.79$). While two-year eGFR in both SCD and ECD groups decreased over time after transplantation, two-year eGFR in AKI group had tendency to improve over time after transplantation especially in AKIN stage 3 (Table 3). In AKIN stage 3 group, two-year eGFR progressively improved from 49.6 ± 27.2 mL/min after transplant to 64.0 ± 29.4 mL/min. However, this change was not statistically different ($P = 0.12$). Univariate regression analysis showed that the use of ECD and presence of DGF were significantly associated with decreased of eGFR at one year by univariate model. However, multivariate regression analysis showed that use of ECD is the only factor that was associated with declining one-year eGFR (Table 4).

Rate of DGF was lowest in SCD group and highest in ECD group. DGF occurred 31.2%, 56.9% and 77.8% for each group ($P = 0.001$). Rate of acute rejection was not differed among the three groups (Table 2). Two-year allograft survival was 100%, 100% and 88.5% for each group (Figure 1, $P = 0.01$). Two-year patient survival rate was similar among three groups (Figure 2). Cardiovascular death was responsible for cause of death in 1, 3 and 1 recipient in SCD, AKI and ECD group respectively. Infection related death was responsible for cause of death in 1 recipient both from SCD and ECD group. Rate of CMV and BK virus infection were not difference among 3 groups (Table 2).

DISCUSSION

Our findings suggest that transplantation from deceased donors with AKI have comparable outcome when compared with SCD. The outcomes include both eGFR and

Table 1 Baseline characteristics

DDKT (<i>n</i> = 111)	SCD (32)	AKI (51)	ECD (28)	^a <i>P</i> -value	^b <i>P</i> -value
Recipients					
Age year (mean ± SD)	42.7 ± 13.8	43.9 ± 12.0	43.1 ± 12.3	0.68	0.67
Male <i>n</i> (%)	19 (59.4)	35 (68.6)	16 (57.1)	0.48	0.55
Pre KT dialysis				1	1
Hemodialysis <i>n</i> (%)	26 (81.3)	42 (82.4)	23 (82.1)		
Peritoneal dialysis <i>n</i> (%)	6 (18.8)	9 (17.7)	5 (17.9)		
Comorbid <i>n</i> (%)					
DM	2 (6.25)	8 (15.7)	3 (10.7)	0.3	0.48
HT	30 (93.8)	49 (96.1)	25 (89.3)	0.67	0.41
CAD	1 (3.1)	1 (1.9)	1 (3.6)	1	1
Cause of ESRD <i>n</i> (%)				0.91	0.73
Unknown (no biopsy)	23 (23.2)	33 (31.8)	18 (18.9)		
Diabetic nephropathy	1 (0.9)	1 (1.3)	1 (0.8)		
IgA nephropathy	1 (1.9)	2 (2.6)	3 (1.5)		
Chronic glomerulonephritis	2 (0.9)	1 (1.3)	0 (0.8)		
Blood group <i>n</i> (%)				0.14	0.38
A	4 (12.5)	13 (25.5)	7 (25.0)		
B	13 (40.6)	13 (25.5)	7 (25.0)		
AB	4 (12.5)	2 (3.9)	3 (10.7)		
O	11 (34.4)	23 (45.1)	11 (39.3)		
PRA - % median (range)	0 (0.85)	0 (0.0)	0 (0.0)	0.03	0.04
Second KT <i>n</i> (%)	2 (6.25)	3 (5.88)	1 (3.57)	1	1
Total HLA mismatch - (mean ± SD)	2.5 (1.2)	2.3 (1.1)	2.1 (1.1)	0.52	0.76
Donors					
Age, year (mean ± SD)	33.9 ± 14.8	41.0 ± 12.0	61.2 ± 7.0	0.02	< 0.001
Male <i>n</i> (%)	24 (75.0)	44 (86.3)	17 (60.7)	0.25	0.04
Terminal serum creatinine (mg/dL) - median (range)	0.91 (0.73, 1.13)	2.22 (1.65, 3.20)	1.28 (0.99, 2.70)		< 0.001
Cold ischemic time, minute (mean ± SD)	1099 ± 291	1129 ± 294	1261 ± 242	0.65	0.5
Immunosuppressive drugs					
Induction <i>n</i> (%)				0.11	0.19
No	16 (50.0)	16 (31.4)	9 (32.1)		
ATG	5 (15.6)	11 (21.6)	11 (39.3)		
Simulect	11 (34.4)	24 (47.1)	8 (28.6)		
Maintenance <i>n</i> (%)				0.05	0.005
Tacrolimus/mycophenolate/prednisolone	16 (50.0)	27 (52.9)	13 (46.4)		
Cyclosporin A/mycophenolate/prednisolone	15 (46.8)	16 (31.4)	5 (17.9)		
Cyclosporin A/everolimus/prednisolone	0	7 (13.7)	8 (28.6)		
Everolimus/mycophenolate/prednisolone	1 (3.1)	0	0		

^a*P*-value compared between SCD and AKI; ^b*P*-value compared among SCD, AKI and ECD. DDKT: Deceased donor kidney transplant; SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; KT: Kidney transplant; DM: Diabetes mellitus; HT: Hypertension; CAD: Cardiovascular disease; ESRD: End stage renal disease; HLA: Human leukocyte antigen; ATG: Antithymocyte globulin.

two year patient survival. In addition, eGFR of AKI group did not decline after two year follow up. In contrast, eGFR in ECD group significantly declined after two year. This finding supports the view that kidneys with AKI may have recovery after a period of time.

In native kidney, after injury subsides, kidney can repair itself and restore normal or sub-normal function over time depends on severity and duration of injury^[8]. Our finding suggests that these processes also occur in transplanted kidney. As shown in AKI group, one-year eGFR had progressive increase from baseline and stable at two-year follow up in all three groups. However, there are difficulties to predict the ability of each kidney allograft regarding the ability to recovery from acute kidney injuries. A calculation of "Kidney Donor Profile Index"^[9,10] has been proposed to predict the risk of graft loss after deceased donor kidney transplantation. The involved donors' parameters include age, height, weight, ethnicity, history of hypertension, history of

diabetes, causes of death, serum creatinine, HCV status and donation after circulatory death status. However, the calculations of KDPI use a single value of serum creatinine and may or may not be indicative the presence of AKI in the donors. Evidences from some studies showed worse allograft function from AKI donor. These suggested that not all kidneys from AKI donor were suitable for transplantation. Researches providing such information are necessary and useful for making decision on which kidney should be used or discarded.

In the recent years, kidneys from AKI donor were underutilization. As shown in some studies that there are high discard rate of deceased donor with high serum creatinine. About 20%-30% of kidneys from AKI donors were discarded and sometime more than 40 percent were discarded when terminal serum creatinine > 2.0 mg/dL^[3,6,11]. In contrast, our study has shown that KT from deceased donors with AKI is associated with comparable clinical outcomes with standard criteria deceased donors.

Table 2 Transplantation outcomes

Outcomes	SCD (32)	AKI (51)	ECD (28)	^a P-value	^b P-value
Cr at discharge - mg/dL (mean ± SD)	1.35 ± 0.51	1.70 ± 0.84	2.41 ± 1.00	0.04	< 0.001
Cr at 1 yr - mg/dL (mean ± SD)	1.35 ± 0.50	1.59 ± 0.75	2.64 ± 1.38	0.14	< 0.001
Cr at 2 yr - mg/dL (mean ± SD)	1.52 ± 0.63	1.68 ± 1.06	3.29 ± 2.12	0.47	< 0.001
eGFR at discharge - mL/min (mean ± SD)	64.1 ± 22.1	52.5 ± 22.9	35.5 ± 17.9	0.03	< 0.001
eGFR at 1 yr - mL/min (mean ± SD)	63.6 ± 19.9	56.6 ± 23.9	33.9 ± 17.3	0.19	< 0.001
eGFR at 2 yr - mL/min (mean ± SD)	58.6 ± 23.2	56.6 ± 24.5	29.9 ± 19.2	0.65	< 0.001
DGF <i>n</i> (%)	10 (31.2)	29 (56.9)	21 (77.8)	0.03	0.001
Length of stay - d (mean ± SD)	24.4 ± 8.3	31.1 ± 14.7	37.9 ± 15.3	0.02	0.002
Nephrectomy <i>n</i> (%)	0	2 (3.9)	2 (7.4)	0.52	0.27
Acute rejection	5 (15.7)	10 (19.6)	6 (27.4)	0.70	0.8
ACR	2 (6.3)	6 (11.8)	2 (7.1)		
ABMR	1 (3.1)	3 (5.9)	3 (10.7)		
ACR + ABMR	2 (6.3)	1 (1.9)	1 (3.6)		
Graft loss <i>n</i> (%)	0	0	3 (11.5)	NS	0.01
Death <i>n</i> (%)	2 (6.3)	3 (5.1)	3 (10.7)	0.63	0.57
CMV <i>n</i> (%)	7 (5.2)	6 (8.3)	5 (4.5)	0.23	0.46
BK virus nephropathy <i>n</i> (%)	1 (3.1)	3 (5.69)	0	1	0.69

eGFR: Estimated glomerular filtration rate; DGF: Delayed graft function; ACR: Acute cellular rejection; ABMR: Antibody mediated rejection; CMV: Cytomegalovirus.

Table 3 Estimated glomerular filtration rate classified by acute kidney injury network stage

eGFR - mean ± SD	SCD (<i>n</i>) (32)	AKIN stage (<i>n</i>)			P-value
		1 (18)	2 (21)	3 (12)	
eGFR at discharge - mL/min	64.1 ± 22.1	49.8 ± 20.7	57.1 ± 23.7	49.6 ± 27.2	0.87, 0.07
eGFR at 1 yr - mL/min	63.6 ± 19.9	52.9 ± 21.2	57.1 ± 21.5	61.9 ± 29.0	0.47, 0.92
eGFR at 2 yr - mL/min	58.6 ± 23.2	53.4 ± 24.3	54.0 ± 21.4	64.0 ± 29.4	0.79, 0.54

AKIN: Acute kidney injury network; eGFR: Estimated glomerular filtration rate; SCD: Standard criteria donor.

Table 4 Univariate and multivariate regression analysis of factors associated with the change of one-year estimated glomerular filtration rate

Factors	Univariate			Multivariate		
	B-coefficient	P-value	95%CI	B-coefficient	P-value	95%CI
Type of donor						
SCD	Reference	NA		Reference	NA	
AKI	-6.73	0.17	-16.41, 2.94	-3.7	0.49	-14.52, 7.13
ECD	-29.76	< 0.001	-41.67, -17.85	-25.43	< 0.001	-38.80, -12.05
PRA > 20%	3.49	0.64	-7.37, 14.34	3.62	0.53	-7.82, 15.05
DGF	12.3	0.008	3.22, 21.39	6.17	0.18	-2.91, 15.25
HLA mismatch ≥ 3	-2.99	0.53	-12.45, 6.46	-7.12	0.11	-15.77, 1.53
CIT > 24 h	-14.72	0.03	-27.99, -1.45	-9.54	0.14	-22.19, 3.12
Received Induction	4.49	0.36	-5.28, 14.25	1.8	0.72	-8.09, 11.70

The B-coefficient values were calculated from univariate and multivariate regression analysis. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; PRA: Panel reactive antibody; DGF: Delayed graft function; HLA: Human leukocyte antigen.

Thus, our results show that kidneys from AKI donor are important source for organ transplantation and should not be discarded.

The limitation of our study is that there may be selection bias regarding the quality of kidneys when compare with other studies^[3,6]. Pre-implantation biopsy and organ perfusion machine are not routinely used in this study for the organ procurement process. These can

lead to more kidneys being used when organ retrieval process was satisfied as judged by the clinician.

In summary, kidney transplantations from deceased donors with variable stages of acute kidney injuries were associated with favorable two-year allograft function and survival. The outcomes were comparable with KT from those of standard criteria deceased donors. This information supports the option that deceased donors

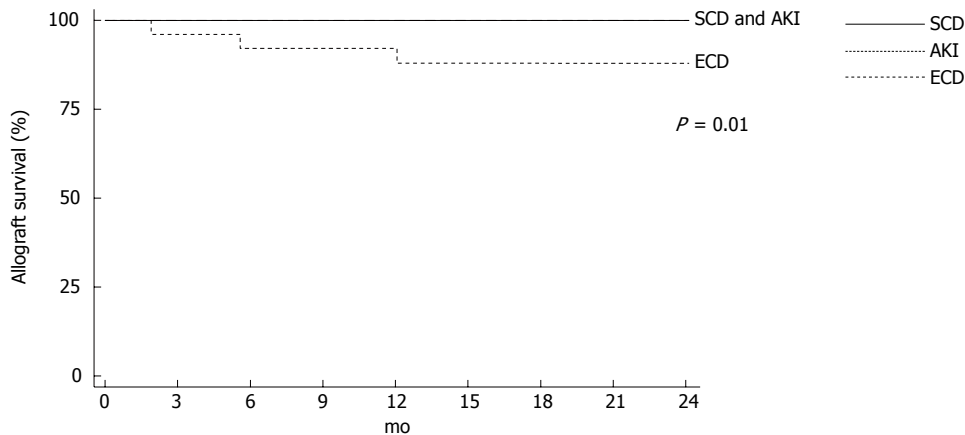


Figure 1 Comparison of two year actuarial allograft survival of standard criteria deceased donor, acute kidney injury donors and expanded criteria donors. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor.

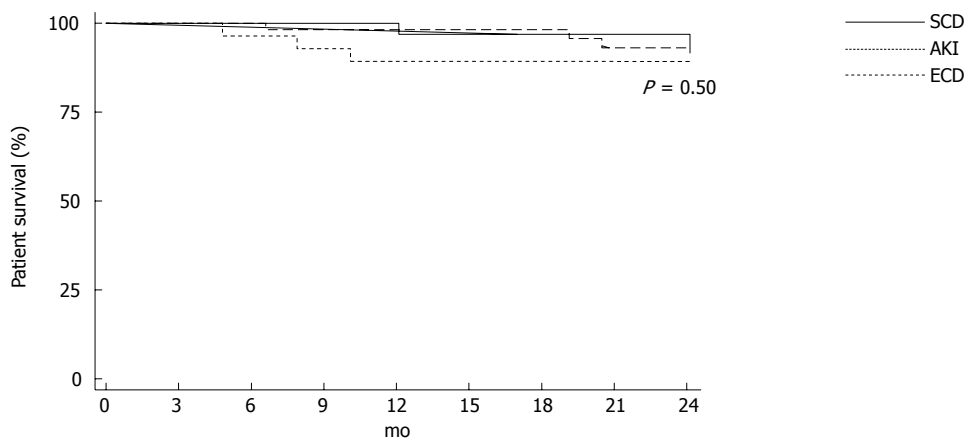


Figure 2 Comparison of two year actuarial patient survival of standard criteria deceased donor, acute kidney injury donors and expanded criteria donors. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor.

with AKI are an important source of organ for kidney transplantation even in the presence of stage 3 AKI. However, not all kidneys from AKI donor may be used for transplantation. Further studies are required to determine and clarify the optimal use of kidneys with AKI and the precise parameters that can identify suitable kidneys from AKI donor suitable for proceeding to transplantation.

COMMENTS

Background

Organ shortage is a common problem worldwide. Kidney transplantations from non-ideal deceased donors are a potential option to minimize this problem. Acute kidney injury (AKI) donor and expanded criteria donor (ECD) are important sources of deceased donors. However, there are several challenging issues about the outcomes of using kidney from AKI donors or ECD. This can lead to the discard of using deceased donors with high terminal serum creatinine (Cr). The "old to old" concept has been proposed to be the model of allocating kidneys from ECD. However, there is no consensus guideline regarding the use of kidneys from AKI donors. The authors therefore evaluate the outcomes of kidney transplant from deceased donors with several stages of AKI and compare with that of standard criteria donors (SCDs) and ECDs.

Research frontiers

Results from some studies showed worse allograft function when trans-

plantation from AKI donor. These suggest that not all kidneys from AKI donor were suitable for transplantation. Researches providing such information are necessary and useful for decision whether which kidney should be used or discarded.

Innovations and breakthroughs

Many kidneys from AKI donors were discarded because of concerning about poor allograft outcomes. This study showed that kidney transplantation from deceased donors with variable stage of acute kidney injuries was associated with equivalent allograft function and survival when compare with SCD.

Applications

Kidneys from AKI donors are important sources of organ for transplantation that can mitigate the problem of organ shortage.

Terminology

KT: Kidney transplant; SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; DM: Diabetes mellitus; HT: Hypertension; CAD: Cardiovascular disease; ESRD: End stage renal disease; PRA: Panel reactive antibody; HLA: Human leukocyte antigen; Cr: Creatinine; eGFR: Estimated glomerular filtration rate; ATG: Antithymocyte globulin; DGF: Delayed graft function; ACR: Acute cellular rejection; ABMR: Antibody mediated rejection; CMV: Cytomegalovirus.

Peer-review

The article is well written and relevant.

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Retrospective Study

Acute antibody-mediated rejection after intestinal transplantation

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Author contributions: Wu GS cared for the patients and collected and summarized the data and wrote the article; Cruz Jr RJ cared for the patients; Cai JC helped for the interpretation of antibody data revising the manuscript.

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Conflict-of-interest statement: The author declares no conflict of interests for this article.

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Abstract

AIM

To investigate the incidence, risk factors and clinical outcomes of acute antibody-mediated rejection (ABMR) after intestinal transplantation (ITx).

METHODS

A retrospective single-center analysis was performed to identify cases of acute ABMR after ITx, based on the presence of donor-specific antibody (DSA), acute tissue damage, C4d deposition, and allograft dysfunction.

RESULTS

Acute ABMR was identified in 18 (10.3%) out of 175 intestinal allografts with an average occurrence of 10 d (range, 4-162) after ITx. All acute ABMR cases were sensitized to donor human leukocyte antigens class I and/or II antigens with a detectable DSA. A positive cross-match was seen in 14 (77.8%) cases and twelve of 18 patients (66.7%) produced newly-formed DSA following ITx. Histological characteristics of acute ABMR include endothelial C4d deposits, interstitial hemorrhage, and severe congestion with focal fibrin thrombin in the lamina propria capillaries. Multivariate analysis identified a liver-free graft and high level of panel reactive antibody

as a significant independent risk factor. Despite initial improvement after therapy, eleven recipients (61.1%) lost transplant secondary to rejection. Of those, 9 (50%) underwent graft removal and 4 (22.2%) received second transplantation following acute ABMR. At an average follow-up of 32.3 mo (range, 13.3-76.4), 8 (44.4%) recipients died.

CONCLUSION

Our results indicate that acute ABMR is an important cause of intestine graft dysfunction, particularly in a liver-exclusive graft and survivors are at an increased risk of developing refractory acute rejection and chronic rejection. More effective strategies to prevent and manage acute ABMR are needed to improve outcomes.

Key words: Intestinal transplantation; C4d deposition; Donor-specific antibody; Acute antibody-mediated rejection

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Core tip: Antibody-mediated rejection (ABMR) has appeared to be an important cause of allograft failure after intestinal transplantation (ITx). This study aimed to evaluate the incidence, risk factors and clinical outcomes of acute ABMR after ITx. The incidence of acute ABMR after ITx was as high as 10.3% in our series, which was closely associated with poor graft and patient survival. Our results indicate that acute ABMR is an important cause of intestinal graft failure, especially in a liver-free allograft and survivors are at an increased risk of developing chronic rejection. Effective strategies to prevent and treat acute ABMR are needed to improve outcomes.

Wu GS, Cruz Jr RJ, Cai JC. Acute antibody-mediated rejection after intestinal transplantation. *World J Transplant* 2016; 6(4): 719-728 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/719.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.719>

INTRODUCTION

Intestinal transplantation (ITx) has increasingly become a viable option for most patients with irreversible intestinal failure. Short-term patient and graft survival have improved to a great extent due to advances in surgical technology and immunosuppressive management^[1,2]. However, long-term outcomes after ITx have been inferior to other solid-organ transplants, especially with less intestinal allograft survival more than 10 years^[3,4]. Allograft failure secondary to acute and chronic rejection remain a significant impediment to the success of ITx^[5].

Traditionally, intestine transplant rejection has been considered as a T-cell-mediated course that can be effectively controlled with T-cell targeted immunosuppressive agents. Harmful effects of antibodies to human leukocyte antigens (HLA) in intestinal allograft rejection have not been studied thoroughly although

these HLA antibodies were often detectable after ITx^[6-8]. To date, HLA antibodies are believed to be a major risk factor for hyperacute rejection, acute antibody-mediated rejection (ABMR) and chronic ABMR after kidney or heart transplantation^[9]. Several case reports imply that HLA antibodies are also associated with lung, liver, or pancreas allograft dysfunction^[10-12]. Increasing data suggest that an early diagnosis and prompt management of acute ABMR are essential for improving patient and graft outcomes^[13,14].

The impact of HLA antibodies has got less attention in the assessment of acute intestinal allograft rejection. Similar to other solid-organ transplantation, many patients who require ITx become sensitized and form alloantibodies that originate either from previous exposure to blood products, pregnancies, transplants, and/or infections or *de novo* formation of donor-specific antibody (DSA) following transplantation^[15,16]. In recent years, we and others have shown that the presence of DSA was closely associated with the incidence and severity of intestinal allograft rejection and decreased the overall graft and patient survival^[17,18]. Although hyperacute rejection, caused by preformed DSA, rarely occurs in highly sensitized recipients after ITx^[19], clinicopathological findings consistent with acute ABMR have increasingly been recognized as an important form of rejection^[20,21]. Currently diagnostic standards for acute ABMR after ITx have not been set up yet and its incidence and clinical significance have remained unknown.

The diagnostic standards for acute ABMR in a kidney or heart transplant have been well-established. According to the guidelines, acute ABMR is defined by circulating DSA, C4d deposition, tissue pathology and clinical allograft dysfunction. In this series, we reviewed our institutional experience to identify recipients with acute ABMR that fulfill the criteria for kidney transplantation, and to evaluate the rate, risk factors and consequences after acute ABMR.

MATERIALS AND METHODS

Patient selection

Since August 2003, patients who received small bowel transplants at the University of Pittsburgh Medical Center have started to have a routine serum DSA specificities determinations, by either the purified HLA antigen-based ELISA or the Luminex single-antigen bead analysis. We performed a retrospective electronic medical records review of patients who underwent a small bowel transplant between August 2003 and May 2010. The clinical charts were reviewed as needed for additional data and the Institutional Review Board approved this study.

Donor and recipient demographics are summarized in Table 1. The transplant type consisted of a liver-exclusive transplant (isolated intestine graft and modified multivisceral graft without liver) and a liver-inclusive full multivisceral transplant. T cell complement-dependent lymphocytotoxic cross-match (CDC-XM) was performed by anti-human globulin (AHG)-enhanced method and

Table 1 Donor and recipient demographic and clinical characteristics

Characteristic	Transplants (<i>n</i> = 175)
Donor characteristics	
Age (yr)	25.4 ± 9.9
Gender (% male)	77.7
Nonwhite race (%)	16.6
Cold ischemic time (h)	7.6 ± 1.5
Recipient characteristics	
Age at transplantation (yr)	43.0 ± 12.5
Gender (% male)	38.9
Nonwhite race (%)	5.9
Primary diagnoses, <i>n</i> (%)	
Vascular occlusion	59 (33.7)
Crohn's disease	34 (19.4)
Neoplastic disorders	28 (16.0)
Motility disorders	21 (12.0)
Others	33 (18.9)
Donor/recipient sex mismatches (%)	56.6
Donor CMV positive/recipient negative (%)	21.9
Type of graft liver-free/liver-inclusive (%)	61.1/38.9
Two mismatches in HLA loci A/B/DR (%)	39.1/82.1/66.9
PRA at transplantation (≥ 10%) Class I (%)	40
Class II (%)	26.3
Positive T/B cell cross-match (%)	25.7
Preformed DSA (%)	30.3
Retransplantation (%)	6.7
Induction, <i>n</i> (%)	
None	41 (23.4)
Zenapax	3 (1.7)
Thymoglobulin	7 (4.0)
Campath-1H	124 (70.9)
Follow-up (mo; range)	37.5 ± 22.7 (0.7 to 81.5)

CMV: Cytomegalovirus; PRA: Panel reactive antibody; HLA: Human leukocyte antigens; DSA: Donor-specific antibody.

B cell CDC-XM was performed by extended-incubation/modified Amos technique. In our practice, a positive CDC-XM was not considered as a contraindication to ITx. HLA panel reactive antibody (PRA) was determined by LAT ELISA assay. The HLA antibodies were checked by the purified HLA antigen-based ELISA prior to April 2007 and have since then been replaced by the Luminex single-antigen bead assay. A value of the mean fluorescence intensity (MFI) ≥ 1000 was considered positive. We did not routinely follow up DSA levels post-transplant and indications for DSA monitoring were usually higher PRA levels, refractory rejection, or suspicious of acute ABMR.

The majority of patients underwent induction therapy with alemtuzumab (Campath-1H; Genzyme, Cambridge, MA) (*n* = 124), administered at day 0 (30 mg each dose) and some patients received antithymocyte globulin (ATG; Genzyme, Cambridge, MA) (*n* = 7), the IL-2 receptor antagonist basiliximab (Simulect; Novartis, East Hanover, NJ) (*n* = 3) or no induction therapy (*n* = 41) during the early period of this study. The basic immunosuppressive regimen was tacrolimus (Prograf; Astellas, Deerfield, IL) and steroids. The 12-h trough levels of tacrolimus during the initial six months were targeted at 10–15 ng/mL with Campath-1H or ATG induction therapy, and 15–25 ng/mL with Simulect induction or without any treatment. Maintenance immunosuppression was similar between a

positive and negative CDC-XM. All patients with a positive preformed DSA were given a single-dose of intravenous immunoglobulin (IVIG) at 2 g/kg body weight on day of transplantation. A 5-d steroid tapering was also given followed by a 10–20 mg daily dose for at least 6 mo. Recipients with acute ABMR underwent steroid boluses and/or OKT3. No patients were given plasmapheresis or anti-B cell treatment for acute ABMR.

Diagnosis of rejection

Surveillance ileal biopsies were routinely performed twice per week for the first 2 to 3 wk after transplantation and then once a week thereafter, with increased frequency in case of clinical indications. A diagnosis of acute ABMR was based upon the criteria, including: (1) clinical evidence of graft dysfunction; (2) histological evidence of tissue damage (vascular congestion, submucosal hemorrhage, neutrophilic margination, and platelet-fibrin thrombi in the lamina propria microvasculature)^[7]; (3) focal (5%–50%) or diffuse (> 50%) linear C4d deposition; and (4) circulating anti-HLA antibodies^[9,22,23]. A C4d staining was done on formalin-fixed paraffin-embedded tissue when acute ABMR was clinically or histologically suspected. The histological criteria for diagnosis of acute cellular rejection (ACR) were as described previously^[24]. A new rejection episode was defined by newly occurred clinical symptoms and histological evidence of acute rejection with at least 1 normal mucosal biopsy between rejection episodes. A determination of chronic rejection was based upon clinical symptoms and was further confirmed by a full-thickness specimen of partially or totally resected allografts to reveal evidence of vasculopathy and mesenteric lymphoid depletion with mesenteric sclerosis^[25].

Statistical analysis

Results are shown as means and ranges, unless otherwise stated. Categorical variables were assessed with the use of the χ^2 test or, when appropriate, Fisher's exact test. Continuous variables were analyzed with the use of the Student's *t*-test. Survival time was analyzed with the Kaplan-Meier method and differences were assessed by log-rank test. All data were analyzed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium).

RESULTS

Diagnosis of acute ABMR after ITx

During the study period, 164 adults underwent 175 consecutive small bowel transplants; 11 (6.7%) patients underwent retransplantation. Donor characteristics, recipient profiles, and perioperative features are summarized in Table 1. We identified 18 cases (10.3%) that fulfilled all the criteria for acute ABMR proposed by the National Conference. Of these, 16 of 164 cases (9.8%) developed acute ABMR after primary transplantation and 2 of 11 cases (18.2%) developed acute ABMR after retransplantation (Figure 1). Recipient age at the time

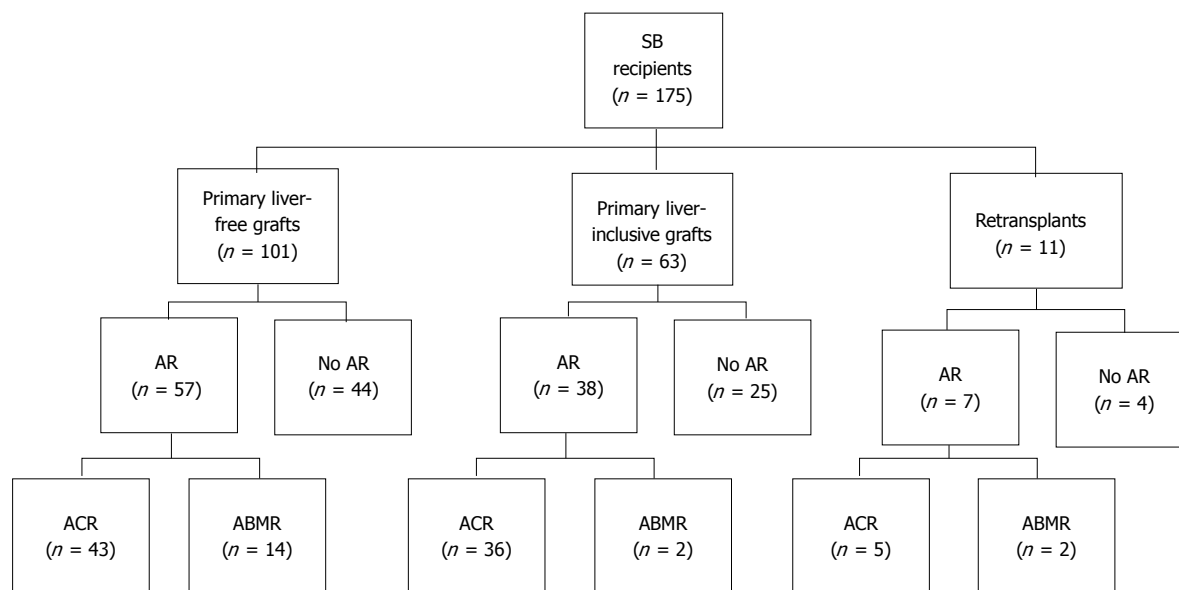


Figure 1 Patient distribution according to graft type and acute rejection type. SB: Small bowel; AR: Acute rejection; ACR: Acute cellular rejection; ABMR: Acute antibody-mediated rejection.

of transplantation was 25.4 ± 9.9 years old and thirteen cases (72.2%) were female. All patients were sensitized to HLA class I (median PRA 78.5%, range 11%-100%) and/or HLA class II antigens (median PRA 67.0%, range 1%-100%). A CDC-XM was positive in 14 (77.8%) recipients, in which anti-donor antibody titer was $\geq 1:8$ in 7 cases (50.0%). Three recipients (16.7%) underwent splenectomy at the time of transplantation (2 at primary transplantation and 1 at retransplantation). Recipients developed acute ABMR at a median time of 10.0 d (range, 4-162 d). Fourteen patients presented within 30 d after transplantation (early acute ABMR) and the remaining 4 presented beyond 30 d (late acute ABMR). Fifteen patients developed a single episode of acute ABMR and three developed repeat episodes of ABMR (Table 2).

In all cases, we established the diagnosis of acute ABMR based on the combination of clinical evidence of graft dysfunction, histological findings, and the presence of DSA. Six (37.5%) patients with acute ABMR occurring within a week displayed evidence of graft dysfunction by severe mucosal vascular congestion and diffuse mucosal hemorrhage during endoscopic examination. The other clinical presentation includes fever, abdominal pain or distention, increased stomal output or other non-specific symptoms. The prominent pathological findings in the cases with early ABMR were vascular congestion, focal hemorrhage with focal platelet-fibrin thrombi, and capillary neutrophilic infiltration in the lamina propria and the submucosa. The mucosal biopsies obtained from acute ABMR occurring within a week showed severe vascular congestion along with diffuse mucosal hemorrhage without any evidence of crypt and epithelial injury or apoptosis. These changes gradually returned to normal by 2 to 3 wk in most cases after treatment. Four cases with late ABMR exhibited less prominent vascular congestion and hemorrhage

but showed significant fibrin thrombi or neutrophilic margination (Table 2). There was no evidence of any significant vasculitis in the biopsies we evaluated. Seventeen cases showed a diffuse C4d deposition in the lamina propria and the submucosal capillaries (Figure 2). One case with a liver-inclusive transplant showed focal C4d deposition of the intestinal allograft but with significant vascular disturbance. Four of the 18 patients had pure acute ABMR without concomitant ACR within a year and the remaining 14 patients had concomitant ACR either before ABMR ($n = 3$), at the time of ABMR ($n = 4$), or after a diagnosis of ABMR ($n = 9$) (Table 2).

All 18 patients had DSA at the time of transplant: 10 to Class I HLA, 1 to Class II HLA only, and 7 to both Class I and Class II HLA. DSA was persistent in 3 cases at the time of the second episode of acute ABMR (Table 2). These antibodies were detected in fourteen cases by the purified HLA antigen-based ELISA and in the remaining four cases by the Luminex single-antigen bead assay.

Treatment, graft loss and patient death

Our treatment approach evolved over time and the regimen was individualized based on severity of illness, clinical course and response to therapy (Table 3). All patients were initially given intravenous steroids. Thirteen patients required additional OKT3 ($n = 10$), ATG ($n = 1$), Campath-1H ($n = 1$), or Campath-1H followed by OKT3 ($n = 1$) to reverse acute ABMR.

During the study period, post-transplant HLA antibodies were checked in 158 (90.3%) cases. *De novo* DSA was detected in twelve of the 18 patients (66.7%): 7 to Class II HLA, 5 to both Class I and Class II HLA. The presence of *de novo* DSA was markedly higher in the cases with acute ABMR compared to 7.6% (5 of 66) in the cases without rejection ($P < 0.0001$) or

Table 2 Characteristics of 18 patients with diagnosis of acute antibody-mediated rejection

Case	Tx type	POD (d)	XM		DSA at time of Tx and/or rejection	<i>De novo</i> DSA	Vascular alterations	C4d	#ACR ≤ 360 d
			T-cell	B-cell					
1	MV + K	4	1:32	1:16	A1, A25, B8, B18	DR51	++	Focal	0
2	SB	5	1:256	1:512	B7, B44, BW4, DQ1, DR10	DR15, DR51	+++	Diffuse	2
3	SB	5	1:2	1:2	B60	DR16	++	Diffuse	3
4 ¹	SB	6	1:8	1:8	B35, B60	A31, DQ7, DR11	+++	Diffuse	0
		109			A24, B60 ⁴	DR14, DR52	++	Diffuse	
5	SB	7	Neg	1:1	A3, B18, DR17	None	++	Diffuse	0
6	MV	7	Neg	1:8	A26, B70, DR52	None	++	Diffuse	1
7	MMV	9	1:32	1:8	A2	None	+++	Diffuse	4
8	SB	10	Neg	Neg	DR52	None	++	Diffuse	1
9	MMV	10	1:2	1:2	B13, BW4, DR7, DR53	DQ1	++	Diffuse	2
10 ¹	SB + K	11	Neg	1:4	A32, B8	A1, DR17	++	Diffuse	4
		52			A32, DQ4 ⁴		++	Diffuse	
11	SB + P	14	Neg	1:4	A3, B64	DQ7	++	Diffuse	0
12 ¹	SB	15	Neg	Neg	A2, B50	DQ8, DR53, DR4	+	Diffuse	5
		112			B7, B50 ⁴		+	Focal	
13	SB	41	1:256	1:8	A3, BW4, B53	DR18	+++	Diffuse	2
14	MMV	84	1:4	1:1	A25, B14, B18	None	++	Diffuse	1
15	SB	140	Neg	Neg	A2 ⁴	A28, B78, A30, DQ7, 9	+	Diffuse	1
16	SB	162	1:2	1:2	A24, B44 ⁴	DQ1, CW5	++	Diffuse	2
17 ²	SB	4	Neg	Neg	A28, B78, A30, DQ7, 9	B44, B58, DR4	+++	Diffuse	2
18 ³	MMV	18	1:1	1:8	A11, B7, DR12, DR17, DQ2	None	++	Diffuse	1

Type of transplant: SB: An isolated small bowel; MMV: A modified multivisceral graft; MV: A full multivisceral graft; P: Pancreas; K: Kidney. ¹Patients with repeat ABMR; ²Patient with a history of ABMR after prior transplant; ³Patient with prior transplant; ⁴DSA detected at time of rejection. POD: ABMR days post-transplant; XM: Cross-match; DSA: Donor-specific antibody; ACR: Acute cellular rejection.

Table 3 Treatment and outcome of 18 patients with acute antibody-mediated rejection

Case	Treatment	Graft status/survival (mo)	Re-Tx/graft type	Patient status/survival (mo)
1	ST/IVIG/OKT3	CHR/30.5	None	Dead (liver failure)/30.5
2	ST/IVIG/OKT3/Campath	CHR/13.5	None	Dead (ruptured pseudo-aneurysm)/18.6
3	ST/OKT3	Functioning/75.9	None	Alive/75.9
4 ¹	ST/OKT3	CHR/5.4	Yes/MV	Dead (pneumonia)/43.0
5	ST/OKT3	Functioning/17.7	None	Alive/17.7
6	ST/OKT3	Functioning/56.4	None	Alive/56.4
7	ST/OKT3/Campath	ACR/31.7	None	Dead (pneumonia)/31.7
8	ST/Campath	Functioning/30.3	None	Dead (unknown)/30.3
9	ST	CHR/35.4	Yes/MV	Alive/55.4
10 ¹	ST/OKT3	ACR/13.2	None	Dead (sepsis)/15.5
11	ST	Functioning/52.6	None	Alive/52.6
12 ¹	ST/ATG	CHR/22.6	None	Alive/22.6
13	ST	AHR/2.7	Yes/MV	Alive/76.4
14	ST	Functioning/22.5	None	Alive/22.5
15	ST/OKT3	CHR/4.8	None	Dead (sepsis)/32.8
16	ST	CHR/12.3	None	Alive/46.2
17 ²	ST/OKT3	CHR/12.6	Yes/SB	Dead (GI bleeding)/13.3
18 ³	ST/OKT3	Functioning/37.6	Yes/MV	Alive/37.6

Type of transplant: SB: An isolated small bowel; MV: A full multivisceral graft. ¹Patients with repeat ABMR; ²Patient with a history of ABMR after prior transplant; ³Patient with prior transplant. ST: Steroids; IVIG: Intravenous immunoglobulin; ACR: Acute cellular rejection; CHR: Chronic rejection.

21.6% (16 of 74) in the cases with ACR ($P < 0.001$). Graft failure occurred in 12 (66.7%) of the 18 patients with acute ABMR. The causes of graft loss were chronic rejection in 8 cases, severe ACR in 2, persistent ABMR in 1, and unknown etiology in 1 (Table 3). Nine cases underwent enterectomy due to rejection. Of those, *de novo* DSA was detectable in 7 cases prior to enterectomy and was persistent after graft removal. Two cases had undetectable levels of *de novo* DSA by the ELISA

assay before enterectomy but became detectable after enterectomy. The presence of a newly-formed DSA was closely associated with graft loss ($P < 0.0001$). Compared with no rejectors, intestinal graft survival was significantly lower in patients with acute ABMR ($P = 0.0001$) or ACR ($P = 0.0009$). Graft survival was lower in acute ABMR than in ACR but the differences between them did not reach statistical significance ($P = 0.088$) (Figure 3A). Patient survival was worse in acute ABMR

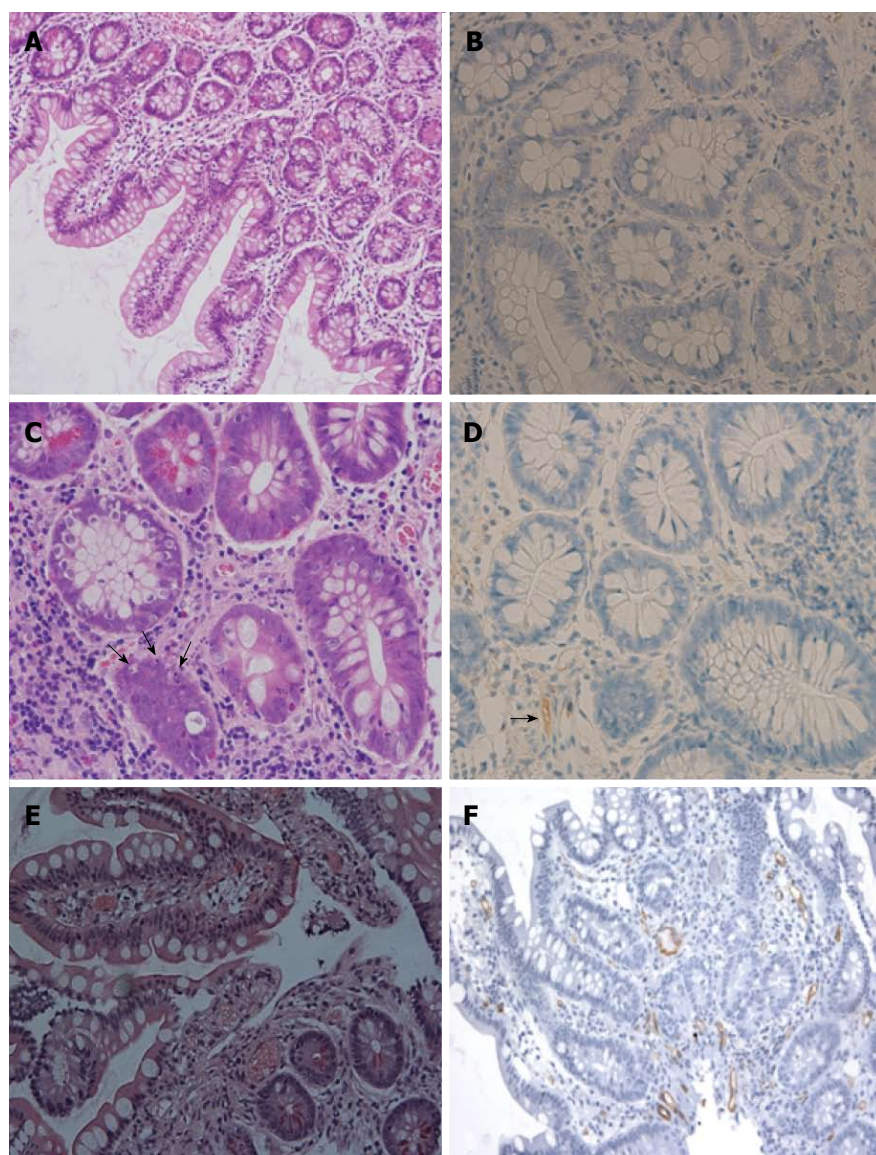


Figure 2 Histopathology of intestinal allograft. A and B: No rejection: normal mucosal architecture of small bowel biopsy after transplantation. No staining for C4d is seen in the capillaries of the lamina propria; C and D: Acute cellular rejection (ACR): There is mononuclear infiltration, crypt epithelial injury, and apoptotic bodies (arrows) in the lamina propria. Weak and focal staining for C4d (arrows) is sometimes present in a patient with ACR; E and F: Acute antibody-mediated rejection (ABMR): There is prominent hemorrhage and congestion with scattered fibrin thrombin in the lamina propria. Widespread and bright staining for C4d is present in the capillaries of the lamina propria. Magnifications: $\times 200$ in A, E and F; $\times 400$ in B, C and D. A, C, E: H and E; B, D, F: C4d.

or ACR than in no rejectors ($P = 0.0264$). There were no statistical differences in patient survival between ABMR and ACR (Figure 3B).

A total of five patients underwent repeat transplants, including a liver-inclusive graft in 4 and a liver-free intestinal graft in 1 patient. Of those, three patients had acute ABMR after primary transplantation and two patients had acute ABMR after retransplant. Four patients underwent repeat transplants after a diagnosis of acute ABMR and one with prior history of a liver-free graft developed acute ABMR after a liver-inclusive retransplant.

At an average follow-up of 32.3 mo (range, 13.3-76.4 mo), eight of the 18 (44.4%) patients died. The causes of patient death were sepsis in 4, massive gastrointestinal bleeding in 2, chronic liver failure in 1 and unknown etiology in 1 (Table 3). Patient 1 with a positive CDC XM developed acute ABMR in the intestinal allograft 4 d after a liver-inclusive intestine combined with kidney transplant. He responded well with a combination of steroids, IVIG and OKT3 therapies. One year after transplantation, he had progressively elevated liver enzymes with circulating

de novo DSA and he subsequently died due to chronic liver failure. In three patients undergoing liver-inclusive retransplants after primary graft loss, one patient died secondary to *Aspergillus* pneumonia, while the other three patients were alive with a well-functioning graft at the time of last follow-up. Two patients (#17 and #18) with a prior history of primary graft loss due to rejection developed acute ABMR after retransplantation. Patient 17 had acute ABMR 4 d after a liver-free retransplant and soon developed chronic rejection within a year with persistent *de novo* DSA and subsequently died due to massive lower GI bleeding. Patient 18 had acute ABMR 18 d after liver-inclusive retransplant, which was successfully treated with steroids and OKT3. The higher levels of preformed DSA gradually declined in this case after transplantation and she was well with functioning graft with no evidence of *de novo* DSA by the Luminex assay at the time of the last follow-up.

Risk factors for acute ABMR

In the univariate analysis, younger recipients at the time

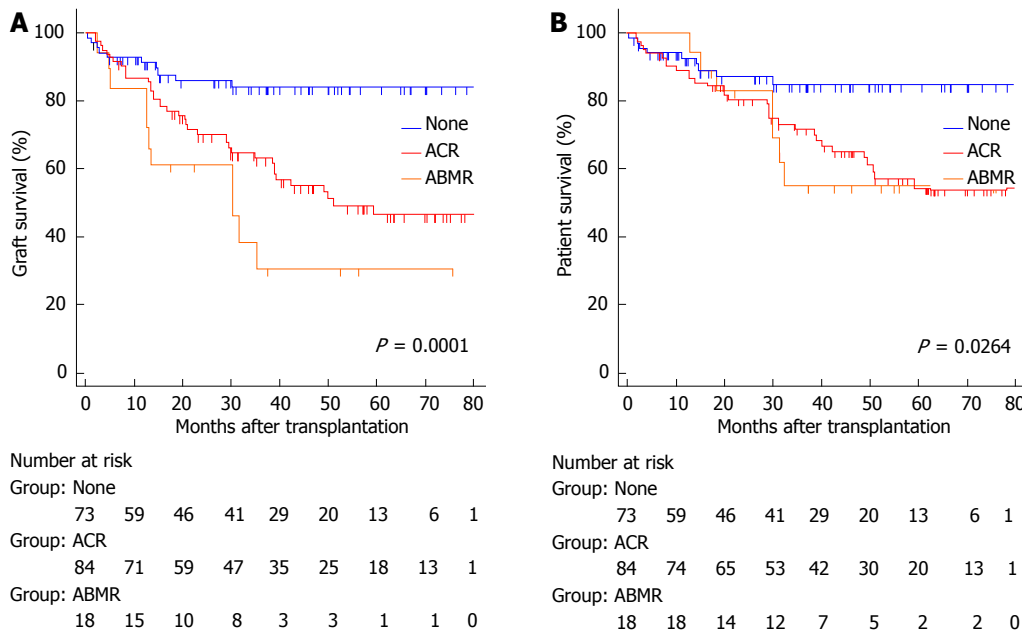


Figure 3 The Kaplan-Meier graft (A) and patient (B) survival for no acute rejection (none) (solid line), acute cellular rejection (dotted heavy line), and acute antibody-mediated rejection (dotted light line). The overall comparison was significantly different in graft (log-rank $P = 0.0001$) and patient survival (log-rank $P = 0.0264$). The patients with antibody-mediated rejection (ABMR) or acute cellular rejection (ACR) had significantly lower graft and patient survival than those without rejection. The graft survival was worse in ABMR than in ACR but the differences between them did not reach statistical significance ($P = 0.088$).

of transplant, liver-free graft, and the presence of DSA were closely related to acute ABMR. The presence of the spleen in recipients tended to be associated with acute ABMR but no statistical significance was observed ($P = 0.071$) (Table 4). In the multivariate analysis, only the presence of DSA and a liver-free graft were significantly associated with the development of acute ABMR (Table 5). Donor age, gender, cold ischemic time, cytomegalovirus donor and recipient serology, HLA mismatches, previous transplants, and induction therapy were not significantly associated with acute ABMR, and their inclusion in the multivariate model did not exclude liver-free grafts or the presence of DSA as independent risk factors.

DISCUSSION

We believe that the established diagnostic standards for acute ABMR in kidney or heart transplants can help identify acute ABMR after ITx. We found the incidence of acute ABMR of the intestinal allograft is 10.3%, on the basis of the presence of circulating DSA, evidence of C4d deposit, acute tissue injury, and clinical graft dysfunction. To our knowledge, this is the first largest series of investigation to date to retrospectively assess the incidence of acute ABMR after ITx. The most important finding in this study was that acute ABMR is closely associated with increased graft loss and poor outcomes. Our rate of intestinal graft acute ABMR is comparable to the incidence of acute ABMR reported in the kidney transplant that ranges from 7.7% to 41%, depending on the level of pre-transplant recipient sensitization status^[9,26,27]. Given the frequency and poor prognosis of acute ABMR after ITx, every effort should

be made to set up or eliminate this diagnosis in the setting of graft dysfunction to more specifically direct immunosuppressive management.

C4d deposition along graft capillaries has become a critical component to the diagnosis of acute ABMR in a kidney or heart transplant. However, the clinical relevance of a positive C4d staining in an intestinal allograft remains uncertain. Post-transplant microvascular lesions in a small intestinal allograft at early time periods might be related to higher pre-transplant PRA levels or a positive CDC-XM^[7]. Other studies concluded that C4d deposition had no clinical significance when assessing acute ABMR in a small bowel allograft^[28,29]. Unfortunately these studies either did not correlate with the HLA antibody levels or they did not detect DSA in small-sized heterogeneous populations. Therefore, the above studies did not have sufficient evidence to include or exclude C4d as a useful marker to detect acute ABMR in intestinal allografts. Our previous publications showed that a diffuse C4d deposition was very common in CDC-XM positive recipients with the presence of DSA, while focal and trace C4d deposition was often seen in CDC-XM negative recipients in the setting of no histological evidence of ACR or evidence of ACR but in absence of DSA^[18]. Our current study further demonstrates that a diffuse C4d deposition is strongly associated with vascular disturbances after ITx, indicating that it is a useful marker for a diagnosis of ABMR after ITx. Our results suggest that a diffuse C4d staining, in conjunction with the presence of DSA, clinicopathological findings and significant clinical improvement after initial treatment, strongly supports a diagnosis of acute ABMR. The clinical relevance of focal and weak C4d staining in an intestinal

Table 4 Pretransplant risk factors for acute antibody-mediated rejection (univariate analysis)

Variables	Non-ABMR (<i>n</i> = 157)	ABMR (<i>n</i> = 18)	OR	95%CI	<i>P</i>
Donor age (yr)	25.6 ± 10.2	24.0 ± 6.5	0.98	0.93-1.04	0.549
Female donor, <i>n</i> (%)	33 (20.8)	6 (37.5)	0.44	0.15-1.29	0.133
Cold ischemic time (h)	7.72 ± 1.52	7.58 ± 1.11	0.94	0.66-1.33	0.711
Recipient age	43.7 ± 12.4	36.9 ± 12.0	0.96	0.92-0.99	0.028
Female recipient, <i>n</i> (%)	94 (59.8)	13 (72.2)	0.57	0.19-1.69	0.299
Donor CMV positive/recipient negative, <i>n</i> (%)	34 (21.4)	4 (25.0)	1.12	0.69-1.81	0.642
Donor/recipient sex mismatches, <i>n</i> (%)	88 (55.3)	11 (68.7)	1.78	0.59-5.35	0.308
HLA mismatches ≥ 4, <i>n</i> (%)	107 (67.3)	12 (75.0)	1.46	0.45-4.74	0.531
Prior transplant, <i>n</i> (%)	9 (5.7)	2 (12.5)	2.38	0.47-2.12	0.296
Campath-IH induction, <i>n</i> (%)	113 (71.1)	12 (75.0)	0.72	0.30-1.73	0.468
Liver-free graft, <i>n</i> (%)	92 (58.5)	15 (83.3)	3.53	1.08-12.7	0.031
Presence of spleen, <i>n</i> (%)	99 (63.1)	15 (83.3)	2.93	0.81-10.55	0.071
Anti-HLA antibodies					
Positive CDC-XM, <i>n</i> (%)	30 (19.1)	14 (77.8)	21.17	5.76-77.81	< 0.0001
PRA I ≥ 10%, <i>n</i> (%)	53 (33.8)	18 (100)	33.36	4.32-257.52	< 0.0001
PRA II ≥ 10%, <i>n</i> (%)	32 (20.4)	14 (77.8)	13.67	4.21-44.36	< 0.0001
Presence of DSA, <i>n</i> (%)	37 (23.6)	18 (100)	55.14	7.09-428.38	< 0.0001

ABMR: Antibody-mediated rejection; CMV: Cytomegalovirus; CDC-XM: Complement-dependent lymphocytotoxic cross-match; PRA: Panel reactive antibody; DSA: Donor-specific antibody.

Table 5 Pretransplant risk factors for acute antibody-mediated rejection (multivariate analysis)

Variables	OR	95%CI	<i>P</i>
Liver-free graft	8.791	2.011-38.480	0.004
PRA class I	16.302	3.092-85.801	0.001
PRA class II	6.023	1.490-24.253	0.012

PRA: Panel reactive antibody.

allograft should be further evaluated in future studies. We suggest that a C4d staining be routinely included in intestinal biopsies in sensitized recipients or in the setting of appearance of a newly-formed DSA after ITx.

The rate of pretransplant sensitization in our current study was 30.3%, higher than 10%-15% in kidney or heart transplant recipients, indicating that intestinal recipients are an high immunological risk group. The causes of sensitization may be from previous operations, multiple blood transfusions, infections, pregnancies or retransplantation. Sensitization increases the risk of a positive CDC-XM and is associated with rejection and poor outcomes. Our results further showed that a positive CDC-XM significantly increases the risk of acute ABMR and is closely associated with graft loss, particularly in a liver-free transplant recipient. In the setting of anti-donor antibody titer ≥ 1:8, all four recipients lost grafts early on after a liver-free transplant. Similar to other solid organ transplants, our findings confirmed a close association between preformed DSA and acute ABMR, indicating that preformed DSA is a prerequisite for the occurrence of acute ABMR after ITx. In our series the majority of patients had preformed class I DSA prior to transplantation, often directed at the A, B locus, whereas the majority of *de novo* DSA post-transplant were against class II, which were often associated with late graft failure. The mechanisms underlying the difference between class I and class II in

this clinical setting is unknown. Based on our results, we suggest special attention should be paid to recipients with high immunological risk in terms of implementing pre-transplant desensitization strategies, avoiding positive cross-match transplantation, increasing maintenance immunosuppression, and frequently monitoring DSA post-transplant.

In our series, younger recipient age was associated with acute ABMR, but this significance no longer exist when we adjusted for other factors. Our analysis identified a liver-free transplant as an independent risk factor for the occurrence of acute ABMR after ITx. Our previous paper demonstrated that the liver is relatively insensitive to antibody-mediated damage and the inclusion of a liver graft with the intestine appears to be protective in recipients of high immunological risk^[30]. In contrast to a liver-free graft, no or only a single mild episode of rejection occurred in three highly sensitized recipients after a liver-inclusive transplant. Our findings further confirmed that the liver as a component of multivisceral transplants might ameliorate or prevent early and late intestinal allograft loss. A liver-inclusive transplant may offer a better long-term patient and graft survival in immunological high-risk recipients. As optimized approaches for depleting HLA antibodies have not yet been set up in ITx, the use of a liver-inclusive graft may be a valuable option in highly sensitized recipients, especially in the setting of retransplantation after primary graft loss due to rejection.

Despite the initial clinical improvement in many patients, long-term outcomes were dismal because of a high incidence of chronic rejection. In this series, although a combination of steroids and T-cell targeted OKT3 achieved the initial resolution after a diagnosis of acute ABMR, the majority of grafts failed due to subsequent severe ACR or chronic rejection. Clearly, additional studies are required to identify effective strategies to control acute ABMR. The antibody-directed regimens, such as

IVIg and anti-CD20, should be routinely implemented in highly sensitized recipients prior to transplantation. A new therapy, such as the proteasome inhibitor "bortezomab" and complement-targeted treatment with C1 or C5 inhibitor, has yielded encouraging preliminary results, but the long-term efficacy and safety remain to be seen.

Our study has several important limitations, including its retrospective nature, inconsistent antibody detection methods, and experience at a single institution. Although we identified 18 cases among 175 transplants over a 7-year period, the true incidence of acute ABMR may be higher after ITx. It is likely that less severe cases were unrecognized due to our evolving antibody detection methods, lack of standardized definition, and our unawareness of the importance of acute ABMR during the early study period. An additional important limitation of this study is that C4d staining of biopsies was not routinely performed in recipients with ACR to evaluate its sensitivity and specificity. Furthermore, the lack of consistent DSA monitoring post-transplantation limits our ability to assess sub-clinical acute ABMR. However, we sought to characterize a convincing series of cases to develop a preliminary definition that may serve as a foundation for future studies.

Our results indicate that acute ABMR is an important cause of intestinal graft dysfunction, particularly in a liver-exclusive transplant. After acute ABMR, patients are at an increased risk of developing refractory acute rejection and chronic rejection. Preventive and effective therapeutic approaches are needed to manage acute ABMR in intestinal transplant recipients.

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COMMENTS

Background

Antibody-mediated rejection (ABMR) is the major cause of kidney transplant failure. The incidence and clinical significance of acute ABMR after intestinal transplantation (IATx) remain unknown.

Research frontiers

ABMR has increasingly become an important area for research and clinical investigation. The aim of this study aimed to assess the incidence, risk factors and clinical outcomes of acute ABMR after ITx.

Innovations and breakthroughs

This is the first largest series of study to retrospectively examine the incidence of acute ABMR after ITx. The incidence of acute ABMR after ITx is 10.3% in the series. Both a liver-free graft and a high level of panel reactive antibody were identified as a significant independent risk factor for acute ABMR. Without appropriate management, acute ABMR was closely associated with increased

graft loss and poor clinical outcomes.

Applications

The results suggest that acute ABMR must be included in the differential diagnosis of acute rejection after ITx. The prevention of acute ABMR should include desensitization, avoiding a positive cross-match donor, and considering the liver as part of an intestinal graft in highly sensitized recipients. Future studies are required to develop the optimal approaches to managing acute ABMR in ITx recipients.

Terminology

Acute ABMR is identified on the basis of circulating donor-specific antibody, C4d deposition, tissue injury and clinical allograft dysfunction after ITx.

Peer-review

This is a well written, well designed retrospective review study on acute ABMR after intestinal transplantation, put through an electronic analysis of medical records of 18 patients diagnosed with ABMR out of 175 liver-free small bowel and modified multivisceral graft transplantations, during a 7-year period.

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Observational Study

Persistent Epstein-Barr viral load in Epstein-Barr viral naïve pediatric heart transplant recipients: Risk of late-onset post-transplant lymphoproliferative disease

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Abstract

AIM

To examine the risk of late-onset post-transplant lymphoproliferative disorder (PTLD) in the presence of persisting high Epstein-Barr virus (EBV) in EBV naïve pediatric heart transplant (HT) recipients.

METHODS

A retrospective review of the medical records of the 145 pediatric HT recipients who had serial EBV viral load monitoring at our center was performed. We defined EBV naïve patients whose EBV serology either IgM or IgG in the blood were negative at the time of HT and excluded passive transmission from mother to child in subjects less than 6 mo of age.

RESULTS

PTLD was diagnosed in 8 out of 145 patients (5.5%); 6/91 (6.5%) in those who were EBV seropositive and 2/54 (3.7%) in the EBV naïve group at the time of HT ($P = 0.71$). We found 32/145 (22%) patients with persistently high EBV load during continuing follow-up; 20/91 (22%) in EBV seropositive group vs 12/54 (22%) in EBV naïve group ($P = 0.97$). There was no significant association between pre-HT serostatus and EBV load after transplant ($P > 0.05$). In the EBV seropositive group, PTLD was diagnosed in 15% (3/20) of patients with high EBV vs 4.2% (3/71) of patients with low or undetectable EBV load ($P = 0.14$) whereas in EBV naïve patients 8.3% (1/12) of those with

high EBV load and 2.3% (1/42) with low or undetectable EBV load ($P = 0.41$). There was a highly significant association between occurrence of PTLD in those with high EBV load and duration of follow up (4.3 ± 3.9 years) after HT by Cochran-Armitage test for the entire cohort ($P = 0.005$). At least one episode of acute rejection occurred in 72% (23/32) of patients with high EBV *vs* 36% (41/113) patients with low or undetectable EBV after HT ($P < 0.05$).

CONCLUSION

There is an association between persistently high EBV load during post-HT follow up and the occurrence of late-onset PTLD in pediatric HT recipients irrespective of serostatus at the time of transplant. The occurrence of allograft rejection increased in patients with high EBV load presumably due to reduction in immunosuppression.

Key words: Pediatric heart transplantation; Epstein-Barr virus; Post-transplant lymphoproliferative disorder; Immunosuppression; Allograft rejection

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Core tip: Post-transplant lymphoproliferative disorder (PTLD) after heart transplantation is a severe complication where there is still limited information is available. There are many publications on estimations of PTLD frequency in different settings and types of patient, as well as the factors associated with its appearance and prognosis. But, most studies do not take into account the length of follow-up which may be misleading given that patients are exposed to the risk of immunosuppression over a long period of follow-up. This study is unique that, it is a single center study span over a period of 18 years in which maintenance immunosuppression therapy and management of rejection episodes remained same throughout. Although, a single center study result cannot be generalized, however it adds to the existing literature for risk stratification of these patients based on whole blood Epstein-Barr virus (EBV) polymerase chain reaction (PCR) after accounting for the time since transplant and patients' pre-transplant EBV serostatus. This paper also highlights the risk of acute rejection after reduction or alteration in immunosuppression in patients with high EBV load by PCR without any effect on the occurrence of PTLD.

Das B, Morrow R, Huang R, Fixler D. Persistent Epstein-Barr viral load in Epstein-Barr viral naïve pediatric heart transplant recipients: Risk of late-onset post-transplant lymphoproliferative disease. *World J Transplant* 2016; 6(4): 729-735 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/729.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.729>

INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) is the most common malignancy occurring in 3.5%-9%

of pediatric HT recipients^[1-4]. It is characterized by uncontrolled proliferation of lymphoid lineage cells, the vast majority of which are B-cell lymphomas, in a context of posttransplant immunosuppression. In some situations, reducing the immunosuppression can reverse the proliferation, thus differentiating it somewhat from truly irreversible malignancies. Most but not all PTLD cases have a strong relationship with Epstein-Barr virus (EBV).

The development of PTLD is influenced by a variety of factors including the type, intensity, and cumulative amount of immunosuppression, and the EBV status of the donor and recipient. Children are at greatest risk for the development of PTLD since they are often seronegative at transplant and acquire a primary EBV infection post-heart transplant (HT) in the setting of immunosuppression. Nevertheless, the factors that account for whether or not a particular child develops EBV-associated PTLD are undetermined. Diagnosis and effective treatment of PTLD is hampered by our inability to determine which children are at risk of developing EBV-associated PTLD.

The onset of PTLD is usually preceded by an elevated EBV load in the peripheral blood which is highly sensitive but not a specific marker for development of PTLD in renal transplant recipients^[5,6]. Routine long-term post-HT EBV monitoring identifies a group of children who carry persistent viral loads for months to years after solid organ transplants^[5-8]. Patients with a persistently elevated level of circulating EBV may have an increased risk of PTLD^[2,9]. Previously, a single center study reported that a high EBV load did not predict PTLD in early post-heart and heart-lung transplant period^[10]. However, another single center study suggested that exposure to EBV and higher intensity immunosuppression was associated with increased risk of PTLD in pediatric HT recipients^[11]. A more recent study has shown that early onset PTLD in solid organ transplant recipients appears mainly as an EBV-driven disease especially favored by insufficient immunosurveillance^[12,13]. These contradictory findings leave the long-term clinical significance of chronic high EBV load unknown. We hypothesized that patients with persistently high EBV viral load at any time after their transplant in the setting of immunosuppression may be at increased risk for development of PTLD.

The objective of the study was to examine the risk of late-onset PTLD (> 1 year post-transplant) in the presence of persisting high EBV and to determine whether patients' serostatus at the time of HT changed the risk.

MATERIALS AND METHODS

All pediatric HT patients transplanted between 1995 and 2013 who had known EBV serology at the time of transplant were included in this retrospective descriptive study. For this study, we defined EBV naïve patients

whose EBV serology either IgM or IgG in the blood were negative at the time of HT and excluded passive transmission from mother to child in subjects less than 6 mo of age. Data collection included demographics, clinical data, pre-HT EBV serological status, serial post-HT EBV load, diagnosis of PTLD and acute rejection episodes during post-HT follow-up. The presence of EBV virus in whole blood was measured by quantitative polymerase chain reaction (PCR) using a cut-off of 1000 copies/mL according to our institution protocol.

Viral (EBV) load testing was done in whole blood using PCR every 2 wk for 3 mo, every month for 3 mo, every 3 to 6 mo for a duration of 1 year after HT, and thereafter annually. Additional EBV PCR levels were drawn if EBV PCR was rising, with any increased immunosuppression for treatment of allograft rejection, or if clinically indicated by symptoms such as protracted fever, gastrointestinal symptoms, unexplained elevated liver enzymes, lymphadenopathy, tonsillar hypertrophy, obstructive sleep apnea, unexplained anemia, pancytopenia, atypical lymphocytes or eosinophilia, persistent headache or focal neurological symptoms.

Our protocol for follow-up of patients based upon EBV PCR positivity included: (1) EBV PCR < 1000 copies/mL: No change in immunosuppression, routine follow-up as per above protocol; (2) EBV PCR 1000-9999 copies/mL: No change in immunosuppression, repeat EBV PCR every 2 wk; and (3) EBV PCR \geq 10000 copies/mL (> 2 consecutive tests and remains positive for > 12 mo): We reduced immunosuppression with a goal for tacrolimus trough level 3-5 ng/mL, cyclosporine trough level 50-75 ng/mL, and decreased mycophenolate mofetil/azathioprine dose to half of the initial dose and closely monitored for any signs of acute rejections. For this study analysis, we divided all patients into 3 groups based on EBV viral load as a continuous value anytime during post-TX follow-up: group I : Negative EBV or EBV PCR < 1000 copies/mL; group II : EBV PCR 1000-9999 copies/mL; and group III : EBV PCR \geq 10000 copies/mL (persistently positive for > one year). During follow-up, patients who had transiently increased EBV PCR in excess of 10000 but did not persist for a year were included in group II.

PTLD was defined according to the 2008 World health Organization (WHO) classification system, but early lesions such as lymphoid hyperplasia with scattered positive in situ hybridization using EBV encoded RNA detected by the Epstein-Barr early region (EBER) immunostaining assay^[14] was excluded for this study as PTLD. All biopsy proved polymorphic, monomorphic and classical Hodgkin lymphoma-type PTLD patients were evaluated by our oncology service and treatment was guided as per oncology protocol.

Acute Allograft rejection was defined as ISHLT grade 2R or higher or an episode of clinically significant decline in cardiac function treated with steroid bolus or anti-T cell therapies. Endomyocardial biopsy was performed per our institutional protocol for all patients and frequency of biopsy was not modified based on high EBV load or reduction of immunosuppression. However, patients

whose immunosuppression was decreased as a result of high EBV load were monitored closely clinically and by echocardiogram for any graft dysfunction.

All patients received basiliximab (simulect) and methyl prednisone for induction at the time of transplant as per our institution protocol since 2001. Between 1995-2000, our induction therapy was only methyl prednisone. Maintenance immunosuppression includes triple therapy of tacrolimus/cyclosporine, mycophenolate mofetil (MMF), and steroids. Steroids were withdrawn after one year routinely unless there are more than one rejection episode within first year after transplant. This study was approved by our institutional IRB.

Statistical analysis

Descriptive analyses of the continuous and categorical data were performed using mean, standard deviation, median, quartiles, frequency and proportion as appropriate. Fisher's exact test and χ^2 tests were used to test binary variables between two groups. Cochran-Armitage test and logistic regression were used to test the association between post-HT EBV load, duration of follow-up and incidence of PTLD. The statistical analyses were performed with SAS 9.3.

RESULTS

A total of 145 patients were followed from 1995 to 2013 for mean 4.3 ± 3.9 years (interquartile range 1.5 to 6.0 years) post-HT. Mean age at HT was 6.6 ± 6.3 years, median age 4.8 years with interquartile range 0.69 to 12.0 years. EBV was first detected at a median of 1 year (range 0.1 to 16 years) post-HT. Patients were then subgrouped based on age at transplant into 0-6 mo, 6 mo to 1 year, 1-7 years and 7-20 years vs EBV load as shown in Table 1. The proportions of high EBV load are 38.8%, 27.4%, 18.4% and 15.4% in age group 0-6 mo, 6 mo to 1 year, 1-7 years and 7-20 years, respectively. Cochran-Armitage test with square root transformation to age and the logistic regression showed that patients' age at HT was negatively associated with high EBV load ($P = 0.03$), which means patients with younger age had high risk for high EBV during follow up. One year old was chosen as the threshold for younger patients. χ^2 test showed that patients 1 year old or younger were more likely to have high EBV during follow up than patients older than 1 year old ($P = 0.01$). The relative risk for developing high EBV load in patients having transplant at 1 year old or younger is 2.16 (95%CI: 1.19-3.92) over patients having transplant at older age irrespective of their pre-HT EBV serological status.

The clinical characteristics of individual PTLD patient are described in Table 2. All patients were treated by reducing immunosuppression; five patients received rituximab, two patients received chemotherapy and one patient received chemotherapy plus radiation therapy. Three patients underwent tumor resection and all patients survived the treatment of PTLD. One patient died two years after treatment of PTLD due to non-cardiac cause.

Table 1 Outcomes (post-transplant lymphoproliferative disorder and number of rejections) by Epstein-Barr virus serological status at heart transplant

	Group I (EBV PCR negative or < 1000) (n = 66)	Group II (EBV PCR 1000-9999) (n = 47)	Group III (EBV PCR ≥ 10000) (n = 32)
EBV Naïve at HT	29/66 (44%)	13/47 (28%)	12/32 (37%)
Age at transplant			
0 up to < 6 mo	9	10	12
6 mo < 1 yr	3	5	3
1 yr up to < 7 yr	28	8	8
≥ 7 yr up to 20 yr	26	24	9
Post-HT Follow-up (yr)	4.5 ± 3.2	4.8 ± 4.2	4.6 ± 5.3
No of PTLD	1	3	4
No of total Rejections	40	42	48
Number of patients with ≥ 1 episodes of rejections	19	22	23

EBV: Epstein-Barr virus; PCR: Polymerase chain reaction; HT: Heart transplant; PTLD: Post-transplant lymphoproliferative disorder.

Table 2 Characteristics of individual patient diagnosed with post-transplant lymphoproliferative disorder

Patient (gender)	Year of HT	HT to PTLD (yr)	EBV serology at HT	EBV load at PTLD	Organ involved in PTLD	CD20 positivity	EBER status of PTLD	Histological diagnosis	Treatment
1 (M)	1995	16	Positive	< 10000	Retroperitoneal lymph node	Neg	Neg	Hodgkin Lymphoma	Chemotherapy
2 (F)	1996	14	Positive	< 10000	Cervical Lymph node	Neg	Neg	Diffuse large B-cell lymphoma	Chemotherapy
3 (M)	1999	12	Positive	> 10000	Retroperitoneal lymph node	Pos	Neg	Polymorphic PTLD	Rituximab
4 (M)	2001	3	Positive	> 10000	Pharynx	Neg	Pos	Intermediate between Hodgkin and large cell lymphoma	Chemotherapy plus Radiation
5 (F)	2009	3	Negative	> 10000	Cervical Lymph node	Pos	Pos	Polymorphic PTLD	Rituximab
6 (F)	2000	14	Positive	< 1000	Brain-Temporal Lobe	Pos	Pos	Polymorphic PTLD	Rituximab
7 (M)	2000	3	Negative	< 10000	Small intestine	Pos	Pos	Polymorphic PTLD	Rituximab
8 (F)	2005	6	Positive	> 10000	Retroperitoneal lymph node	Pos	Pos	Large B-cell Lymphoma	Rituximab

HT: Heart transplant; Neg: Negative; Pos: Positive; EBER: Epstein-Barr virus encoded small RNA; PTLD: Post-transplant lymphoproliferative disorder.

Figure 1 describes the distribution of patients' EBV serological status at the time of HT, EBV viral load by PCR post-HT, and number of patients who developed PTLD for the entire cohort. Out of 145 patients, 54 (37%) were EBV seronegative and 91 (63%) were EBV seropositive at the time of transplant and 22% from each group developed persistently high EBV viral load during follow-up after HT ($P = 0.97$). There were 6 cases (6.4%) of PTLD in EBV seropositive group vs 2 cases (3.9%) in EBV naïve group ($P = 0.71$). In the EBV seropositive group, PTLD was diagnosed in 15 % (3/20) of patients with persistently high EBV vs 4.2% (3/71) of patients with low or undetectable EBV load ($P = 0.14$) whereas in EBV naïve patients PTLD was diagnosed in 8.3% (1/12) who had persistently high EBV load and 2.3% (1/42) with low or undetectable EBV load ($P = 0.41$). There was no significant association between pre-HT serostatus and post-HT EBV viral load after transplant ($P > 0.05$).

For the entire cohort of 145 patients, we found 65/145 (44.8%) had negative or EBV < 1000 copies/mL (Group

I), 48/145 (33%) had EBV load between 1000-9999 copies/mL (Group II), and 32/145 (22%) patients had EBV load ≥ 10000 copies/mL (Group III) during follow-up irrespective of initial serological status at the time of transplant. PTLD was diagnosed in 8 out of 145 patients (5.5%) at a median of 4.4 years (mean 7.5 ± 6.5 years, interquartile range 2.7 to 13.7 years) after heart HT. PTLD was diagnosed in 12.5% (4/32) of patients with persistently high EBV vs 3.5% (4/113) of patients with low or undetectable EBV load ($P = 0.07$ by Fisher's exact test). High viral load could predict PTLD with sensitivity 50% (95%CI: 15.7%-84.30%), specificity 79.66% (95%CI: 71.8%-85.97%), positive likelihood ratio 2.45 (95%CI: 1.14-5.27), negative likelihood ratio 0.63 (95%CI: 0.31-1.26) and positive predictive value 12.5% (95%CI: 3.51%-2.88%). There is a significant association between persistently high EBV load during a sum of follow up over 11 ± 7 years after HT and the occurrence of PTLD by Cochran-Armitage test ($P = 0.005$).

There was at least one episode of acute rejection

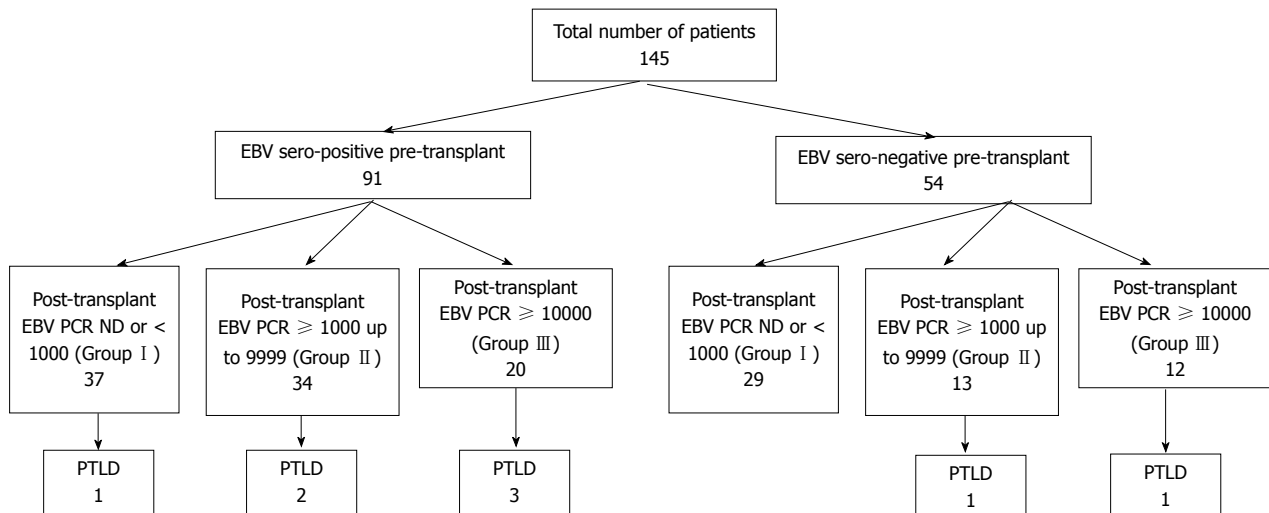


Figure 1 Patients' Epstein-Barr virus serostatus pre-heart transplant, post-transplant Epstein-Barr virus viral load as determined by whole blood polymerase chain reaction and incidence of post-transplant lymphoproliferative disorder for the entire cohort. EBV: Epstein-Barr virus; PTLD: Post-transplant lymphoproliferative disorder; PCR: Polymerase chain reaction.

(Grade 2R) in 23 patients with high EBV load after reduction of their immunosuppression (Table 1). On the other hand, 41 patients with low or negative EBV load who had no change in their immunosuppression had at least one episode of rejection. Thus, a larger proportion of patients 72% (23/32) with persistently high EBV load had acute rejections vs 36% (41/113) patients with low or negative EBV load ($P < 0.05$). Furthermore, there was an increase in frequency of total rejection episodes in patients with persistently high EBV load by 150% (48/32) vs 72.5% (82/113) in patients with low or negative EBV load ($P < 0.05$).

DISCUSSION

The incidence of PTLD in our study at 5.5% is similar with other series reported^[1]. The occurrence of PTLD is dependent on the transplanted organ type and patient-specific risk factors. The strongest risk factor for PTLD is the development of primary EBV infection after transplantation^[2,12,15,16]. Schubert *et al.*^[2] have reported 8.2% incidence of PTLD in pediatric HT recipients and the EBV association was 83% as a risk factor for development of PTLD. EBV monitoring in peripheral blood using PCR has been reported to have variable sensitivity and lack of specificity as an indicator of risk for developing PTLD^[5,10,17-19]. Among pediatric HT recipients studied by Bingler *et al.*^[20] those with high EBV load were more likely to develop late-onset PTLD, occurring as long as 8.4 years after HT. In this study, we showed that patients who underwent HT at younger age (Table 1) are at higher risk for development of high EBV load over time ($P = 0.05$) irrespective their serological status at the time of HT. This observation is of clinical importance because many potential risk factors for development of PTLD such as persistent EBV viremia and overall immunosuppression are a function of duration of follow-up and may not be observed in early post-HT period. The

occurrence of PTLD is highest in younger patients; age may not be an independent risk factor but may depend upon the likelihood of the recipient being exposed to long-term immunosuppression.

One of the limitations of the current study is the fact that we had incomplete data on the donor EBV status. Therefore, we could not determine whether high EBV load was the result of primary infection derived from community exposure or related to donor transmission. Asymptomatic high EBV load also predicts other adverse outcomes, such as graft dysfunction or acute rejection^[20,21]. Jabs *et al.*^[21] showed that EBV viremia occurring immediately after renal transplant was associated with subsequent rejection episodes, and they speculate that T cell responses to viral infection might cross-react with the graft. In another study, Smith *et al.*^[22] have showed that subclinical cytomegalovirus and EBV viremia occurring in the early post-transplant period was associated with higher incidence of allograft injury. The authors did not find evidence of significant viral replication in the renal allograft at 2 years after transplant, suggesting that graft dysfunction is not related to chronic infection^[22]. We found a higher rate of rejection episodes (mostly grade 2R) in pediatric HT recipients with persistently high EBV load compared to those patients with low or negative EBV PCR. The mechanisms of rejection are not clear from this study but may include viral cytopathic effects, increased expression of alloantigen, adhesion molecule expression by endothelial cells, or indirect inflammatory effects due to cytokine release, or a combination of multiple mechanisms leading to allograft injury.

In our practice, we do reduce maintenance immunosuppression in patients who have persistently raised EBV PCR ≥ 10000 copies/mL of whole blood. A link between EBV load and level of immunosuppression in adult HT patients was noted^[23]. We hypothesize that reduction of immunosuppression has probably contributed for higher allograft rejection episodes in patients with high

EBV load. Therefore, we recommend close monitoring for allograft rejection must be done after reduction of immunosuppressive therapy.

In this study, we have used methyl prednisone as induction therapy from 1995 through 2000 and basiliximab and methyl-prednisone as induction therapy from 2001 through 2013. Our standard maintenance immunosuppression (tacrolimus/cyclosporine, MMF or azathioprine and steroids) and consistent decrease in immunosuppression strategy in response to a high EBV support to the notion that it is overall immunosuppression exposure during the life time of the patient which compromises anti-tumor and anti-viral immunosurveillance capacity and thus facilitate development of PTLD. We have not used sirolimus or everolimus routinely and we cannot comment regarding the effect of proliferation signal inhibitors on EBV PCR or PTLD from this study.

This study must be viewed in light of some limitations. It was a single-center retrospective study and thus findings may not be generalizable. Some patients were transferred to another center and also transitioned to an adult HT program, thus complete follow-up data for a small portion of patients were not available. However, this is a well-studied patient population in which maintenance immunosuppression therapy and management of rejection episodes remained same throughout and we followed our standardized institutional protocol strictly.

In conclusion, there is an association between persistently high EBV load and the occurrence of late-onset PTLD in pediatric HT recipients especially considering cumulative incidences at different lengths of follow-up. Patients ≤ 1 year of age at the time of HT are more likely to have persistently high EBV PCR during follow up than patients > 1 year of age at the time of transplant irrespective of their EBV serological status. Reduction of immunosuppression in the face of persistently high EBV load did not change the proportions of patient who had late-onset PTLD but did increase the risk of allograft rejection significantly. Based on our findings, there is a need for research to better determine other factors that might be predictive of PTLD. Currently, there is a multi-center study sponsored by National Institute of Allergy and Infectious Diseases examining the role of viral (EBV) and immunological biomarker associated with development of PTLD after transplantation^[24]. This study will provide further insight to identify surrogate markers that can predict development of PTLD.

COMMENTS

Background

Post-transplant lymphoproliferative disorder (PTLD) is a significant complication after heart transplantation. Most but not all PTLD cases have a strong relationship with Epstein-Barr virus (EBV). This condition straddles the disciplines of transplantation, immunology, oncology, and virology. PTLD presents significant problems for the clinician because it is difficult to predict and has high morbidity and mortality rates. In addition, it has the potential for graft loss due to disease itself or the need to reduce immunosuppression, which increases the risk of graft rejection.

Research frontiers

The goal of this study is to review a single center experience of late-onset PTLD in the presence of persisting high EBV in pediatric heart transplant recipients.

Innovations and breakthroughs

This study showed that there is an association between persistently high EBV load during post-transplant follow up and the occurrence of late-onset PTLD in pediatric heart transplant recipients. The occurrence of PTLD is highest in younger patients; age may not be an independent risk factor but may depend upon the likelihood of the recipient being exposed to long-term immunosuppression. The incidence of allograft rejection increased in patients with high EBV load presumably due to reduction in immunosuppression.

Applications

Late-onset PTLD is less likely to be associated with patients' EBV serostatus at the time of transplant. Also, late-onset PTLD may be more likely extra-nodal and heterogeneous.

Terminology

Detection of persistently high EBV viral DNA by polymerase chain reaction (PCR) from the peripheral blood is associated with late-onset PTLD in pediatric heart transplant recipients.

Peer-review

The research is methodological well performed, clearly written, and the data is honestly presented.

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Observational Study

Psychological perspective of medication adherence in transplantation

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Abstract

AIM

To identify the risk factors and the post-transplant psychological symptoms that affect adherence to therapy in a population of kidney transplant recipients.

METHODS

The study examined the psychological variables likely responsible for the non-adherent behavior using a psychological-psychiatric assessment, evaluation of the perception of patients' health status, and an interview regarding the anti-rejection drug therapy assumption. The study included 74 kidney transplant recipients.

RESULTS

Individuals with a higher level of education and more years since transplantation showed better mental balance. Regarding gender, women appeared to be less adherent to therapy. Further, the years since transplantation adversely affected the proper pharmacological assumption. Adherence to therapy did not significantly change with the mental health index.

CONCLUSION

The biopsychosocial illness model provides a conceptual

frame of reference in which biological, psychological, and social aspects take on the same importance in the adherence to treatment protocols. For effective management, it is necessary to understand the patients' personal experiences, their assumptions about the disease, health status perception, and mood, and to identify any "barriers" that could cause them to become noncompliant.

Key words: Transplantation; Adherence; Mental health; Psychological assessment; Psychiatric assessment

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Core tip: Therapeutic Adherence after transplantation is of fundamental importance for the patient's short- and long-term well-being and assumes a set of adaptations to a new lifestyle. The authors in this study analyzed the psychological characteristics of a sample of transplant recipients and different temperament styles, yet not studied in other research on transplantation. The results suggested that different temperaments influence in different ways the treatment compliance and showed that the transplant experience change behaviors and quality of life based on the personality and temperament characteristics. In conclusion, post-transplant psychological support positively affects adherence to treatment, and coping strategies of the subject.

De Pasquale C, Veroux M, Fornaro M, Sinagra N, Basile G, Gozzo C, Santini R, Costa A, Pistorio ML. Psychological perspective of medication adherence in transplantation. *World J Transplant* 2016; 6(4): 736-742 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/736.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.736>

INTRODUCTION

The theme of therapeutic adherence (TA) plays a central role in research on education and health promotion^[1]. Adherence to immunosuppressive therapy after transplantation is of fundamental importance for the patients' well-being both short- and long-term and assumes a set of adaptations to a new lifestyle. The treatment effectiveness and transplant success not only depend on the correct choice of immunosuppressive drugs, but also on the patients' active participation in the therapeutic program that often includes psychological support and appropriate motivation^[2,3].

Non-adherence to therapy in transplant patients is one of the emerging causes of early and late graft loss. Patients with an organ transplant must take immunosuppressive drugs daily for the prevention of acute and chronic rejection. There is an obvious relationship between the discontinuity in the use of immunosuppressive drugs and the incidence of transplant failures in the medium and long term^[4]. Non-adherence to the transplant medication

regimen can lead to graft rejection, post-transplant mortality, increase in healthcare costs, and decrease in quality of life^[5,6]. One meta-analysis found non-adherence to medication across all organ transplants to be 22.6%^[7]. An estimated 50% of late acute rejections and 15% of graft losses are associated with non-adherence^[8]. An essential aspect to ensure full adherence to the treatment is the assessment of transplant recipient needs and his/her expectations while establishing a good therapeutic alliance^[9]. Many studies evaluating the relationship between the healthcare team and the patient highlighted the need for a relationship based on trust and clarity for the sharing of information regarding the treatment course^[10,11]. Even psychological and psychosocial aspects can alter the response to treatment^[12,13]: Mood disorders, high levels of anxiety, hostility, and the presence of "unstable" personality traits are associated with an increased risk of non-adherence to medical prescriptions in kidney transplant recipients^[14-16].

Adherence to therapy thus is a complex variable and influenced by many factors: Socio-demographics, psychological characteristics, transplant recipient self-efficacy, factors related to immunosuppressive therapy, and the doctor-patient relationship. The aim of this study is to identify the risk factors and post-transplant psychological symptoms that affect adherence to therapy in a population of kidney transplant recipients.

MATERIALS AND METHODS

The study examined the psychological variables that are likely responsible for the non-adherent behavior using a psychological-psychiatric assessment, evaluation of the perception of patients' health status, and an interview regarding the anti-rejection drug therapy assumption. The psychological-psychiatric assessment involved the use of the following tests:

The Symptom Checklist-90-Revised (SCL-90-R) evaluated psychological symptoms. It is a relatively brief self-report psychometric instrument (questionnaire) published by the Clinical Assessment division of the Pearson Assessment and Information group. It is one of the most widely used measures of psychological distress in clinical practice and research and is designed to evaluate a broad range of psychological problems and symptoms of psychopathology. According to the overview given by the publisher, the SCL-90-R is normed on individuals 13 years and older. It consists of 90 items and takes 12-15 min to administer. The following primary symptom dimensions are assessed: Somatization (SOM), obsessive-compulsive (OBS), interpersonal sensitivity (INT), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR), psychoticism (PSY), and a category of "additional items" that helps clinicians assess other aspects of the patient's symptoms^[17,18]. A large number of studies have been conducted demonstrating the reliability, validity, and utility of the instrument^[19-22].

Personality study has provided an analysis of the

Table 1 Demographic data (*n* = 74 kidney transplant responders)

Years since transplantation procedure, mean ± SD (range)	5.39 ± 3.74 (1.00-14.00)
Education	
Basic	36%
High school	56%
University	8%
Occupation	
Employed	31.17%
Unemployed	56.82%
Retired	12.01%

temperament variables by the TEMPS-A (Temperament Evaluation of Memphis, Pisa and San Diego Autoquestionnaire). The features of temperament as well as its intensity may exert a constructive or destructive impact on the quality of life^[23]. The TEMPS-A contains 110 items (109 in the version for males) measuring affective temperament traits occurring throughout life of the subject, as represented by five dimensions: Depressive (DT), cyclothymic (CT), hyperthymic (HT), irritable (IT), and anxious (AT). Questions about the various types are grouped together. The TEMPS-A measures the severity of the temperament traits ranging from 0 to 1. The calculation of points for each temperament is done by dividing the sum of points obtained in a given subscale by the number of questions contained therein^[24,25].

Quality of life was examined with the Short Form Health Survey (SF-36) that assesses the degree of self-perceived psychological well-being. The SF-36 consists of eight subscales: Vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health. Subscales are presented as scores between 0 and 100; a lower score indicates more disability and a higher score less disability. The two considered variables in this study were the physical index score (PIS) and mental index score (MIS). The validity and reliability of the SF-36 has been confirmed in patients with renal disease^[26,27].

Therapeutic adherence was studied through the Basel Assessment of Adherence to Immunosuppressive Medication instrument (BAASIS), which was developed to assess adherence to immunosuppressive medication in adult transplant patients. The instrument measures patients' taking, skipping, timing (± 2 h from the prescribed time, TM), and dose reduction of drugs. The recall period is limited to four weeks. The BAASIS comprises four questions with a 6-point scale for responses ranging from never (0) to every day (5). In addition, the BAASIS has a visual analogue scale (VAS) ranging from 0% (medication never taken as prescribed) to 100% (medication always taken as prescribed)^[28,29].

The current study included 74 kidney transplant recipients (32 females, 43.25%), with a mean age of 48.3 ± 13.6 years (range 22-75). Demographic data regarding years since transplant procedure (first transplantation), occupation, level of education are presented in Table 1. All

patients underwent a standardized immunosuppressive protocol with tacrolimus, mycophenolate mofetil, and steroids. The basic psychological-psychiatric assessment excluded the presence of lifetime psychiatric disorders (axis I) according to the Diagnostic and Statistical Manual for Mental Disorders (5th ed., DSM-5) or concomitant use of drugs that could influence cognitive and emotional aspects^[30]. All patients provided written informed consent after the procedures were fully explained by a trained physician (MD, psychiatrist) or a psychologist.

The data were examined for normality and transformed if necessary. Pearson's R correlation test was performed using the "Statistical Package for Social Sciences" (SPSS, Version 17). The *P* value of less than 0.05 ($P < 0.05$) was considered statistically significant. In addition, we applied multivariate linear regression analysis to predict the outcome variable (BAASIS total score, BT) from predictor variables (patterns of personality and demographic characteristics).

RESULTS

The current study included 74 kidney transplant recipients (32 females, 43.25%), with a mean age of 48.3 ± 13.6 years (range 22-75). Demographic data regarding years since transplant procedure (first transplantation), occupation, level of education are presented in Table 1. All patients underwent a standardized immunosuppressive protocol with tacrolimus, mycophenolate mofetil, and steroids. Correlations by the Pearson coefficient between results of the SCL-90-R, SF-36 (physical and mental index score), and demographic characteristics of the sample are shown in Figure 1. Individuals with a higher level of education (E) and with more years of transplantation (YT) showed higher mental balance (E/MIS $r = 0.61$; YT/MIS $r = 0.48$). Specifically, the level of education was negatively correlated with anxious, obsessive-compulsive, and depression aspects (E/OBS $r = -0.81$; E/DEP $r = -0.67$; E/ANX $r = -0.59$).

Correlations by the Pearson coefficient between results of the BAASIS, SF-36 (physical and mental index score), and demographic characteristics of the sample are shown in Figure 2. Regarding gender, women (female sex, FS) appeared to be less adherent to therapy in our study (FS/BT $r = 0.46$), while years of transplantation adversely affected the proper pharmacological assumption (YT/BT $r = 0.34$). In addition, as the index of subjective physical well-being increases, compliant behavior increases as well (PIS/BT $r = -0.47$), especially with regards to the treatment assumption of correct timing (PIS/TM $r = -0.27$). Adherence to therapy was not significantly correlated with the mental health index (MIS/BT $r = -0.01$).

Correlations by the Pearson coefficient between the results of the TEMPS-A, BAASIS of the sample are shown in Figure 3. The temperament variables measured with the TEMPS-A were correlated with treatment adherence. Specifically, the cyclothymic, irritable, and depression personality adversely affected adherent behavior (BT/CT

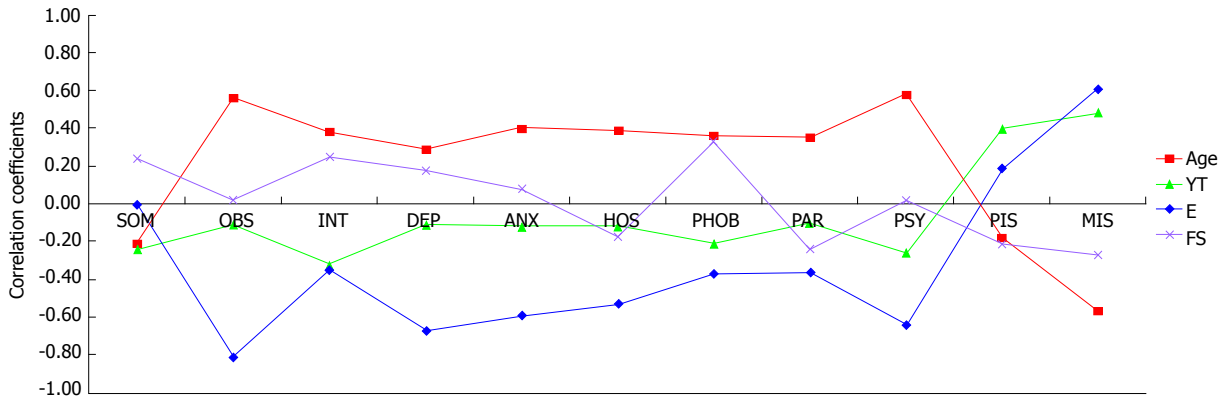


Figure 1 Correlations between symptom Checklist-90-R, short form health survey, and demographic characteristics. SF-36: Short form health survey; SOM: Somatization; OBS: Obsessive-compulsive; INT: Interpersonal sensitivity; DEP: Depression; ANX: Anxiety; HOS: Hostility; PHOB: Phobic anxiety; PAR: Paranoid ideation; PSY: Psychoticism; PIS: Physical index score of SF-36; MIS: Mental index score of SF-36; YT: Years since transplant procedure; E: Education; FS: Female sex. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7 strong correlation.

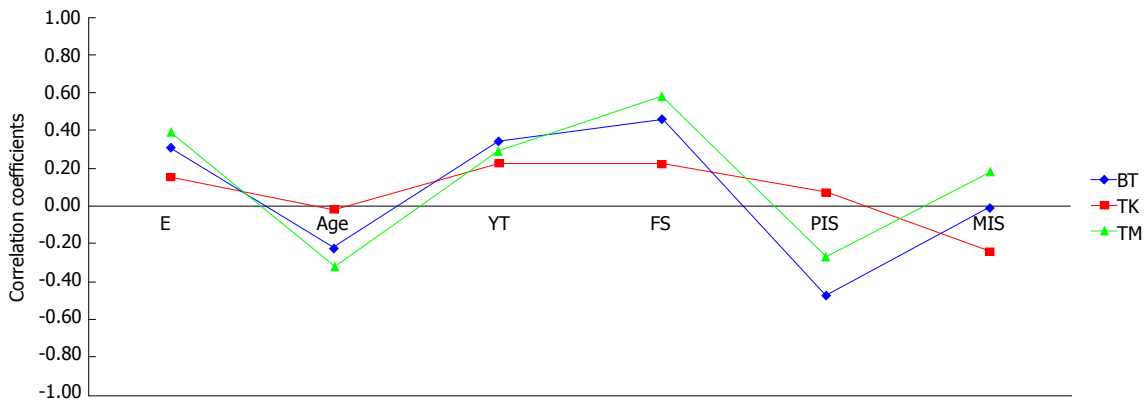


Figure 2 Correlations between basal assessment of adherence to immunosuppressive medication instrument, short form health survey, and demographic characteristics. BAASIS: Basel assessment of adherence to immunosuppressive medication instrument; BT: BAASIS total score; TK: BAASIS taking dimension; TM: BAASIS timing dimension; SF-36: Short form health survey; PIS: Physical index score of SF-36; MIS: Mental index score of SF-36; E: Education; YT: Years since transplant procedure; FS: Female sex. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7 strong correlation.

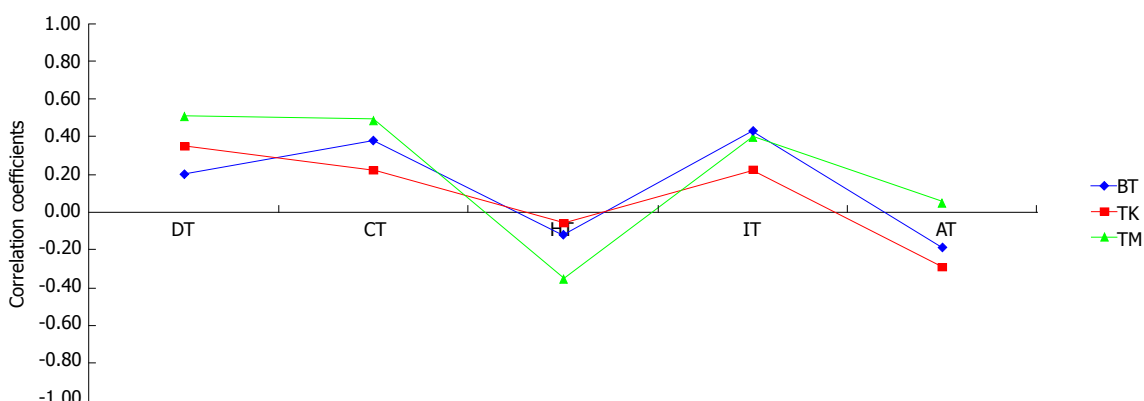


Figure 3 Correlations between temperament evaluation of memphis, pisa and san diego autoquestionnaire and basal assessment of adherence to immunosuppressive medication instrument. BAASIS: Basel assessment of adherence to immunosuppressive medication instrument; DT: Depressive temperament; CT: Cyclothymic temperament; HT: Hyperthymic temperament; IT: Irritable temperament; AT: Anxious temperament; BT: BAASIS total score; TK: BAASIS taking dimension; TM: BAASIS timing dimension. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7, strong correlation.

$r = 0.39$; BT/IT $r = 0.44$; BT/DT $r = 0.21$); however, a moderate positive correlation was found between the timing scale of the BAASIS and depressive temperament

variable (TM/DT $r = 0.52$), suggesting time management difficulties for patients with a depressive personality.

Multivariate linear regression analysis showed high

Table 2 Linear model of predictors sex, age, and years since transplant on basel assessment of adherence to immunosuppressive medication instrument total score

	B	SE B (SE)	β	P
Constant	-0.98 (-3.44 to 1.47)	1.13	0.00	0.39
Sex	1.53 (0.33 to 2.74)	0.55	0.75	0.01
Age	0.01 (-0.03 to 0.05)	0.02	0.14	0.58
YT	0.15 (0.03 to 0.27)	0.06	0.58	0.01

YT: Years since transplant procedure; Linear model with 95% bias corrected and accelerated confidence intervals (in parentheses).

associations between predictor variables (sex and years since transplant procedure, cyclothymic temperament, and anxious temperament) and outcome variable (BAA SIS total score), whereas no consistent associations between other predictor variables (age, irritable temperament, IT) and outcome variable (BAASIS total score) were detected (Tables 2 and 3).

DISCUSSION

Similar studies on the subject have revealed significant psychological and behavioral differences between adherent and non-adherent transplanted patients, differences that express a greater vulnerability of the latter and which lead to consider that, next to drug therapy, psychological therapy is required^[5,31]. Still not considered in other studies on the transplant topic is the temperament. "Temperament has been contemporarily defined as a biologically determined, hereditary core of the personality, being stable and relatively unchangeable throughout life, which determines the basic level of reactivity, mood and energy of given individual"^[24].

Based on these assumptions, this study has allowed us to analyze different temperament styles and suggested that different temperaments can influence in different ways the treatment compliance and quality of life of transplant recipients.

In this study, the biopsychosocial illness model (BIM) provided a conceptual frame of reference within which biological, psychological, and social aspects took on the same importance in explaining the adherence to treatment protocols^[32]. For good treatment management, it is necessary to understand the patients' personal experiences, their beliefs about the disease, health status perception, and mood, and to identify any "barriers" that could make them noncompliant. The analysis of the variables that are responsible for the behavior of not adhering to the treatment regimen should provide suggestions for psychological support and psychiatric treatment. Treatment adherence towards the prescribed medication is critical for the safe and successful delivery of efficacious interventions, especially for complex tasks such as the management of transplant patients^[33,34].

The study revealed that years of transplantation positively affected mental health, but on the other hand,

Table 3 Linear model of temperament predictors on basel assessment of adherence to immunosuppressive medication instrument total score

	B	SE B (SE)	β	P
Constant	0.87 (-0.14 to 1.88)	0.39	0.00	0.06
CT	0.51 (0.10 to 0.93)	0.16	1.92	0.02
IT	-0.17 (-0.58 to 0.23)	0.16	-0.48	0.32
AT	-0.28 (-0.46 to -0.09)	0.07	-1.44	0.01

CT: Cyclothymic temperament; IT: Irritable temperament; AT: Anxious temperament; Linear model with 95% bias corrected and accelerated confidence intervals (in parentheses).

adversely affected therapeutic adherence, while the level of education was positively correlated with good mental balance. Studies examining the non-pharmacological risk factors that influence therapeutic adherence thus need further confirmation^[5,35]. Another finding that requires careful reflection concerns the long-term negative impact that the cyclothymic and anxious personalities could have on adherent behavior, while the presence of post-transplant psychological symptoms (mental health index) did not affect treatment adherence^[36-39]. This finding could be related to the notion that while people with mental problems feel the need to be cared for and are more willing to properly follow the treatment protocol, patients with mood swings (cyclothymic temperament) and those in an alert and apprehensive state (anxious temperament) are not prepared to calmly accept the rigorous therapeutic protocol and require constant attention by healthcare staff^[40]. Thus, the quality of care is not just about the correct prescription but also about the patients' active participation through an assessment of their expectations and preferences. Patients adopt adherent behavior when they accept the type of care in terms of the therapeutic project^[41]. Helpful in this regard is cognitive behavioral therapy aimed at increasing transplant recipients' awareness^[42].

Immediately after transplant, patients must be assisted to increase the capacity for self-efficacy and resilience necessary to achieve the correct lifestyle for maintaining the graft. In a next step, it would be desirable to establish a cognitive and psychosocial rehabilitation plan to improve coping strategies and strengthen the patients' resources in order to positively influence the final outcome of the transplant process. These efforts therefore must operate simultaneously at several levels on the basis of an integrated strategy that organizes and coordinates the various types of treatment-medication, psychotherapy, assistance-and the operators' different functions, in an intervention program formulated on the basis of the characteristics and needs of each individual patient^[43,44].

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COMMENTS

Background

Adherence to immunosuppressive therapy after transplantation is of fundamental importance for the patients' well-being both short- and long-term and assumes a set of adaptations to a new lifestyle. I pazienti trapiantati devono assumere quotidianamente farmaci immunosoppressori per la prevenzione del rigetto acuto e cronico (infezioni, complicanze secondarie). The treatment effectiveness and transplant success not only depend on the correct choice of immunosuppressive drugs, but also on the patients' active participation in the therapeutic program that often includes psychological support and appropriate motivation. Adequate adherence to doctor's orders is a resource for both patients and the health care system and society, as it reduces the costs for therapies, for minor complications associated with the disease, the health care interventions, morbidity and mortality. However various social, cultural, financial and psychological aspects affect adherence to immunosuppressive therapy.

Research frontiers

Although most research has focused on adherence to drug treatment, the concept of adherence must include other behaviors related to health protection involving the doctor-patient relationship, the service delivery system and change their living habits. The communication characteristics of the doctor, the kind of language used and the setting are essential to strengthen the motivation and awareness of the need for a cure. Future programs should provide the ability to support the transplanted in transplant experience, helping him to properly follow treatment, help him to learn cognitive and behavioral strategies of self-regulation.

Innovations and breakthroughs

Studies of the Italian population have revealed significant differences in psychological and personality traits among transplant patients adherent and non-adherent to therapy, differences that express a greater vulnerability of the latter and which lead to consider that, next to drug therapy, you are required psychological therapy. This study also allows to analyze different temperament styles, yet not studied in other research on transplantation and suggests that different temperaments influence in different ways the treatment compliance.

Applications

The data in this study suggested that psychological and psycho-educational support to the transplanted patient could yield favorable outcomes about adherence to immunosuppressive therapy. Furthermore, this study also provided readers with important informations about psychological problems that could highlight on transplanted subject.

Terminology

TA is the patient's ability to be able to follow precisely the prescribed cure. Specifically, the concept of adherence to therapy includes the compliance and persistence: Compliance reflects the acceptance of the patient to medical prescription (number of daily dose), the persistence instead indicates the time period between the start and the interruption of the treatment. BIM provided a conceptual frame of reference within which biological, psychological, and social aspects took on the same importance in explaining the adherence to treatment protocols. You must operate simultaneously at several levels in an intervention program formulated on the basis of the characteristics and needs of each individual patient.

Peer-review

Studies concerning the influence of temperament to the therapeutic adherence are scarce. The authors in this study analyzed the psychological characteristics of a sample of transplant recipients followed as outpatients at a transplant center. The results showed that the transplant experience change behaviors and quality of life based on the personality and temperament characteristics.

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Observational Study

Time spent in hospital after liver transplantation: Effects of primary liver disease and comorbidity

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Abstract

AIM

To explore the effect of primary liver disease and comorbidities on transplant length of stay (TLOS) and LOS in later admissions in the first two years after liver transplantation (LLOS).

METHODS

A linked United Kingdom Liver Transplant Audit - Hospital Episode Statistics database of patients who received a first adult liver transplant between 1997 and 2010 in England

was analysed. Patients who died within the first two years were excluded from the primary analysis, but a sensitivity analysis was also performed including all patients. Multivariable linear regression was used to evaluate the impact of primary liver disease and comorbidities on TLOS and LLOS.

RESULTS

In 3772 patients, the mean (95%CI) TLOS was 24.8 (24.2 to 25.5) d, and the mean LLOS was 24.2 (22.9 to 25.5) d. Compared to patients with cancer, we found that the largest difference in TLOS was seen for acute hepatic failure group (6.1 d; 2.8 to 9.4) and the largest increase in LLOS was seen for other liver disease group (14.8 d; 8.1 to 21.5). Patients with cardiovascular disease had 8.5 d (5.7 to 11.3) longer TLOS and 6.0 d (0.2 to 11.9) longer LLOS, compare to those without. Patients with congestive cardiac failure had 7.6 d longer TLOS than those without. Other comorbidities did not significantly increase TLOS nor LLOS.

CONCLUSION

The time patients spent in hospital varied according to their primary liver disease and some comorbidities. Time spent in hospital of patients with cancer was relatively short compared to most other indications. Cardiovascular disease and congestive cardiac failure were the comorbidities with a strong impact on increased LOS.

Key words: Length of stay; Hospital stay; Comorbidity; Liver transplantation

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Core tip: Time patients spent in hospital in transplant admission and in later admissions after liver transplantation may reflect the success of liver transplantation. By analysing a linked United Kingdom Liver Transplant Audit - Hospital Episode Statistics database between 1997 and 2010, we found that average transplant length of stay (LOS) was 24.8 d, and mean LOS of all admissions in the first two years after transplantation was 24.2 d. Primary liver disease and comorbidities had a significant impact on LOS. Patients transplanted for cancer has shorter LOS compared to other indications. Cardiovascular disease and congestive cardiac failure were associated with increased LOS.

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INTRODUCTION

The short-term and long-term survival after liver

transplantation has been improving over the last few decades. However, length of stay (LOS) is another outcome that reflects the success of liver transplantation. Transplant LOS (TLOS) has also been one of the main outcomes in investigating health care resource use in organ transplantation. Identifying variables predicting longer LOS will help clinicians plan hospital resources in advance, and maximise the resource utilisation. LOS in transplant admission can also reliably reflect the cost of liver transplant admission^[1].

There are several studies report risk factors predicting longer transplant LOS^[2-9]. Recipient factors (e.g., age, sex, liver disease severity, retransplantation, pre-transplant nutritional status, pre-transplant renal support), donor factors (e.g., age, weight, non-local donor centre) and early post-transplant complications and graft dysfunction have been shown to be associated with prolonged transplant LOS. LOS also varies between liver transplant centres^[4,5]. Nevertheless, primary liver disease and comorbidities have rarely been investigated in terms of their effects on LOS. Moreover, the previous studies were based on only single or few centres, and the cohort sizes were often limited. In this study, we used a national clinical database linked to an administrative hospital database in England to investigate LOS in a larger national cohort of liver transplant patients.

LOS in later admissions after liver transplantation may reflect quality of life after liver transplantation and represent the success of liver transplantation. Moreover, it can reflect the healthcare resource use in the maintenance period after liver transplantation. To the best of our knowledge, there has not been any study in this topic.

In this study, we aim to investigate the effect of primary liver disease and pre-transplant comorbidities on TLOS as these two important factors have rarely been studied. The secondary aim is the effect of these factors on LOS in later admissions (LLOS) in the first two years after liver transplantation.

MATERIALS AND METHODS

The linked UKLTA-HES database

Records from the United Kingdom Liver Transplant Audit (UKLTA) database linked at a patient level to Hospital Episode Statistics (HES) records were used in this study. The UKLTA database prospectively collects liver transplant-specific data for all patients undergoing liver transplantation in the United Kingdom for audit purposes^[10]. The HES database is an administrative hospital database of all admissions to National Health Service (NHS) hospitals in England^[11]. A HES record contains the tenth revision of the International Classification of Diseases (ICD-10) diagnosis codes^[12], procedure codes, admission method as well as length of hospital stay based on date of admission and date of discharge. The linkage process was based on hierarchical deterministic linkage criteria, including NHS number, sex, date of birth, postcode, date of transplant and a

procedure code for liver transplantation or a diagnosis code relevant to liver disease. A detailed description of the linkage process has been published elsewhere^[13].

This linked database contained records of patients receiving a first liver transplant in England between 1st April 1997 and 31st March 2010. We excluded linked records of paediatric liver transplantation (younger than 17 years), multi-organ transplantation, living-donor liver transplantation and domino liver transplantation. To avoid the interference from the short LOS in patients who died early after transplantation, the patients who died within the first two years after liver transplantation (718 patients) were also excluded from the primary analysis. However, a sensitivity analysis for the whole cohort was also performed. At least two years follow-up was available for all included patients.

LOS information

LOS information was obtained from the HES database. TLOS was calculated from date of transplant to date of discharge, while LOS of a later admission was calculated from date of admission to date of discharge. LOS of all later admissions in the first two years after transplantation (LLOS) was defined as a sum of LOS of every admission in any NHS hospital in England that had an admission date within the first two years from the date of transplant.

Statistical analysis

Unadjusted TLOS and LLOS of patients in each primary liver disease group and of patients with each comorbidity were compared using unpaired two-tailed Student's *t*-test and analysis of variance (ANOVA) as appropriate. Primary liver diseases were categorised into ten indication groups according to Roberts *et al.*^[14], including one group with less common indications grouped together as the other liver diseases group. Eight comorbidities were identified from ICD-10 diagnosis codes in HES based on the adaptation of the Royal College of Surgeons Charlson Score for liver transplantation^[15]. Cardiovascular comorbidity comprises of a history of myocardial infarction, peripheral vascular disease and cerebrovascular disease.

Multivariable linear regression analysis was performed to determine the effects of the individual variables on TLOS and LLOS, taking into account other baseline characteristics, severity of liver disease and transplant centres. Variables included in the model were 10-group primary liver disease, all eight comorbidities, recipient age, sex, serum bilirubin, creatinine, sodium and international normalised ratio (INR) of prothrombin time (factors reflecting the severity of liver disease), liver transplant centre and time period of liver transplantation. Serum bilirubin and creatinine were log-transformed before inputting into the model to improve the linearity of the relationship between these factors and LOS. The comorbidities and sex were included as binary variables, while primary liver disease groups, transplant centre and time period of liver transplantation

were entered as categorical variables. The remaining variables were included as continuous variables.

LOS may not only depend on disease and patient factors, such as type and severity of liver disease and comorbidities, but also on hospital policy that may change over time. Therefore, we included the information about individual transplant centre and time period of liver transplantation in the models.

The ten primary liver disease groups were mutually exclusive. In the multivariable models, coefficients of primary liver disease groups were compared to cancer group as a reference group because it was one of the most common and shortest LOS groups. To make the comparison easier to interpret, we also presented adjusted mean LOS, which reflects LOS for these groups after taking other variables into account. The adjusted mean TLOS and LLOS for patients in each of the primary liver disease groups were calculated based on the prediction from multivariable linear regression models and presented along with their 95% confidence intervals (CI). This represents the LOS according to primary liver disease groups with an average case-mix profile of other variables in the model.

To ensure that patients with missing values were not excluded from the analyses, missing values were imputed with ten plausible data sets using multiple imputation with chain equations technique^[16]. The ten completed data sets were individually analysed, and estimates were then pooled to give final estimates using Rubin's rules^[17]. However, all of the variables in the model had missing values for less than 5% of the patients. All statistical analyses were performed using Stata version 11.2 (StataCorp, College Station, TX, United States). A *P*-value of less than 0.05 was considered statistically significant.

Sensitivity analysis

The primary analysis included only patients who survived the first two years after transplantation as the patients who died early after transplantation would shorten the average of LOS. A sensitivity analysis for TLOS was performed using the whole cohort including patients who died in the first two years after transplantation (4490 patients).

RESULTS

Demographic characteristics

The data used in this study were from 3772 adult patients who had a first liver transplant in England from April 1997 to March 2010 and survived the first two years after liver transplantation. The median (interquartile range: IQR) age was 52 (42 to 59) years, and 58.7% of the patients were male. The most common indication for liver transplantation was alcoholic cirrhosis (20.0%), followed by cancer (13.9%) and primary biliary cirrhosis (13.4%). The most common comorbidity was diabetes mellitus with a prevalence of 20.8%, followed by chronic

Table 1 Demographic characteristics of the adult recipients of a first liver transplant in England who survived the first two years after transplantation

Characteristic	Value	Missing (n)
Number	3772	
Age (yr)	52 (42-59)	0
Sex (%)		0
Male	2214 (58.7)	
Female	1558 (41.3)	
Primary liver disease (%)		0
Cancer	525 (13.9)	
Acute hepatic failure	455 (12.1)	
Hepatitis C cirrhosis	392 (10.4)	
Primary sclerosing cholangitis	354 (9.4)	
Hepatitis B cirrhosis	98 (2.6)	
Primary biliary cirrhosis	507 (13.4)	
Alcoholic cirrhosis	753 (20.0)	
Autoimmune and cryptogenic cirrhosis	348 (9.2)	
Metabolic liver disease	107 (2.8)	
Other liver disease	233 (6.2)	
Comorbidities (%)		0
Cardiovascular disease	200 (5.3)	0
Congestive cardiac failure	82 (2.2)	0
Connective tissue disease	134 (3.6)	0
Dementia	159 (4.2)	0
Diabetes mellitus	784 (20.8)	0
Non-hepatic malignancy	40 (1.1)	0
Chronic pulmonary disease	344 (9.1)	0
Chronic renal disease	247 (6.6)	0
Era of liver transplantation (%)		0
April 1997 - September 2000	841 (22.3)	
October 2000 - September 2003	899 (23.8)	
October 2003 - September 2006	897 (23.8)	
October 2006 - March 2010	1135 (30.1)	
Bilirubin (μmol/L)	54 (27-124)	20
Creatinine (μmol/L)	89 (74-109)	2
INR	1.4 (1.2-1.8)	162
Sodium (mmol/L)	137 (134-140)	8
UKELD score	55 (51-59)	184

Results are numbers (percentages) or medians (interquartile ranges). INR: International normalised ratio; UKELD: United Kingdom End-stage Liver Disease.

pulmonary disease (9.1%) and chronic renal disease (6.6%) (Table 1). In terms of pre-transplant status, 74.0% of the patients were at home, while 16.0% were in hospital but not ventilated, and 10.0% were hospitalised and ventilated at the time of transplantation. Some 4.7% of the patients received a liver graft from donation after cardiac death.

Overall LOS

Overall, patients spent an average of 24.8 d (95%CI: 24.2 to 25.5) in hospital during their transplant admission, and 24.2 d (22.9 to 25.5) in later admissions in the first two years after transplantation (Table 2).

LOS according to primary liver diseases

Primary liver disease groups were significant predictors of both TLOS and LLOS (Table 2). Using cancer group as a baseline, the multivariable analysis demonstrated that patients in acute hepatic failure (6.1 d longer), other liver disease (5.9 d longer), metabolic and non-

alcoholic fatty liver disease (4.3 d longer) and hepatitis C cirrhosis (3.9 d longer) groups had significantly longer TLOS than the baseline. As for LLOS, other liver disease (14.8 d longer) and primary sclerosing cholangitis (8.4 d longer) were significantly associated with longer LLOS than the baseline (Table 3).

Figure 1 presents the same above results using adjusted means for TLOS and LLOS, and further illustrates that patients with acute hepatic failure, hepatitis C cirrhosis, metabolic and non-alcoholic fatty liver disease and other liver disease had longer than average TLOS and LLOS, although not all significantly so. Whereas, patients with liver diagnosis of cancer, primary biliary cirrhosis, alcoholic cirrhosis and autoimmune hepatitis and cryptogenic cirrhosis groups had shorter than average TLOS and LLOS. Of note, primary sclerosing cholangitis was associated with shorter TLOS, but significantly longer LLOS (Figure 1).

LOS according to comorbidities

Patients with atherosclerotic cardiovascular disease had the longest unadjusted TLOS at 33 d, and those with chronic renal disease had the longest unadjusted LLOS at 32 d (Table 2). The multivariable linear regression analysis demonstrated that cardiovascular disease and congestive cardiac failure were significantly associated with longer TLOS. Patients with cardiovascular disease spent an average of 8.5 d longer in transplant admission than those without the comorbidity, and those with a history of congestive cardiac failure spent 7.6 d longer than those without, confirming what previously observed in unadjusted LOS (Table 3).

Patients with cardiovascular disease spent significantly longer time in hospital in the first two years after transplantation than those without the comorbidity (6.0 d longer). Those with chronic renal disease and chronic pulmonary disease spent 4.8 d and 4.3 d longer than those without the comorbidities, respectively, albeit not statistically significant (Table 3).

Sensitivity analysis of TLOS including patients who died in the first two years after liver transplantation

After including 718 patients who died within the first two years after transplantation, the sensitivity analysis of 4490 patients found that primary liver disease, cardiovascular disease and congestive cardiac failure remained statistically significant in predicting TLOS. In terms of primary liver disease groups, patients in other liver disease group had the longest TLOS, followed by acute hepatic failure group. Cardiovascular disease were associated with 8.7 d longer TLOS than those without the comorbidity, while patients with congestive cardiac failure had 7.7 d longer TLOS than those without, which were similar to those in the primary analysis (Table 4).

DISCUSSION

Liver transplant recipients spent in total 49 d in hospital during the first two years after transplantation,

Table 2 Unadjusted transplant length of stay and length of stay in later admissions in the first two years after liver transplantation regarding primary liver disease and comorbidities

Variable	n (%)	Unadjusted TLOS			Unadjusted LLOS		
		(d)	95%CI	P-value	(d)	95%CI	P-value
Overall average	3772	25	24-26	N/A	24	23-26	N/A
Primary liver disease groups							
Cancer	525 (13.9)	22	20-24	< 0.001	22	18-25	< 0.001
Acute hepatic failure	455 (12.1)	33	32-35		26	22-30	
Hepatitis C cirrhosis	392 (10.4)	27	25-29		26	22-30	
Primary sclerosing cholangitis	354 (9.4)	23	20-25		27	23-31	
Hepatitis B cirrhosis	98 (2.6)	22	18-26		21	13-29	
Primary biliary cirrhosis	507 (13.4)	21	20-23		20	16-23	
Alcoholic cirrhosis	753 (20.0)	24	23-26		22	19-25	
Autoimmune and cryptogenic	348 (9.2)	22	20-24		23	18-27	
Metabolic liver disease	107 (2.8)	26	22-30		29	21-37	
Other liver disease	233 (6.2)	28	26-31		37	32-42	
Comorbidities							
Cardiovascular disease	200 (5.3)	33	28-39	< 0.001	31	24-38	0.02
Congestive cardiac failure	82 (2.2)	32	24-39	0.003	18	13-24	0.18
Connective tissue disease	134 (3.6)	22	20-25	0.13	25	18-32	0.91
Dementia	159 (4.2)	25	21-28	0.87	26	21-32	0.54
Diabetes mellitus	784 (20.8)	24	23-26	0.42	26	23-29	0.18
Non-hepatic malignancy	40 (1.1)	23	17-29	0.65	24	10-37	0.91
Chronic pulmonary disease	344 (9.1)	27	25-30	0.04	28	24-33	0.06
Chronic renal disease	247 (6.6)	27	23-30	0.17	32	25-38	0.003

TLOS: Transplant length of stay; LLOS: Later length of stay; N/A: Not applicable.

Table 3 Multivariable linear regression analysis for transplant length of stay and later length of stay in the first two years after liver transplantation

Variable value	Coefficient for TLOS			Coefficient for LLOS		
	(d)	95%CI	P-value	(d)	95%CI	P-value
Primary liver disease groups ¹						
Cancer	0	Reference	< 0.001	0	Reference	< 0.001
Acute hepatic failure	6.1	2.8, 9.4		4	-3.3, 11.3	
Hepatitis C cirrhosis	3.9	1.3, 6.4		5.4	-0.1, 10.9	
Primary sclerosing cholangitis	0.2	-2.5, 3.0		8.4	2.6, 14.3	
Hepatitis B cirrhosis	1.9	-2.3, 6.1		1	-7.9, 9.9	
Primary biliary cirrhosis	-0.5	-3.2, 2.1		0.2	-5.6, 5.9	
Alcoholic cirrhosis	0.6	-1.6, 2.8		0.6	-4.1, 5.4	
Autoimmune and cryptogenic	0.1	-2.6, 2.9		1.4	-4.4, 7.2	
Metabolic liver disease	4.3	0.3, 8.4		7.1	-1.5, 15.7	
Other liver disease	5.9	2.8, 9.1		14.8	8.1, 21.5	
Comorbidities ²						
Cardiovascular disease	8.5	5.7, 11.3	< 0.001	6	0.2, 11.9	0.04
Congestive cardiac failure	7.6	3.4, 11.8	< 0.001	-5	-14.0, 3.9	0.27
Connective tissue disease	-1.4	-4.8, 2.0	0.42	2.3	-4.9, 9.5	0.54
Dementia	0.2	-2.9, 3.4	0.87	3.6	-3.1, 10.4	0.29
Diabetes mellitus	1	-0.6, 2.6	0.22	2.6	-0.8, 6.0	0.13
Non-hepatic malignancy	-0.7	-6.7, 5.3	0.82	-0.5	-13.2, 12.2	0.94
Chronic pulmonary disease	1.6	-0.5, 3.8	0.14	4.3	-0.2, 8.9	0.06
Chronic renal disease	1	-1.6, 3.5	0.47	4.8	-0.7, 10.3	0.09

¹A coefficient for primary liver disease groups represents a difference in days between LOS of patients in the primary liver disease group and the reference group (cancer); ²A coefficient for comorbidities represents a difference in days between LOS of patients with and without the comorbidity. The multivariable models were adjusted for primary liver disease group, comorbidities, recipient age, sex, log bilirubin, log creatinine, INR, sodium, transplant centre and time of transplantation. TLOS: Transplant length of stay; LLOS: Later length of stay.

approximately half in the transplant admission and the other half in subsequent admissions. However, the LOS was affected by primary liver disease and some comorbidities. Patients with liver diagnosis in acute hepatic

failure, hepatitis C cirrhosis and other liver disease groups had a longer TLOS than those in any other groups, while patients in primary sclerosing cholangitis and other liver disease groups had longer LLOS. Patients with

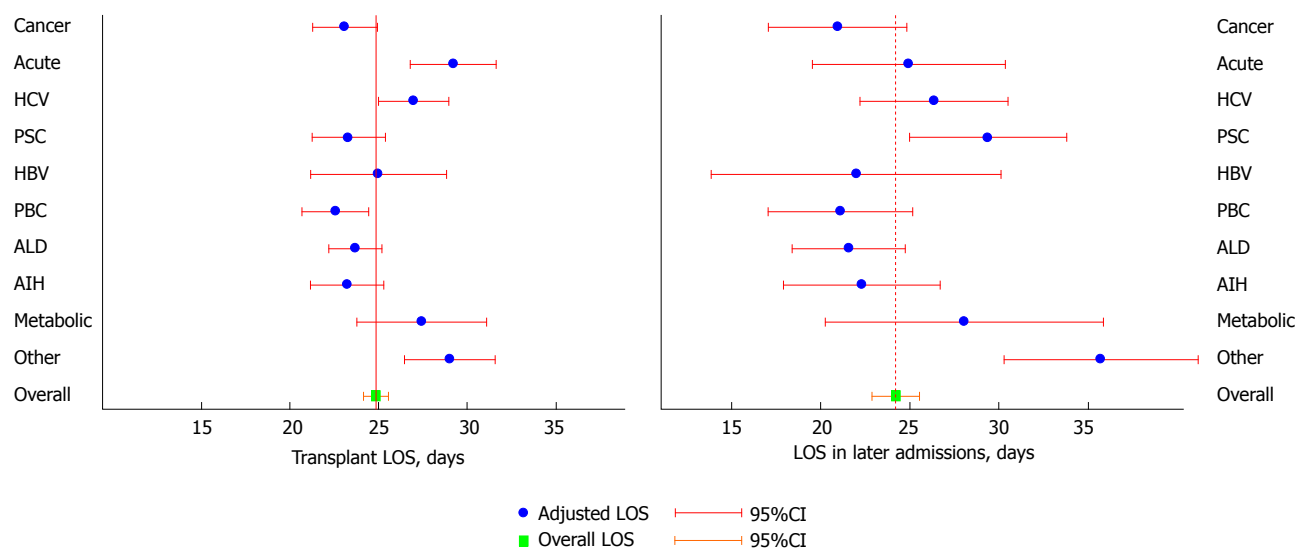


Figure 1 Adjusted transplant length of stay and length of stay in later admissions in the first 2 years after liver transplantation according to primary liver disease. HCV: Hepatitis C cirrhosis; PSC: Primary sclerosing cholangitis; HBV: Hepatitis B cirrhosis; PBC: Primary biliary cirrhosis; ALD: Alcoholic liver disease; AIH: Autoimmune hepatitis and cryptogenic cirrhosis; LOS: Length of stay.

Table 4 Sensitivity analysis of the multivariable linear regression for transplant length of stay including patients who died within the first two years after liver transplantation (4490 patients)

Variable	Coefficient for TLOS		
	(d)	95%CI	P-value
Primary liver disease groups ¹			
Cancer	0.0	Reference	0.001
Acute hepatic failure	3.7	0.2, 7.2	
Hepatitis C cirrhosis	3.3	0.6, 6.1	
Primary sclerosing cholangitis	-0.3	-3.2, 2.7	
Hepatitis B cirrhosis	0.6	-4.0, 5.2	
Primary biliary cirrhosis	-1.5	-4.4, 1.4	
Alcoholic cirrhosis	1.1	-1.3, 3.5	
Autoimmune and cryptogenic	0.5	-2.5, 3.4	
Metabolic liver disease	3.3	-0.9, 7.6	
Other liver disease	5.9	2.5, 9.2	
Comorbidities ²			
Cardiovascular disease	8.7	5.8, 11.5	< 0.001
Congestive cardiac failure	7.7	3.6, 11.8	< 0.001
Connective tissue disease	-0.3	-4.0, 3.3	0.86
Dementia	1.1	-2.4, 4.6	0.54
Diabetes mellitus	1.1	-0.6, 2.8	0.21
Non-hepatic malignancy	0.9	-4.6, 6.3	0.76
Chronic pulmonary disease	1.2	-1.1, 3.5	0.3
Chronic renal disease	1.7	-1.0, 4.4	0.22

¹A coefficient for primary liver disease groups represents a difference in days between LOS in the primary liver disease group and the reference group (cancer); ²A coefficient for comorbidities represents a difference in days between LOS of patients with and without the comorbidity. The multivariable model was adjusted for primary liver disease group, comorbidities, recipient age, sex, log bilirubin, log creatinine, INR, sodium, transplant centre and time period of transplantation. TLOS: Transplant length of stay; INR: International normalised ratio.

cardiovascular disease and cardiac failure also had longer TLOS than those without these comorbidities, and patients with cardiovascular disease spent longer time in later admissions than those without.

In terms of primary liver disease, acute hepatic failure, hepatitis C cirrhosis and other liver disease were associated with longer TLOS. Patients with acute hepatic failure are mostly intubated and ventilated and on renal replacement therapy^[18]. Therefore, it is not unexpected that they required more time to recover from the liver transplant operation. Hepatitis C cirrhosis may be related to more complications after liver transplantation, and this is probably the reason why patients transplanted for this indication spent longer time in transplant admission. The group of patients with other liver disease is the most heterogeneous group of patients with a wide range of liver diagnoses, consisting mainly of Budd-Chiari syndrome, secondary biliary cirrhosis and polycystic liver disease. Thus, the reason why these patients had longer LOS needs further investigation.

Patients with primary sclerosing cholangitis had relatively shorter TLOS, but relatively longer LLOS. These discrepancies may be a result of a higher rate of recurrent disease, vascular complications or conditions related to ulcerative colitis that need admissions for interventions or procedures^[19]. Nevertheless, the reasons for readmissions can be either transplant-related or non-transplant-related, and they were not explored in this study.

With respect to comorbidities, common comorbidities, such as diabetes, chronic pulmonary and renal disease, showed no impact on TLOS, while less common comorbidities, such as cardiovascular disease and congestive cardiac failure, were found to have an impact on TLOS. Cardiovascular disease, which is the grouping of three comorbid conditions in the same disease spectrum (myocardial infarction, peripheral vascular disease and cerebrovascular disease), was significantly associated with longer TLOS as was congestive cardiac failure. We have shown elsewhere that a previous history of cardiovascular disease and cardiac failure were also associated with

higher 90-d mortality^[15]. This study further showed that these groups of high-risk patients also used more health resources during their transplant admission. This is probably because of a higher risk of cardiac complications following a hemodynamically stressful liver transplant operation in these already compromised patients.

A previous single-centre study carried out in the United States with only 83 patients found that multi-vessel coronary artery disease is associated with higher mortality, increased LOS and post-operative vasopressor requirements^[20], which is in line with the results found in our national cohort in England. In addition, we demonstrated that the LOS in later admissions in the first two years in patients with atherosclerotic cardiovascular disease was also longer, particularly in those who survived the first two years. This reflects that these patients with cardiovascular comorbidity have a higher mortality risk and require more healthcare resources during transplant admissions as well as the early period after liver transplantation.

Our results have a number of implications for clinical practice. First, LOS of the transplant admission and of later admissions can be an alternative marker of outcomes after liver transplantation, especially in the era that graft and patient survival after liver transplantation have been excellent. LOS in later admissions after liver transplantation may also reflect the quality of life and functional status of a patient after transplantation. A successful liver transplantation should return a patient back to the healthy status with as few admissions after transplantation as possible. Second, the ability to estimate LOS may be beneficial to the pre-transplant counselling process as it can help to inform patients and their relatives what to expect after liver transplantation. Third, another benefit of estimating LOS is that it would help clinicians and hospitals plan their resource utilisation and bed management. For example, patients transplanted for cancer spent, on average, a total of 44 d in the first two years after transplantation, whereas patients who were transplanted with an indication in other liver disease group, such as Budd-Chiari syndrome, had a total LOS that was 50% longer (66 d) (Figure 1). Fourth, our results can improve economic evaluations of liver transplantation as it provides more accurate estimates of LOS for patients with comorbidities.

We note a number of limitations of this study. Firstly, we have not explored the reasons for later admissions. It may be beneficial to understand the indication for readmissions in particular groups of patients, and this may warrant further research. Secondly, this study includes only patients who had a first liver transplant. It is known that the outcomes of retransplantation are much different to those of first liver transplantation^[21]. Retransplantation has also been shown to be associated with longer transplant LOS^[4].

Conclusion

We have shown that the time patients spent in hospital after liver transplantation is linked to primary liver

disease and comorbidities. LOS was relative short for patient who had a liver transplant for cancer whereas the opposite was true for patients with atherosclerotic cardiovascular disease and congestive cardiac failure.

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COMMENTS

Background

Time patients spent in hospital after transplantation is directly related with health care resource use and partly reflects the success of liver transplantation. Identifying factors predicting longer length of stay will help clinicians and hospitals to plan and maximise the resource utilisation.

Research frontiers

Several recipient and donor factors have been found to be associated with prolonged transplant length of stay. However, primary liver disease and comorbidities have rarely been investigated in this issue. Length of stay in later admissions after transplantation also reflects quality of life after liver transplantation and can represent the success of liver transplantation. This has never been investigated in liver transplantation.

Innovations and breakthroughs

Based on the United Kingdom national liver transplant cohort, the authors demonstrated that transplant length of stay was affected by primary liver disease and comorbidities. Patients with acute hepatic failure, hepatitis C cirrhosis, atherosclerotic cardiovascular disease and a history of congestive cardiac failure stayed longer in hospital in their transplant admissions, while patients with primary sclerosing cholangitis spent more time in subsequent admissions in the first two years after liver transplantation.

Applications

Estimating length of stay will help clinicians and hospitals plan their health care resource utilisation including bed management. Moreover, knowing the estimated length of stay will be beneficial to the pre-transplant counselling process. It can help inform patients and relatives what they expect after liver transplantation. Finally, in the era that graft and patient survival after liver transplantation have been excellent, length of stay of the transplant admission and of later admissions can be a surrogate of outcomes after liver transplantation. Length of stay in later admissions after liver transplantation also specifically reflects the quality of life of patients after transplantation and the success of liver transplantation as it should return a patient back to the healthy status with as few admissions after transplantation as possible.

Terminology

Transplant length of stay (TLOS) was calculated from date of transplant to date of discharge, not including time patients spent in hospital in the pre-

transplant period. Length of stay in later admissions was a sum of length of stay of every admission in any National Health Service hospital in England that had an admission date within the first two years from the date of transplant. Cardiovascular comorbidity comprises of a history of myocardial infarction, peripheral vascular disease and cerebrovascular disease coded in the administrative hospital database in any previous admission in the preceding year before the transplant. Congestive cardiac failure is defined by a history of congestive cardiac failure coded in the administrative hospital database in any previous admission in the preceding year before the transplant. Other liver disease is a group of indications for liver transplantation that is consisted of less common indications grouped together. It is the most heterogenous group of indications, including mainly Budd-Chiari syndrome, secondary biliary cirrhosis and polycystic liver disease.

Peer-review

This study investigated the time after surgery after liver transplantation. The aim was clear, and methods were appropriate.

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Prospective Study

Magnetic resonance imaging of the transplanted pediatric heart as a potential predictor of rejection

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Abstract

AIM

To evaluate cardiac magnetic resonance imaging (CMR) as a non-invasive tool to detect acute cellular rejection (ACR) in children after heart transplant (HT).

METHODS

Thirty pediatric HT recipients underwent CMR at the time of surveillance endomyocardial biopsy (EMB) and results were compared to 14 non-transplant controls. Biventricular volumes, ejection fractions (EFs), T2-weighted signal intensities, native T1 times, extracellular volumes (ECVs) and presence of late gadolinium enhancement (LGE) were compared between patients and controls and between

patients with International Society of Heart and Lung Transplantation (ISHLT) grade \geq 2R rejection and those with grade 0/1R. Heart rate (HR) and brain natriuretic peptide (BNP) were assessed as potential biomarkers.

RESULTS

Significant ACR (ISHLT grade \geq 2R) was an infrequent event in our population (5/30, 17%). Ventricular volumes, EFs, LGE prevalence, ECVs, native T1 times, T2 signal intensity ratios, HR and BNP were not associated with the presence of \geq 2R ACR.

CONCLUSION

In this pilot study CMR did not reliably identify ACR-related changes in pediatric HT patients.

Key words: Heart; Pediatric; Transplantation; Magnetic resonance imaging; Rejection

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Core tip: After heart transplantation the diagnosis of significant acute cellular rejection (ACR) changes management and is associated with adverse outcome. Endomyocardial biopsy is the gold standard for the detection of ACR but has important limitations. This prospective trial examined the use of cardiac magnetic resonance imaging (CMR) for the diagnosis of ACR in pediatric heart transplant recipients. Significant rejection was a rare event in our cohort and was not associated with changes in CMR parameters in this pilot study.

Greenway SC, Dallaire F, Kantor PF, Dipchand AI, Chaturvedi RR, Warade M, Riesenkauff E, Yoo SJ, Grosse-Wortmann L. Magnetic resonance imaging of the transplanted pediatric heart as a potential predictor of rejection. *World J Transplant* 2016; 6(4): 751-758 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/751.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.751>

INTRODUCTION

Acute cellular rejection (ACR) is an immune-mediated process leading to allograft damage and decreased graft survival. It is a serious and potentially lethal complication after heart transplant (HT). The gold standard for the detection of rejection is an endomyocardial biopsy (EMB). However, EMB is an invasive procedure, exposes the patient to ionizing radiation and carries a small but important risk of serious complications^[1-4].

Cardiac magnetic resonance imaging (CMR) has been proposed as a non-invasive method for the detection of rejection in adults after HT. However, CMR measurements used in adults for the detection of rejection or myocardial inflammation, including T2-weighted imaging^[5,6], native T1 times and extracellular volume fractions (ECVs) derived from T1 mapping^[7], myocardial thickness,

ventricular volumes and ejection fraction (EF)^[8,9] have not been systematically evaluated in pediatric HT recipients with EMB-proven ACR.

In this pilot study we sought to assess the utility of parameters of ventricular function and myocardial tissue characterization for the non-invasive detection of ACR in children and adolescents after HT.

MATERIALS AND METHODS

Patients and study design

This single center, prospective, cross-sectional study was approved by the institutional research ethics board and included pediatric (age < 18 years) HT patients who were scheduled for a clinically-indicated EMB between April 2010 and March 2011. All consecutive and eligible patients without contraindications to contrast-enhanced CMR during the study period were invited to participate. In patients who underwent more than one CMR/EMB procedure during the study period only the first set of investigations was analyzed for this study. Following written informed consent, CMR was performed immediately prior to cardiac catheterization and EMB. Control subjects were asymptomatic relatives of patients diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC) who had normal echocardiograms, electrocardiograms (ECGs), signal-averaged ECGs as well as CMR scans and who were negative for ARVC-associated mutations if testing had been performed. Control subjects did not receive gadolinium as part of their CMR study. Heart rate (HR) was obtained from the average HR during the short axis CMR cine acquisition for ventricular volumetry.

Standardized immunosuppression post-transplantation for all patients included the use of thymoglobulin for induction (1-5 doses depending on risk factors), tacrolimus and mycophenolate mofetil. Perioperative steroids were discontinued 6 mo post-HT until 2007 and thereafter were discontinued at 5 d post-HT. Routine surveillance for rejection included serial echocardiograms, ECGs and cardiac catheterization with decreasing frequency over time post-transplantation.

EMB

At the authors' institution right ventricular EMBs are obtained at 1, 6 and 12 mo and then annually up to 5 years post-HT; thereafter only if there is clinical or echocardiographic suspicion for rejection. During the EMB five or six tissue samples were obtained from the right ventricular surface of the interventricular septum, stained with hematoxylin and eosin and evaluated using light microscopy. Samples were graded by a hospital pathologist who was blinded to the CMR and biochemistry results (below) and reported according to the International Society of Heart and Lung Transplantation (ISHLT) Standardized Cardiac Biopsy Grading Criteria^[10]. Congruent with clinical practice grades 2R and 3R were classified as significant ACR and grades 0R and 1R as

non-significant ACR. Tissue samples were also evaluated for the presence of antibody-mediated rejection (AMR) by C4d immunohistochemical staining.

CMR

CMR was performed using a 1.5 Tesla scanner (Magnetom Avanto, Siemens AG Healthcare Sector, Erlangen, Germany) and a phased-array multi-channel surface receiver coil.

Ventricular volumetry and late gadolinium enhancement

A stack of multiphase short axis slices was acquired using the steady state free precession technique for left and right ventricular volumes, as described previously^[11,12]. Ventricular volumes were extracted from the cine short axis stack in end-diastole and end-systole in the routine clinical fashion using commercially available software (QMass, version 7.2, Medis, Leiden, The Netherlands). Ventricular volumes were reported as indexed to recipient body surface area. EFs for both ventricles were calculated using end-diastolic and end-systolic volumes. The presence of late gadolinium enhancement (LGE) was determined qualitatively on standard long-axis (4-chamber, 2-chamber and 3-chamber) and short-axis slices using phase-sensitive inversion-recovery acquisitions > 10 min after the administration of 0.2 mmol/kg gadopentetate dimeglumine (Magnevist®, Bayer, Leverkusen, Germany).

T1 mapping and extracellular volumes

We previously described our T1 mapping approach for these patients in detail^[13]. In short, a modified Look-Locker inversion recovery sequence (MOLLI) with inversion pulses of 100 msec and 150 msec, respectively, as well as 3 and 5 single-shot images after these inversion pulses was used to measure native and post-contrast longitudinal relaxation T1 times of myocardium and blood. Images were acquired in diastole at a single mid-ventricular short axis slice orientation before and > 10 min after administration of contrast (same injection as described above for LGE). Breathholds were used in cooperative patients and all other patients were scanned during free breathing. Longitudinal relaxation times (T1 times) were measured using commercially available software (CVI42, Circle Cardiovascular Imaging, Calgary, AB, Canada). Contours were drawn in the interventricular septum, the left ventricular (LV) free wall and in a region encompassing the entire LV myocardium. T1 times in the blood pool were measured in the LV cavity. The ECV was calculated using pre- and post-gadolinium T1 times of blood and myocardium as well as the patient's hematocrit, obtained at the time of the scan^[14].

T2-weighted imaging

An ECG-gated turbo spin-echo readout sequence without fat saturation pulse preceded by a double inversion recovery dark-blood preparation and the following parameters was obtained in a single midventricular short axis slice^[15]: Inplane spatial resolution 1.6 mm, slice

thickness 6-10 mm, TE 59 ms. Imaging was performed in diastole during every other or every third heartbeat, depending on the HR, to achieve a TR of at least 1000 ms. The scanner's body coil was used for a homogeneous signal reception within the field of view. Myocardial signal intensity was measured around the circumference of the short axis slice and normalized to that of skeletal muscle using a dedicated module within the CVI42 software^[16].

Brain natriuretic peptide levels

A blood sample was drawn upon insertion of the peripheral intravenous cannula needed for the CMR and analyzed for brain natriuretic peptide (BNP) levels (Modular Analytics, Roche Diagnostics, Laval, QC, Canada).

Statistical analysis

CMR data from transplant patients were stratified according to the presence (grade \geq 2R) or absence (grade 0R or 1R) of significant ACR. Most variables were not normally distributed and results are thus presented as medians, 10th and 90th percentiles. Medians between groups were compared using a non-parametric Wilcoxon two-sample test or the Kruskal-Wallis test where appropriate. A *P*-value < 0.05 was considered statistically significant. All analyses were performed using SAS for Windows 9.4 (SAS Institute Inc., Cary, NC, United States). Statistical review of the study was performed by a biomedical statistician (FD).

RESULTS

Patient demographics and non-imaging biomarkers of rejection

The CMR studies from 14 non-transplant pediatric controls and 30 pediatric HT recipients were included in this study. The EMBs from 25 HT patients (83%) showed no significant ACR (ISHLT grades 0R or 1R) while 5 (17%) demonstrated significant rejection (ISHLT 2R). No patient had ISHLT grade 3R ACR. None of our HT patients were identified as having AMR. There were no statistically significant differences between the transplant groups with < 2R and \geq 2R ISHLT rejection with respect to age at CMR or for time since transplant (Table 1). Patients with grade 2R rejection were younger than the controls and "no rejection" groups at the time of CMR but this difference was not statistically significant. HR and BNP were significantly increased in both groups of HT patients compared to controls but there were no statistically significant differences between the "no rejection" and "rejection" HT groups.

Ventricular function, volumes and mass

Biventricular end-diastolic volumes were decreased in the rejection group compared to the controls but not in the group without rejection (Figure 1). LVEF was decreased and LV mass increased only in the "no rejection" group compared to controls (Table 2). However, no significant

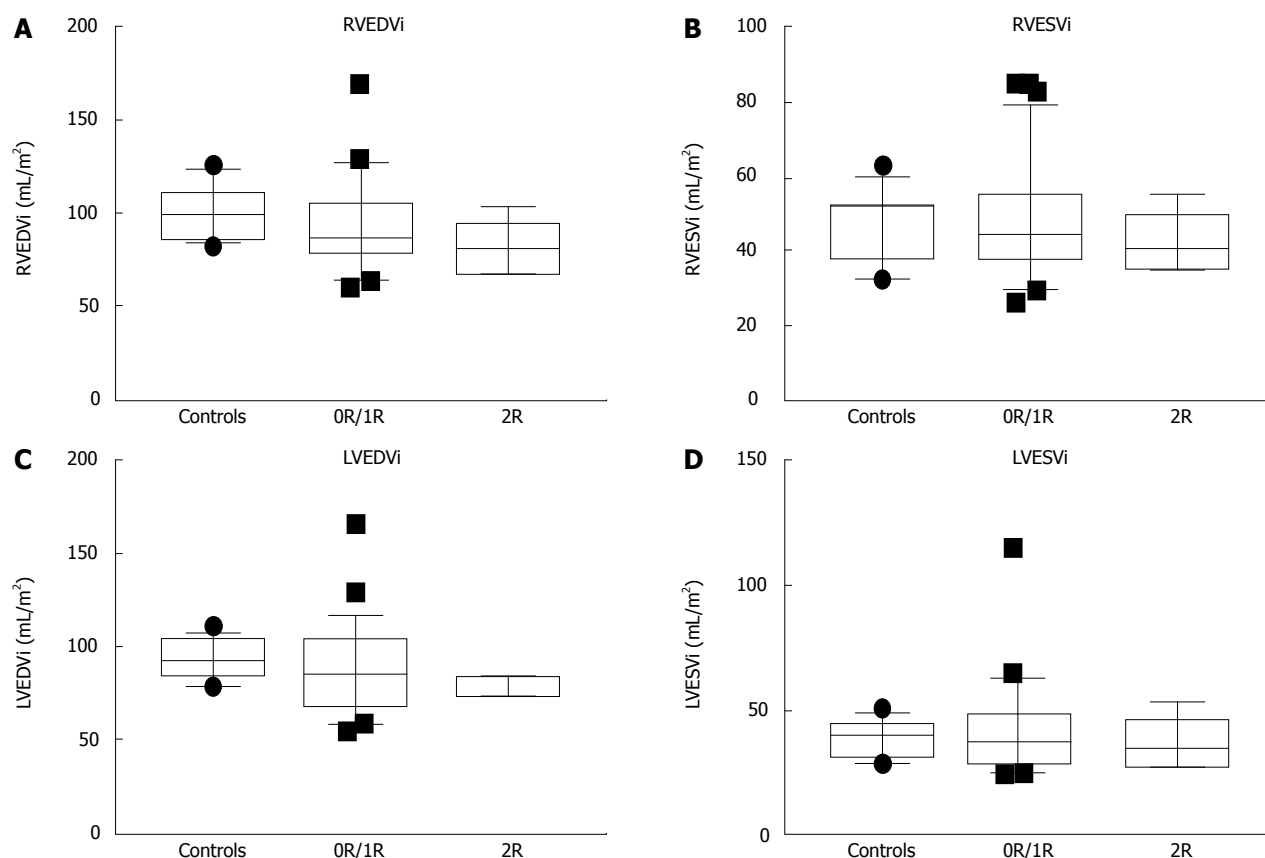


Figure 1 Box and whiskers plots for end-diastolic and end-systolic ventricular volumes of controls and transplant patients without (OR/1R) and with (2R) significant rejection. A: Right ventricular end-diastolic volume indexed to body surface area (RVEDVi); B: Right ventricular end-systolic volume indexed to body surface area (RVESVi); C: Left ventricular end-diastolic volume indexed to body surface area (LVEDVi); D: Left ventricular end-systolic volume indexed to body surface area (LVESVi).

Table 1 Patient characteristics

	Controls	No rejection (OR/1R)	Rejection (2R)
Number	14	25	5
Female (%)	8 (57)	10 (40)	1 (20)
Days post-transplant	-	702 (78, 1797)	160 (12, 800)
Age at CMR (yr)	12.8 (9.2, 15.3)	13.6 (2.2, 17)	7.7 (1.6, 17.5)
HR	71 (57, 84)	96 ^b (82, 126)	108 ^d (101, 130)
BNP	9.2 (5, 12.9)	38.7 ^f (5, 81.6)	59.9 ^e (14.9, 202)

Data shown as number (percentage) or median (10th, 90th percentiles). Significantly different compared to controls, ^b*P* = 5.76E-05, ^d*P* = 0.005, ^f*P* = 0.005, ^e*P* = 0.03. There were no significant differences between the transplanted rejection groups. HR: Heart rate in beats per minute; BNP: Brain natriuretic peptide in ng/L; CMR: Cardiac magnetic resonance imaging.

differences between HT patients with and without clinically important rejection were observed with regards to ventricular volumes, ejection fractions, LV mass or LV mass/volume ratio. The absence of a significant change in ventricular volumes with rejection may be confounded by the increase in ventricular size with age (Figure 2). There was no significant association between BNP and CMR parameters.

Native T1 times, myocardial extracellular volume fraction and LGE

The MOLLI sequence for T1 mapping became available to us after study enrollment had begun and therefore this data was available only in a subgroup of patients (Table 3). With regards to patient demographics there were no significant differences between this patient subset and the entire cohort. There were no significant differences in native T1 times and ECV fraction between patients with < 2R and ≥ 2R ISHLT rejection. LGE was not observed in any of the HT patients. Native T1 times and ECV were not quantified and LGE imaging was not obtained in controls who did not receive contrast.

Myocardial T2-weighted imaging

The global ratios of myocardial:skeletal muscle T2 signal intensities on a mid-ventricular short axis slice were similar between groups and did not differ between controls (median 1.37, 10th percentile 1.29, 90th percentile 1.67) and transplant patients with no rejection (median 1.3, 10th percentile 1.02, 90th percentile 1.6) or with rejection (median 1.3, 10th percentile 1.12, 90th percentile 1.47). There were no significant differences between transplant patients with < 2R and ≥ 2R ACR rejection.

Table 2 Ventricular volumes, function and mass

	Controls	No rejection (0R/1R)	Rejection (2R)
Number	14	25	5
RVEDVi (mL/m ²)	98.5 (85.7, 120.4)	86.6 (64.1, 124.4)	80.6 ^a (68.1, 102.7)
RVESVi (mL/m ²)	50.5 (33.9, 56.1)	44.1 (30.7, 77)	41 (34.9, 55.4)
RVEF (%)	53.4 (48, 60)	50 (41, 57)	47.4 (40, 56)
LVEDVi (mL/m ²)	93 (79.2, 104)	85.2 (58.9, 112)	74.1 ^c (73.7, 85)
LVESVi (mL/m ²)	40.3 (29.8, 45)	37.5 (24.6, 60.9)	33.8 (27.2, 52)
LVEF (%)	58.8 (53.2, 63)	54 ^e (46, 64)	56 (36, 63)
LV mass (g/m ²)	53.5 (45.8, 61)	61.5 ^b (50, 84.6)	66.1 (48, 80)

Data shown as median (10th, 90th percentiles) except for number. Significantly different compared to controls, ^a*P* = 0.03, ^c*P* = 0.02, ^e*P* = 0.05, ^b*P* = 0.003. There were no significant differences between the transplant rejection groups. RVEDVi: Right ventricular end-diastolic volume indexed to recipient body surface area; RVESVi: Right ventricular end-systolic volume indexed to recipient body surface area; RVEF: Right ventricular ejection fraction; LVEDVi: Left ventricular end-diastolic volume indexed to recipient body surface area; LVESVi: Left ventricular end-systolic volume indexed to recipient body surface area; LVEF: Left ventricular ejection fraction; LV mass: Left ventricular mass indexed to recipient body surface area; CMR: Cardiac magnetic resonance imaging.

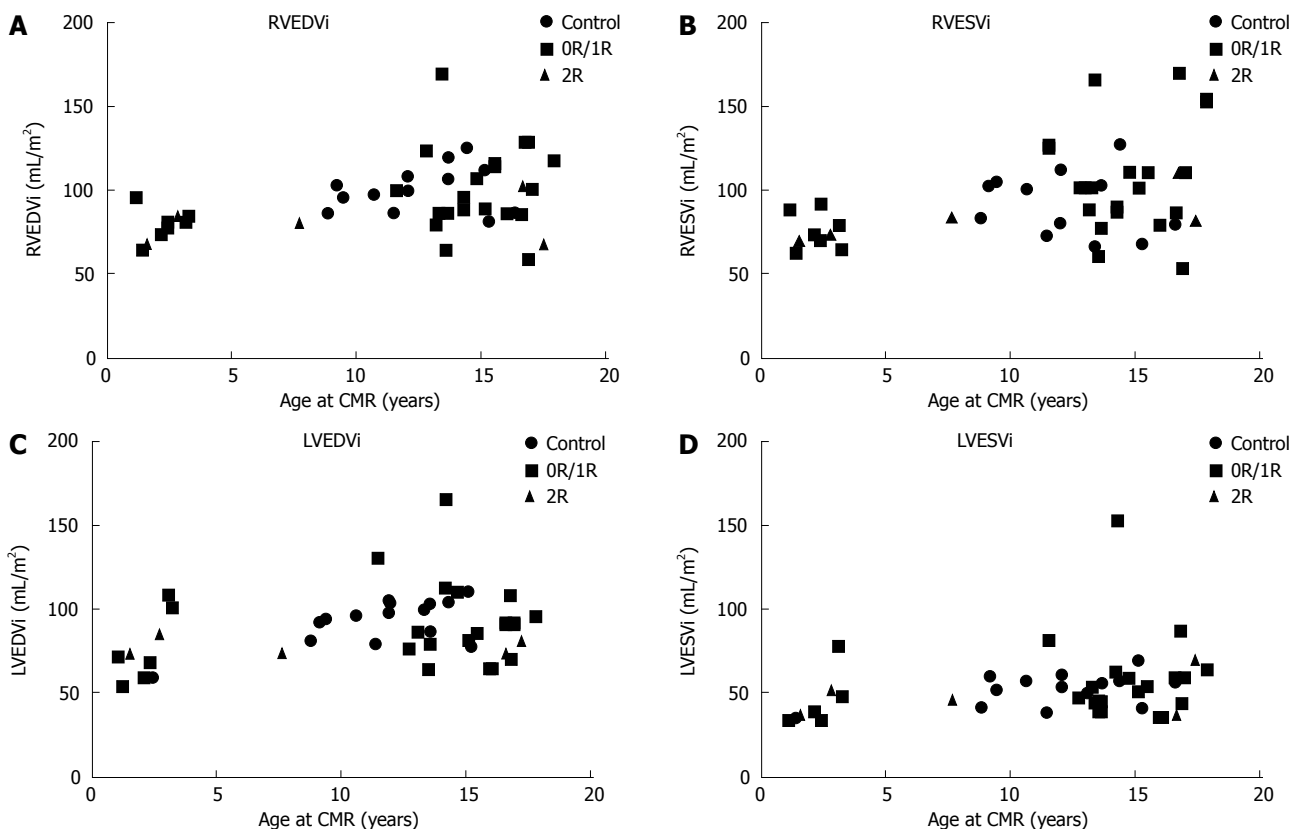


Figure 2 Ventricular volumes plotted as a function of age for controls and transplant patients without (0R/1R) and with (2R) significant rejection. A: Right ventricular end-diastolic volume indexed to body surface area (RVEDVi); B: Right ventricular end-systolic volume indexed to body surface area (RVESVi); C: Left ventricular end-diastolic volume indexed to body surface area (LVEDVi); D: Left ventricular end-systolic volume indexed to body surface area (LVESVi).

DISCUSSION

Despite a growing body of evidence in adult HT patients and important information from animal experiments the role of CMR for the detection of ACR in children has not been explored^[17-19]. CMR tissue characterization overcomes important limitations of EMB such as the potential of containing scar from a previous EMB in the

histological sample and the fact that specimens are collected from the RV surface of the interventricular septum and may not be representative of the remainder of the myocardium^[17]. The current study compared descriptors of myocardial edema, expansion of the myocardial extracellular space, presence of patchy myocardial scarring as well as ventricular size and function between controls and HT recipients as well

Table 3 T1 and extracellular volume data for heart transplant patients

	No rejection (0R/1R)	Rejection (2R)
Number	18	4
Female (%)	8 (44)	1 (25)
Days post-transplant	485 (13, 1818)	142 (12, 800)
Age at CMR (yr)	13.2 (1.4, 16.9)	5.3 (1.6, 16.8)
Native T1 (ms)		
IVS	1008 (963, 1067)	976 (967, 1026)
LV free wall	988 (903, 1018)	978 (924, 1016)
Entire LV	991 (930, 1031)	978 (944, 1020)
Hematocrit	0.37 (0.26, 0.44)	0.35 (0.29, 0.38)
ECV		
IVS	0.3 (0.26, 0.34)	0.29 (0.26, 0.33)
LV free wall	0.27 (0.24, 0.34)	0.28 (0.25, 0.31)
Entire LV	0.29 (0.26, 0.33)	0.29 (0.27, 0.32)

Data shown as number (percentage) or median (10th, 90th percentiles). There were no significant differences between the groups. ECV: Extracellular volume; IVS: Interventricular septum; LV: Left ventricle.

as between HT patients with < 2R and \geq 2R ISHLT rejection. However, in contrast to the experience in adult HT populations for several of these parameters, we were unable to demonstrate an association of any of them with ACR in pediatric HT recipients^[17,19].

None of the 30 patients in our study displayed patchy myocardial scarring as evidenced by LGE. This finding is in contrast to studies in adult HT recipients which found myocardial scarring on LGE imaging in a sizeable proportion (although this was not correlated with rejection)^[19,20]. The reason for this discrepancy remains unclear, but may be related to the younger age of the donor hearts used for pediatric HT^[21,22]. While LGE reflects patchy myocardial scarring of a certain size native T1 and ECV are regarded as measures of expansion of the extracellular matrix. Both are elevated in states of increased myocardial fibrosis or edema. Acute rejection is characterized histologically by inflammation of the myocardium while chronic or repeated episodes of rejection have been associated with fibrotic remodeling^[7,23,24]. Native T1 and ECV have been explored as markers of ACR in a pilot study in adults after HT but an association with rejection has yet to be demonstrated^[8]. In the current study, albeit in a limited number of patients, ECV and native T1 times did not distinguish between < 2R and \geq 2R ISHLT rejection. T2-weighted imaging is an established approach to detect tissue edema in inflammatory conditions and in the heart it is used as a marker for myocardial edema in myocarditis^[6]. Studies that employed T2 signal intensity for the non-invasive detection of rejection have yielded mixed results in adult HT patients^[5,9,25]. Our early results did not reveal increased signal intensity on T2-weighted imaging in patients with ACR. T2 mapping is another approach to myocardial edema which has yielded promising results in adult ACR^[19,26-28], but this technique was not available to us at the time of the study. When discussing the lack of agreement between CMR markers and histological

indicators of ACR, important shortcomings of EMB as the gold standard for the detection of ACR must be considered. Marie *et al.*^[26] found T2 mapping CMR to be "positive" for significant rejection several weeks before a follow-up EMB confirmed it suggesting a lack of sensitivity for EMB.

Ventricular size, LV myocardial mass, and function did not distinguish between patients with < 2R and \geq 2R ISHLT rejection in our study. An increase in indexed right ventricular end-diastolic volume has emerged as a potential predictor of rejection in adults^[19], but the trend in our patients was in the opposite direction for both right and left ventricular end-diastolic volumes. The use of ventricular volumes as a biological marker is potentially problematic for two reasons: Firstly, there is often a size mismatch between the donor and the recipient which can be up to two-fold in children. This mismatch is fairly random and quite possibly obscures any association between ventricular size and the presence of rejection. Secondly, indexing to body surface area, although standard practice, is a crude strategy for normalizing ventricular volumes in children. Z-scores are more reliable in ensuring comparability across a spectrum of ages, body sizes and genders, but universally accepted Z-scores for CMR volumes are missing.

Another potential sign of inflammation is myocardial swelling as evidenced by increased LV "mass". Studies in adults have shown an increase in LV wall thickness during episodes of rejection^[28,29]. However, an increase in LV mass in HT patients also occurs unrelated to rejection due to myocardial hypertrophy either as an adverse effect of medications^[30], myocardial TNF- α expression^[31] or hypertension. In our study there was no significant difference between HT patients with and without \geq 2R ACR with regards to LV mass.

With regards to non-CMR parameters, higher HRs were noted in the HT recipients as compared to controls due to denervation during the transplant operation. However, in our small cohort HR did not differ significantly between patients with and without significant rejection. An elevated BNP has also been proposed as a marker for rejection in pediatric cardiac transplant patients^[32] and, although elevated in the transplant patients, there was no significant difference between the transplant rejection groups.

The most important limitation of this pilot study is the small number of patients with \geq 2R rejection which may have obscured associations of EMB with CMR parameters. The number of patients with available T1 mapping data, in particular, was very small. The small numbers may have also augmented the effects of potential confounders, for example donor:recipient size mismatch in HT patients, and thereby affected the comparability of ventricular volumes. The relatively low prevalence of ACR in the current era is related to improved immunosuppression regimes and, consistent with contemporary outcomes^[33], none of the patients in our study had severe grade 3R rejection. The incidence of moderate (grade 2R) ACR (17%) was similar

to the 13%-23% found by others^[8,19,20]. T1 relaxometry and T2-weighted imaging were based exclusively on measurements in a single mid-ventricular short axis slice. Many experts now recommend a wider representation of all regions of the LV in tissue characterization. Since many of the measures we assessed are associated with intramyocardial edema, which is rare in 2R rejection, it is perhaps unsurprising that the studied CMR parameters were unchanged. It is possible that, rather than detecting acute rejection, CMR may have a greater role in identifying long-term changes in the myocardium perhaps associated with cardiac allograft vasculopathy.

Studies in adults have produced mixed results with regards to the use of CMR as a screening tool for rejection and our pilot study did not identify CMR parameters altered by the presence of 2R rejection. However, myocardial tissue characterization by CMR is undergoing continuous refinement. Given the conceptual association between ACR and myocardial inflammation and the multiple disadvantages of EMB, CMR should continue to be evaluated for its ability to non-invasively detect rejection. Larger trials producing sizable cohorts of patients with clinically-significant rejection episodes and including T2 relaxometry are recommended.

COMMENTS

Background

Cardiac magnetic resonance imaging (CMR) has been proposed as a non-invasive method for the detection of rejection in adults after heart transplant (HT). However, CMR measurements used in adults for the detection of rejection or myocardial inflammation have not been systematically evaluated in pediatric HT recipients with biopsy-proven acute cellular rejection (ACR). In this pilot study, the authors sought to assess the utility of parameters of ventricular function and myocardial tissue characterization for the non-invasive detection of ACR in children and adolescents after HT.

Research frontiers

CMR tissue characterization overcomes important limitations of endomyocardial biopsy (EMB) such as the potential of containing scar from a previous EMB in the histological sample and the fact that specimens are collected from the RV surface of the interventricular septum and may not be representative of the remainder of the myocardium.

Innovations and breakthroughs

CMR has shown potential utility in adult heart transplant recipients. However, in this pilot study CMR did not reliably identify ACR-related changes in pediatric heart transplant patients.

Applications

Given the multiple disadvantages of EMB, CMR should continue to be evaluated for its ability to non-invasively detect rejection. Larger trials producing sizable cohorts of patients with clinically-significant rejection episodes and including T2 imaging are recommended.

Terminology

EMB: Invasive procedure used to sample the endomyocardium of the right ventricle to diagnose rejection; ACR: Damage created by T-cell mediated immune response directed by the recipient against the transplanted organ; T1- and T2-weighted imaging: MRI sequences that are used to differentiate tissues based mainly on their composition of fat and water.

Peer-review

The authors have produced an interesting study evaluating the use of CMR scanning as a means to diagnose acute cellular rejection in paediatric HT recipients.

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Corticosteroid minimization in renal transplantation: Careful patient selection enables feasibility

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Abstract

AIM

To explore the benefits and harms of corticosteroid (CS) minimization following renal transplantation.

METHODS

CS minimization attempts to improve cardiovascular risk factors (hypertension, diabetes, dyslipidemia), to enhance growth in children, to ameliorate bone disease and to lead to better compliance with immunosuppressive agents. Nevertheless, any benefit must be carefully weighed against the reduction in net immunosuppression and the potential harm to renal allograft function and survival.

RESULTS

Complete CS avoidance or very early withdrawal (*i.e.*, no CS after post-transplant day 7) seems to be associated with better outcomes in comparison with later withdrawal. However, an increased incidence of CS-sensitive acute rejection has been observed with all CS minimization strategies. Among the prerequisites for the safe application of CS minimization protocols are the administration of induction immunosuppression and the inclusion of calcineurin inhibitors in maintenance immunosuppression regimens.

CONCLUSION

Transplant recipients at low immunological risk (primary transplant, low panel reactive antibodies) are

thought as optimal candidates for CS minimization. CS avoidance may also be undesirable in patients at risk for glomerulonephritis recurrence or with severe delayed graft function and prolonged cold ischemia time. Thus, CS minimization is not yet ready for implementation in the majority of transplant recipients.

Key words: Acute rejection; Corticosteroid withdrawal; Corticosteroid minimization; Corticosteroid avoidance; Immunosuppression; Renal transplantation

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Core tip: Although corticosteroids have been traditional components of immunosuppressive regimens in renal transplantation, corticosteroid minimization strategies are developed in an attempt to mitigate their many side-effects. The benefit from this approach must be balanced against the risk of acute rejection due to insufficient immunosuppression and the potential harm to allograft survival. We present an overview of these strategies and their impact on clinical outcomes analyzing the key clinical trials performed. Furthermore, we focus on patient selection according to the immunological risk and the induction immunosuppression, the principal factors that determine the success of corticosteroid withdrawal and avoidance protocols.

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INTRODUCTION

Corticosteroids (CS) have been ubiquitously included in immunosuppressive regimens since the early days of renal transplantation (Tx). They have significantly contributed to the successful transformation of a highly experimental intervention into a universally adopted clinical treatment. However, their use is associated with a plethora of adverse events due to their non-specific mode of action. The negative impact of CS on cardiovascular disease risk factors such as hypertension, diabetes mellitus, and dyslipidemia is well known. Non-cardiovascular adverse events such as growth retardation, impaired wound healing, subcapsular cataract, bone problems (osteoporosis, fractures, avascular necrosis) and cosmetic effects leading to patient non-compliance are equally established^[1-4]. An increasing interest in minimizing the exposure to CS in transplant recipients with stable allograft function has been manifested by renal transplant clinicians to reduce the morbidity burden associated with their use. In the United States, CS avoidance regimens were administered to 23% of

all first renal transplant recipients in 2004^[5]. Among the remaining 77% who were discharged on CS, roughly 10% had CS withdrawn during the first post-transplant year. Nonetheless, this policy has to be carefully balanced against the risk of acute rejection due to insufficient immunosuppression and should not jeopardize renal allograft function and survival.

MATERIALS AND METHODS

Definitions

Strategies for CS minimization can be categorized as: (1) CS avoidance; and (2) CS withdrawal following a period after Tx. The latter can be further divided as early withdrawal (weeks or months after Tx, usually 3-6 mo after Tx) or late withdrawal (at least 6 mo after Tx). Overlapping between these categories has been reported in the literature leading to a degree of uncertainty over the exact terminology. For instance, very early withdrawal (< 2 wk) has been classified under both CS avoidance and CS withdrawal strategies. For the purpose of this manuscript, we will include very early withdrawal under the CS avoidance strategy. The overall efficacy of CS minimization regimens depends on the extent to which the rest of the immunosuppressive agents can suppress the alloimmune response and on the immunological risk stratification. In general, induction immunosuppression is required for the safe application of CS minimization as well as the inclusion of calcineurin inhibitors in maintenance immunosuppression. Patients at low immunological risk (first transplant, non-sensitized) are considered as ideal candidates for the implementation of CS minimization^[6].

Data from studies on CS minimization have produced conflicting results regarding benefit vs harm. Clinical heterogeneity across these studies is moderate to high, especially regarding the spectrum of induction and maintenance immunosuppression agents used. Some studies have reported reductions in cardiovascular risk factors such as dyslipidemia^[7], but there is no clearly proven reduction of the burden of cardiovascular disease. On the other hand, although CS avoidance or withdrawal studies resulted in increased rates of acute rejection, the impact on allograft survival appears to be neutral. Given the current dilemma over the efficacy vs safety profile of CS minimization strategies, our institution has continued on the traditional strategy of rapid CS tapering after Tx to the lowest possible dose. We reserve CS avoidance or withdrawal for highly selected cases at low immunological risk who present compelling contraindications to CS such as severe osteoporosis. We will try to elaborate on the potential advantages and disadvantages of our protocol focusing on the comparison with the CS minimization practices mentioned above. Our goal is to identify the optimal management strategy, which will allow for the maximum benefit of different patient subsets without compromising safety and will likely improve Tx outcomes.

Table 1 Characteristics of the multi-center, randomized Astellas Corticosteroid Withdrawal Study with a follow-up time of 5 years^[8]

	CS withdrawal at day 7 arm (n = 191)	Standard CS arm (n = 195)	P-value
Baseline demographic, immunological risk and immunosuppressive therapy data			
Age (mean \pm SD, yr)	46.6 \pm 12.2	46.2 \pm 12.7	NS
Female gender (%)	30.9	36.4	NS
African American (%)	17.8	21.5	NS
Deceased donor (%)	43.5	42.6	NS
Cold ischemic time (mean \pm SD, h)	18.4 \pm 5.7	17.2 \pm 7.3	NS
HLA mismatch (mean)	3.5	3.5	NS
Current PRA (mean \pm SD)	1.6 \pm 5.3	1.8 \pm 5.5	NS
Induction immunosuppression (%)			NS
Thymoglobulin	65.4	69.7	
Basiliximab	31.4	27.2	
Daclizumab	3.1	3.1	
Maintenance immunosuppression	TAC, MMF	TAC, MMF	
Main outcomes			
Biopsy-proven acute rejection (%)	17.8	10.8	0.04 (with Kaplan-Meier analysis)
Allograft survival (%)	94.2	93.3	NS
Patient survival (%)	94.2	96.4	NS
Creatinine clearance (Cockcroft-Gault equation, mean \pm SD, mL/min)	58.6 \pm 19.7	59.8 \pm 20.5	NS

CS: Corticosteroid; HLA: Human leukocyte antigens; MMF: Mycophenolate mofetil; NS: Not significant; PRA: Panel-reactive antibodies; SD: Standard deviation; TAC: Tacrolimus.

RESULTS

Low CS dose as maintenance therapy

We advocate the immunosuppressive protocol, which involves the administration of three daily intravenous pulses of 500, 250 and 250 mg methylprednisolone intraoperatively and on postoperative days 1 and 2 respectively. We attempt to rapidly taper CS dose to 20 mg oral methylprednisolone per day by 2-4 wk following Tx. Thereafter, we further reduce CS dose with the aim of 4 mg methylprednisolone per day at 3 mo in the absence of acute rejection. This dose is continued indefinitely. Data from randomized clinical trials (RCT) argue that maintenance CS treatment has still a dominant place in the management of renal transplant recipients. In the RCT with the longest follow-up to date (Astellas Corticosteroid Withdrawal Study), Woodle *et al.*^[8] assigned 386 renal transplant recipients with PRA (panel reactive antibodies) \leq 25% to either very early CS withdrawal at one week post-transplant or CS continuation tapered to 5 mg prednisolone per day at 6 mo (Table 1). All patients received induction immunosuppression; 68% of them with the lymphocyte-depleting agent anti-thymocyte globulin (ATG) and 32% with anti-interleukin-2 receptor monoclonal antibodies. Maintenance immunosuppressive regimen consisted of tacrolimus and mycophenolate mofetil (MMF). After a follow-up of 5 years, no difference was found in the rate of patient death, death-censored allograft loss and moderate/severe acute rejection. Total biopsy-confirmed acute rejection was lower in the CS continuation arm (10.8% vs 17.8%, $P = 0.04$). This result was driven by the increased rates of mild, CS-sensitive acute rejection in the very early CS withdrawal

arm. It is interesting that biopsy-proven acute rejection rates were numerically lower with ATG induction than with anti-interleukin-2 receptor monoclonal antibodies in very early CS withdrawal patients, but that did not reach statistical significance (14.4% vs 24.2%, $P = 0.09$). Serum creatinine and creatinine clearance estimated by the Cockcroft-Gault equation were similar between the two arms at 5 years. However, chronic allograft nephropathy (CAN) incidence at 5 years was more than double (9.9% vs 4.1%, $P = 0.028$) with very early CS withdrawal compared to a continuation. This finding raises an important concern. Although very early CS withdrawal seems to be non-inferior to CS continuation at 5 years concerning patient and allograft survival, it is unknown if the increased incidence of CAN would negatively influence those outcomes beyond that time-point. Clinical trials with extended follow-up time to 10 years are needed to resolve this issue. The effect of very early CS withdrawal on cardiovascular risk factors was mixed. No significant difference was found in hypertension, new-onset diabetes mellitus, total cholesterol and low-density lipoprotein (LDL) levels; very early CS withdrawal led only to improvement in serum triglycerides. As far as it concerns non-cardiovascular adverse events, very early CS withdrawal reduced bone fractures and avascular necrosis but it was paradoxically associated with more frequent subcapsular cataract.

A meta-analysis of 34 studies, which included 5637 patients, produced broadly similar results^[9]. It was found that acute rejection risk was significantly increased with CS avoidance or withdrawal regimens compared to maintenance CS (relative risk: 1.56, 95%CI: 1.31-1.87, $P = 0.0001$). No statistically significant differences

were found for patient or allograft survival, but allograft function was modestly better with maintenance CS (weighted mean difference in creatinine clearance: 3.05 mL/min, 95%CI: 1.45-4.66). In contrast to the abovementioned RCT, occurrence of hypertension, new onset diabetes mellitus and hypercholesterolemia was reduced with CS avoidance or withdrawal regimens. However, the effect on hard cardiovascular endpoints cannot be estimated because included studies underreported cardiovascular events. In conclusion, acute rejection rates are constantly lower when CS maintenance regimens are used. Patient and allograft survival seems not to be influenced by CS minimization, but it is unknown if this remains the same with longer follow-up. Although CS minimization may permit some improvement in cardiovascular risk factors, data are not consistent about it.

CS avoidance

The rationale behind CS avoidance or very early withdrawal is that acute rejection may be triggered more easily with CS withdrawal within weeks or months after Tx. However, it invariably requires the use of potent induction immunosuppression and the selection of low immunological risk recipients. Attempts to use CS avoidance regimens in the absence of induction immunosuppression resulted in unacceptably high acute rejection rates^[10]. In a three-arm multicenter RCT, which included 336 renal transplant recipients with PRA \leq 20%, Vincenti *et al*^[11] used basiliximab as an induction agent and compared no CS at all vs CS withdrawal by day 7 vs standard CS. Maintenance immunosuppression consisted of cyclosporine and enteric-coated mycophenolate sodium. Biopsy-proven acute rejection rates were significantly higher with complete CS avoidance and very early CS withdrawal regimens (31.5% vs 26.1% vs 14.7%) at a follow-up of 12 mo. No difference was found for patient and allograft survival as well as for median 12-mo estimated glomerular filtration rate. A prospective RCT, which included 300 patients, compared very early CS withdrawal at day 2 with standard CS^[12]. It also used basiliximab for induction, but maintenance was a calcineurin inhibitor and mycophenolate mofetil or sirolimus. It found absolutely no difference in patient and allograft survival, acute rejection, incidence of CAN and allograft function between the two arms at 3 years. A lower frequency of new-onset diabetes mellitus was noted in the very early CS withdrawal group.

Induction with a lymphocyte-depleting agent (rabbit anti-lymphocyte globulin, rALG) was explored in the clinical context of CS avoidance for the first time by Laftavi *et al*^[13]. They randomized 60 renal transplant recipients to either very early CS withdrawal at day 7 or CS continuation. Maintenance immunosuppression involved tacrolimus and MMF. No difference in acute rejection and allograft function was demonstrated with very early CS withdrawal at a follow-up time of 12 mo. However, increased interstitial fibrosis was found in protocol biopsies at 12 mo in the group of very early CS

withdrawal. In a case series of 1241 renal transplant recipients with an impressive follow-up time of 10 years, the results of CS withdrawal at day 5 were reported^[14]. All patients received induction immunosuppression with Thymoglobulin while maintenance immunosuppression comprised of a calcineurin inhibitor (tacrolimus or cyclosporine) and a secondary agent (MMF or sirolimus). Despite acute rejection rates of 25% for cadaveric donor Tx and 31% for living donor Tx at 10 years, patient and allograft survival was comparable to that reported in national registry databases. A beneficial effect of very early CS withdrawal was shown for new-onset diabetes mellitus, subcapsular cataract, and avascular necrosis. Till now, induction with lymphocyte-depleting agents seems to be the optimal option for consolidating the benefits of CS avoidance strategies without putting renal allografts at risk of acute rejection. It is not surprising that approximately 90% of United States renal transplant recipients with a steroid-free regimen on discharge have received induction with a lymphocyte-depleting agent^[15]. Anti-interleukin-2 receptor monoclonal antibodies have been used in the remaining 10% of the patients.

The monoclonal lymphocyte-depleting antibody alemtuzumab has lately emerged as a promising CS-sparing agent. In a comparative, multicenter RCT, 852 unselected (both low and high immunological risk) renal transplant recipients were administered either induction with alemtuzumab (followed by reduced-dose tacrolimus and MMF without CS) or with basiliximab (followed by standard-dose tacrolimus, MMF, and CS)^[16]. According to the preliminary results, alemtuzumab halved biopsy-proven acute rejection at 6 mo. Patient and allograft survival were not different between the two groups. Long-term follow-up results of this study are eagerly awaited. In a direct comparison of alemtuzumab with basiliximab (both arms were subjected to CS withdrawal by day 5) in a cohort of 335 low-risk patients, the rate of biopsy-confirmed acute rejection was lower with alemtuzumab (10% vs 22%, $P = 0.003$) at 3 years^[17]. The major studies on CS avoidance are summarized in Table 2. Lastly, an important question is whether patients on CS avoidance regimens should be put in CS maintenance after treatment of an acute rejection episode. A retrospective study found that allograft survival is not affected by the introduction of CS maintenance or not but the lack of CS maintenance is a risk factor for a subsequent second acute rejection^[18].

CS withdrawal

Early CS withdrawal: Initial attempts to apply early CS withdrawal under cyclosporine-based maintenance immunosuppressive regimens did not meet success^[19,20]. The advent of more potent maintenance immunosuppressants like tacrolimus and MMF renewed researchers' interest in assessing the feasibility of early CS withdrawal (Table 3). Vanrenterghem *et al*^[21] studied CS withdrawal 3 mo after Tx in 556 low immunological risk patients enrolled

Table 2 Characteristics of major randomized corticosteroid avoidance trials (the trial by Woodle *et al.*^[8] is described separately in table 1); *P* > 0.05 for all comparisons unless otherwise stated

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Biopsy-proven acute rejection (%)	Allograft/patient survival (%)	Follow-up (mo)
Vitko <i>et al.</i> ^[10]	151 147	Low/moderate (PRA < 50%, first transplant)	Day 1 Standard CS	No	TAC, MMF	30.5 ^f 8.2 ^f	97/99 96/100	6
Laftavi <i>et al.</i> ^[13]	30 30	Low (PRA < 30%, first transplant)	Day 7 Standard CS	rALG	TAC, MMF	13 11	NR	12
Kumar <i>et al.</i> ^[12]	150 150	Low (PRA < 10%)	Day 2 Standard CS	Basiliximab	TAC or CsA, MMF or sirolimus	16 14	78/91 79/89	36
Vincenti <i>et al.</i> ^[11]	112 115 109	Low (PRA < 20%, first transplant)	No CS Day 7 Standard CS	Basiliximab	CsA, EC-MPS	31.5 ^a 26.1 ^b 14.7 ^{h,a}	96/95 98/98 97/98	12
Hanaway <i>et al.</i> ^[17]	164 171	Low (PRA < 20%, first transplant)	Day 5 Day 5	Alemtuzumab Basiliximab	TAC, MMF	10 ^d 22 ^d	93/95 92/98	36
Haynes <i>et al.</i> ^[16]	426 426	Unselected patients	No CS Standard CS	Alemtuzumab Basiliximab	Low-dose TAC-MMF/ Standard TAC-MMF	7 ^h 16 ^h	96/97 97/99	6

^a*P* = 0.046, ^b*P* = 0.004, ^d*P* = 0.003, ^f*P* < 0.001, ^h*P* = 0.0001. CS: Corticosteroids; CsA: Cyclosporine; EC-MPS: Enteric-coated mycophenolate sodium; MMF: Mycophenolate mofetil; NR: Not reported; PRA: Panel-reactive antibodies; rALG: Rabbit antilymphocyte globulin; TAC: Tacrolimus.

in a multicenter RCT. Maintenance immunosuppression consisted of tacrolimus and MMF. In the follow-up time of only 6 mo, it was shown that acute rejection rates were higher in the CS withdrawal arm during months 3-6. Mean total cholesterol and LDL were reduced in the CS withdrawal arm at the same period. Pascual *et al.*^[22] summarized RCTs in CS withdrawal between 3 and 6 mo in a systematic review including 9 studies with 1820 patients. They concluded that patient and allograft survival is not affected by early Cs withdrawal up to 3 years after Tx. Total acute rejection rates were higher with early CS withdrawal in cyclosporine-treated patients. Although reduction of total cholesterol levels was observed with early CS withdrawal, no significant difference was found for any of the other cardiovascular or non-cardiovascular adverse events. It is worth mentioning that induction immunosuppression was not used in any of the included studies. Overall, evidence about the benefit-risk ratio of early CS withdrawal is weaker than that of CS avoidance and follow-up times are shorter. It is unknown if induction with lymphocyte depleting agents or anti-interleukin-2 receptor monoclonal antibodies were used in any of the studies, it would have any meaningful impact on the results.

Late CS withdrawal: It appears that late CS withdrawal (more than 6 mo and possibly years after Tx) represents the least favorable method of the CS minimization strategies. It is apparent that certain CS-related complications would already have been established by that time. For instance, it is well known that a rapid deterioration in osteoporosis occurs within the first post-transplant year^[23]. Moreover, acute rejection risk is clearly increased upon late withdrawal of immunosuppressants as dictated by cases of non-compliant patients^[3]. In a single-center RCT, Smak Gregoor *et al.*^[24] examined the

effect of CS withdrawal at 6 mo after Tx in 212 renal transplant recipients. Biopsy-proven acute rejection was manifested in 4% of CS withdrawal patients vs 1.4% of controls (*P* > 0.05). Patient and allograft survival was not different after a follow-up of 2 years. Allograft function was also not different. CS withdrawal resulted in reduced mean blood pressure but had no effect on other metabolic risk factors. Interestingly enough, a prospective, observational study from the Collaborative Transplant Study group reported that in renal transplant recipients with CS withdrawal more than 6 mo from Tx, patient and allograft survival was better than retrospectively matched controls over a follow-up time of 7 years with no difference in acute rejection rates^[25]. The reduction was also noted in the incidence of cardiovascular parameters. However, the lack of randomized design remains a significant limitation of this study.

DISCUSSION

Challenges and opportunities of CS minimization strategies

The beneficial effects of CS minimization in selected, low-risk patients have prompted researchers to attempt CS minimization in renal transplant recipients at higher immunological risk. However, available data are sparse (Table 4). In a small RCT, 21 patients with PRA > 20% or retransplantation were assigned to either alemtuzumab and tacrolimus monotherapy without CS or Thymoglobulin with standard tacrolimus, MMF and very early CS withdrawal at day 5^[26]. Biopsy-proven acute rejection rates were quite high at one year; 18.2% with alemtuzumab vs 37.5% with Thymoglobulin. In a more recent, head to head comparison of alemtuzumab with ATG (both

Table 3 Characteristics of major randomized corticosteroid withdrawal trials

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Biopsy-proven acute rejection (%)	Allograft/patient survival (%)	Follow-up (mo)
Vanrenterghem <i>et al</i> ^[20]	252	Low	At month 3	No	CsA, MMF	23 ^b	95/99	12
	248		Standard CS			14 ^b	96/98	
Smak Gregoor <i>et al</i> ^[24]	76	Low	After month 6	No	CsA, MMF	4.0 ^a	98/97	24
	73		Standard CS			1.4	97/97	
Vanrenterghem <i>et al</i> ^[21]	279	Low	After month 3	No	TAC, MMF	5.9 ^{a,d}	93/99	6
	277		Standard CS			0.9 ^d	94/98	

$P > 0.05$ for all comparisons unless otherwise stated. ^aAfter CS discontinuation, ^b $P = 0.008$, ^d $P = 0.004$. CS: Corticosteroids; CsA: Cyclosporine; MMF: Mycophenolate mofetil; TAC: Tacrolimus.

Table 4 Characteristics of corticosteroid avoidance/withdrawal trials in immunologically high-risk and in pediatric patients

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Acute rejection (%)	Allograft/patient survival (%)	Follow-up (mo)
Immunologically high-risk patients								
Thomas <i>et al</i> ^[26]	11	PRA > 20%, or	No CS	Alemtuzumab	TAC	18.2	86/100	12
	10	repeat transplant	Day 5	ATG	TAC, MMF	37.5	88/88	
Hanaway <i>et al</i> ^[17]	164	PRA > 20%, or	Day 5	Alemtuzumab	TAC, MMF	18	91/99	36
	171	black race, or repeat transplant	Day 5	ATG		15	84/91	
Pediatric patients								
Grenda <i>et al</i> ^[28]	98	Low/moderate (PRA < 50%)	Day 4	Daclizumab	TAC, MMF	10.2	97/99	6
Höcker <i>et al</i> ^[29]	98	Moderate/high (PRA < 80%)	Standard CS	No induction		7.1	97/100	
	23		After year 1	No	CsA, MMF	4	100/100	24
	19		Standard CS			10	100/100	

ATG: Antithymocyte globulin; CS: Corticosteroids; CsA: Cyclosporine; MMF: Mycophenolate mofetil; PRA: Panel-reactive antibodies; TAC: Tacrolimus.

arms underwent CS withdrawal by day 5) in a cohort of 139 high-risk patients, there was no difference in biopsy-proven acute rejection at 3 years (18% vs 15%, $P = 0.63$)^[17]. The inference is that CS minimization is not yet ready for prime time in immunologically high-risk patients. It has been hypothesized that CS minimization may increase post-transplant glomerulonephritis recurrence. In a major retrospective study, it was found that recurrence rate was indeed higher with rapid CS discontinuation compared to CS maintenance for all glomerulonephritis types (hazard ratio 4.86, 95%CI: 2.34-10.07, $P < 0.0001$)^[27]. The analysis also showed no difference in patient, allograft, and death-censored allograft survival. Pediatric patients are a subgroup in which CS minimization may be of special interest due to growth retardation that is associated with chronic CS use (Table 4). In a multicenter RCT, Grenda *et al*^[28] assessed the effect of CS withdrawal at day 4 (together with daclizumab induction and tacrolimus, MMF) vs standard tacrolimus, MMF, and CS in a cohort of 196 children. Growth was significantly enhanced at 6 mo by CS withdrawal. Patient survival, allograft survival and allograft function were not different.

The effect of CS withdrawal on total cholesterol and triglycerides was positive. Similar results were obtained by Höcker *et al*^[29] who evaluated CS withdrawal ≥ 1 year after Tx in 42 moderate- to high-risk children (maintenance immunosuppression was cyclosporine and MMF).

In contrast to the perceived benefits of CS minimization in younger transplant recipients, this strategy may not be suitable for elderly patients. Although acute rejection rates may be lower in the elderly, it has been suggested that acute rejection may be more severe and lead to a compromised death-censored allograft survival^[30]. Furthermore, the potentially beneficial effect of CS minimization in cardiovascular disease risk factors in the elderly may not be relevant due to their limited lifespan. For these reasons, it seems that CS minimization in the elderly may result in poor outcomes and should not be exercised except with extreme caution. Finally, CS minimization may not also be suitable for transplant recipients with delayed graft function (DGF) and prolonged cold ischemia time. The ischemic injury in these allografts is strongly associated with the development of acute rejection^[31]. Therefore, it is

prudent to avoid CS minimization in this patient subgroup if possible.

Based on current evidence, we believe that the majority of renal transplant recipients should continue to receive indefinite CS maintenance immunosuppression. However, selected patients can be good candidates for CS minimization protocols. The optimal patient phenotype to undergo CS minimization is that of a young transplant recipient (including children) who has no prior transplants and is unsensitized to HLA alloantigens. A primary disease that caused end-stage renal disease should not be glomerulonephritis. Any severe perioperative ischemic insult to the allograft should discourage the application of CS minimization. As such, CS minimization may be contraindicated with DGF, prolonged cold ischemia time, and donation after cardiac death. Available data indicate that the preferred CS minimization strategy is probably either complete CS avoidance or very early CS withdrawal. ATG (or alemtuzumab) may be preferable to anti-interleukin-2 receptor monoclonal antibodies as induction agents in this clinical scenario whereas maintenance immunosuppression should better contain the calcineurin inhibitor tacrolimus instead of cyclosporine.

In conclusion, CS maintain their position as important components of the therapeutic armamentarium in renal transplantation. A movement towards CS elimination from induction and maintenance immunosuppression regimens has developed to reduce the myriad side effects associated with chronic CS use. CS minimization strategies have resulted in an increased incidence of acute rejection compared to CS continuation. However, these acute rejection episodes are considered mild and amenable to treatment. Moreover, they do not seem to have detrimental effects on patient survival, allograft survival or allograft function at a follow-up until 5 years. Nonetheless, an observed trend towards increased fibrosis is alarming and calls for the conduction of RCTs with longer follow-up to determine the true consequences of CS minimization. Although CS minimization protocols have been associated with a reduction of adverse effects (especially improvement of dyslipidemia), these results are not always reproducible, and it is unclear if they could clinically translate to less cardiovascular events. At present, the implementation of CS minimization cannot be universally recommended to renal transplant recipients.

COMMENTS

Background

Due to their immunosuppressive properties, corticosteroids (CSs) have been extensively used for the prevention and treatment of rejection in solid organ transplantation. However, myriad side-effects have been associated with CS. Recent research attempts to minimize CS use in renal transplantation in an effort to reduce the burden of their side-effects without compromising allograft and patient survival.

Research frontiers

The choice of the induction immunosuppression agent - and of maintenance immunosuppression to a lesser degree - is an extremely important aspect of

CS minimization strategies and the focus of many studies. Studies with follow-up times of more than 5 years and with data on interstitial tissue fibrosis are needed.

Innovations and breakthroughs

Alemtuzumab appears to be a very promising induction agent potentially allowing the combination of CS avoidance with lower doses of tacrolimus and mycophenolate mofetil. Long term results of the 3C study may provide valuable insights on this topic.

Applications

In the United States, CS avoidance regimens were administered to 23% of all first renal transplant recipients in 2004. Although they cannot yet be recommended to the majority of renal transplant recipients, selected patient groups such as primarily those with low immunological risk and low risk for glomerulonephritis recurrence may benefit more from CS minimization.

Terminology

CS avoidance: Either no CS use at all or CS use only until day 7 after transplantation (Tx). CS withdrawal: CS tapering following a period after Tx. It is divided as early withdrawal (weeks or months after Tx, usually 3-6 mo after Tx) or late withdrawal (at least 6 mo after Tx).

Peer-review

This review paper is a well written paper of the impact of corticosteroid minimisation on kidney transplant and has valuable information.

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Systematic review of the negative pressure wound therapy in kidney transplant recipients

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Abstract

AIM

To review negative pressure wound therapy (NPWT) as an

important addition to the conventional methods of wound management.

METHODS

A systematic review, performed by searching the PubMed, EMBASE and Cochrane Library databases, showed 11 case reports comprising a total of 22 kidney transplantation (KT) patients (range, 1 to 9), who were treated with NPWT. Application of NPWT was associated with successful healing of wounds, leg ulcer, lymphocele and urine leak from ileal conduit.

RESULTS

No complications related to NPWT were reported. However, there was paucity of robust data on the effectiveness of NPWT in KT recipients; therefore, prospective studies assessing its safety and efficacy of NPWT and randomised trials comparing the effectiveness of NPWT with alternative modalities of wound management in KT recipients is recommended.

CONCLUSION

Negative pressure incision management system, NPWT with instillation and endoscopic vacuum-assisted closure system are in investigational stage.

Key words: Negative pressure; Wound therapy; Kidney transplantation; Wound infection; Wound dehiscence

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Core tip: Systematic review of the safety and efficacy of negative pressure wound therapy (NPWT) in kidney transplant (KT) recipients revealed 11 case reports, which have shown the effective role NPWT in the management of wound dehiscence, lymphocele, urine leak from ileal conduits and leg ulcers. Because of the lack of robust evidence on the safety and efficacy of NPWT in KT patients, prospective multicentre studies recruiting large number of patients is recommended to examine the role

of NPWT in the treatment of wound-related complications in KT recipients. The efficacy of negative pressure incision management system, NPWT with instillation and endoscopic vacuum-assisted closure system remain in investigational stage.

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INTRODUCTION

Kidney transplantation (KT) represents the best treatment modality for patients with end-stage renal disease, providing the best outcomes for survival, quality of life and cost-effectiveness^[1]. Immunosuppressive agents administered to prevent rejection and prolong transplant survival, not only increase susceptibility to infections, but also delay wound healing. Post-operative wound infection leading to cavitation and dehiscence continue to remain serious problems resulting in extended hospital stay, readmissions, repeated surgical interventions and protracted recovery, thereby imposing extra cost to the healthcare delivery system^[2]. The wound complication rate after KT ranges between 2% to 47%. The risk factors for these complications are advancing age, diabetes mellitus, body mass index, kidney failure, type of surgical incision, re-operation, operator's experience, and immunosuppressive drugs including sirolimus and steroids^[3-6]. The wound-related complications can present as superficial infection, haematomas, lymphocele, and partial or full-thickness wound dehiscence leading to incisional hernias^[7].

Negative pressure wound therapy (NPWT), also referred to as, vacuum-assisted closure therapy (VACT), topical negative pressure therapy or microdeformational wound therapy has been evaluated over last two decades and is considered as an useful adjunct to the management of diverse range of lesions including open abdominal wounds, open fractures, post-traumatic wounds, split-thickness skin grafts and after clean surgery in obese patients^[8-13]. Application of any new form of treatment in KT patients is associated with concerns on the part of clinicians, particularly when robust evidence supporting their safety and efficacy are lacking. A systematic review of the published literature was carried out to evaluate the effectiveness and safety of NPWT in KT recipients presenting with wound-related complications.

MATERIALS AND METHODS

Literature search

A systematic electronic literature search was performed

in PubMed, EMBASE and Cochrane Library databases from inception to March 2016. The search terms "renal transplantation", "kidney transplantation", "negative pressure wound therapy", "vacuum-assisted closure", "wound", and "topical negative pressure therapy" were used. EndNote software (Version X7.5, BLD 9325; Thomson Reuters, Philadelphia, PA, United States) was used to compile pertinent references.

Renal transplantation technique

KT is performed by using classical Gibson's muscle-cutting incision, where the iliac vessels and urinary bladder are accessed extraperitoneally. The renal vessels are anastomosed to the iliac vessels and the ureter to the bladder by the techniques described previously^[14]. Wound infection leading to muscular dehiscence exposes the kidney to the external environment, which predisposes to infection around the kidney, haemorrhage from mycotic aneurysms of the vascular anastomoses, lymph leak, urine leak, and dehiscence of muscle layers leading to incisional herniation.

Principles of NPWT

The beneficial effect of negative sub-atmospheric pressure on the wound results in gradual closure of wound edges by micro- and macrodeformation of the wound surface, and by suction of infectious material and interstitial fluid, reduces tissue oedema. Decompression of tissue increases blood flow and tissue oxygenation, thereby accelerates the wound healing cascade including, angiogenesis, neurogenesis, granulation tissue formation, cellular proliferation, differentiation and migration of appropriate cellular components at the site of healing^[15-20].

Glass *et al*^[21] in a systematic review, evaluated the molecular basis for the promotion of wound healing by NPWT and observed an increase in the expression of cytokines, chemokines and growth factors, which reflected mechanoreceptor and chemoreceptor transduction in response to stress and hypoxia. There was reduction of expression of matrix metalloproteinase-1, -2, -9 and -13, with no changes on the activity of tissue inhibitor of metalloproteinase-1^[21].

The NPWT device comprises of black polyurethane ether foam dressing or white polyvinyl alcohol foam, which is tailored to fit into the dimension of the wound. A tube with multiple perforations is placed within the foam for the evacuation of the wound discharge. The tube together with the foam is then covered with an occlusive drape, which helps to maintain uniform negative pressure. The effluent of the wound is collected in a canister, which is attached to the vacuum pump with an adjustable negative pressure, ranging from 50 and 125 mmHg (Figure 1A and B). At the interval 48-72 h, the soiled dressing is replaced with fresh dressing at the bedside, when progress of healing is assessed. The device can be used in preparation for secondary suture, a skin graft, flap or until full closure of the wound has taken place^[22,23]. The oldest and most popular device in clinical practice is the vacuum-assisted

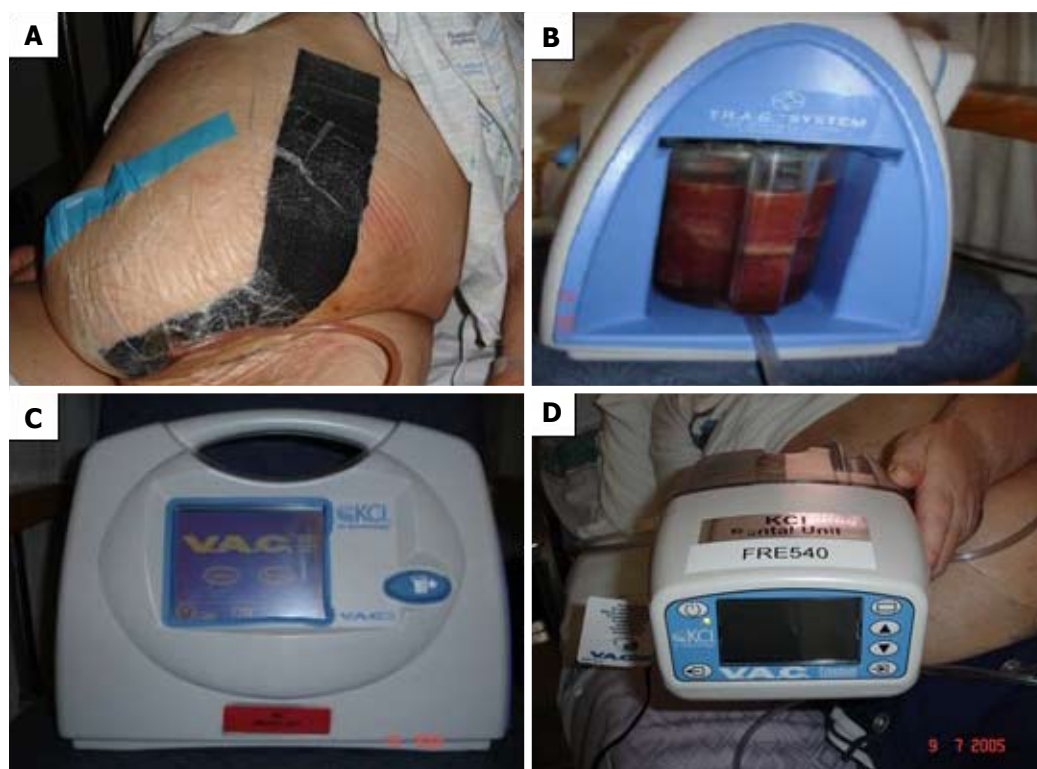


Figure 1 Negative pressure wound therapy device and its components. A: Black polyurethane foam dressing and tubing in the wound; B: Canister containing exudates; C: Standard suction device; D: Portable suction device.

closure (VAC® KCI, San Antonio, Texas) system (Figure 1C and D)^[24].

The contra-indications for the applications of NPWT are excessive pain with NPWT, presence of pus or excessive bleeding and intolerance. The success of NPWT is assessed by the reduction in wound size by at least 10% per week or 50% improvement over 4 wk period, which indicates high probability of success of the therapy^[25].

RESULTS

The literature search identified 11 case reports comprising of a total of 22 KT patients (range, 1 to 9) (Table 1), who were treated with NPWT^[26-36]. Comparison between NPWT and other methods of wound treatment in KT patients has not been reported in any study.

In 2003, Hodzic *et al*^[32], for the first time, reported successful outcome of application of NPWT for 15 d in 2 KT patients prior to secondary suture of wounds. Successful treatment of an infected and dehiscd laparotomy wound following liver and KT in a patient by employing NPWT was reported by Zanus *et al*^[26], where associated complications included acute pancreatitis, abdominal compartment syndrome and wound infection by multi-drug resistant organisms. The patient required 14 successive laparotomies and NPWT for 6 mo for complete closure of the wound^[26]. Similarly, Markić *et al*^[34] have described successful treatment with application of NPWT in 2 KT patients who developed infected and dehiscd wounds. NPWT was

applied for 2 and 3 wk, respectively, which was followed by secondary sutures^[34].

The occurrence of ureteric complications significantly delays the recovery following KT and the incidence of such complication ranges between 1.2% and 8.9%^[37]. Urinary leak rate of 11% requiring re-implantation was reported by Surange *et al*^[38] in a series of KT into ileal conduits. Two cases of urine leak and wound dehiscence following KT into ileal conduits were managed successfully by Heap *et al*^[28] with the application of NPWT. Secondary suture of the wounds was carried out after two and three months in these patients. The renal function was restored in both patients leading to 141 $\mu\text{mol/L}$ and 75 $\mu\text{mol/L}$ of serum creatinine, respectively, at the end of 3 mo^[28]. On the other hand, Ortiz *et al*^[27], had negative experience of NPWT in a KT recipient with peri-renal collection and wound infection. They concluded that NPWT had encouraged and prolonged urine leak, which had healed after 5 d of discontinuation of NPWT^[27]. Iesari *et al*^[36] had applied VAC device in a KT patient who had developed spontaneous rupture of urinary bladder due to gangrenous cystitis and extensive wound dehiscence associated with multidrug resistant *Acinetobacter baumannii* infection. There was significant urine leak following VAC therapy, hence this was discontinued and topical homologous platelet-rich gel was used resulting in complete wound healing^[36].

Infection caused by virulent organisms after skin grafts and reconstructive surgery in KT recipients not only lead to failure of treatment, but also can be life-

Table 1 Characteristics of studies

Ref.	Year	Country	No. of cases	No. of NPWT days	Indications
Iesari <i>et al</i> ^[36]	2015	Italy	1	Not described	Wound dehiscence
Bozkurt <i>et al</i> ^[33]	2015	Turkey	1	5	Primary surgery
Markic <i>et al</i> ^[34]	2014	Croatia	2	14, 21	Wound dehiscence
Franchin <i>et al</i> ^[35]	2014	Italy	1	45	Infected lymphocele
Zanus <i>et al</i> ^[26]	2011	Italy	1	180	Wound dehiscence, pancreatitis
Ortiz <i>et al</i> ^[27]	2011	United States	1	15	Wound infection
Heap <i>et al</i> ^[28]	2010	United Kingdom	2	Not described	Wound dehiscence, Urine leak
Thodis <i>et al</i> ^[29]	2009	Greece	1	Not described	Vibrio infection of leg
Devries <i>et al</i> ^[31]	2009	United States	1	Not described	Leg wound
Shrestha <i>et al</i> ^[30]	2007	United Kingdom	9	3, 5, 5, 5, 8, 10, 10, 15, 30	Wound dehiscence, infection
Hodzic <i>et al</i> ^[32]	2003	Germany	2	15	Wound dehiscence

NPWT: Negative pressure wound therapy.

threatening. Thodis *et al*^[29] treated soft tissue infection caused by *Vibrio vulnificus* with NPWT, which involved the leg in a KT recipient. Autologous platelet concentrate spray further enhanced granulation tissue formation leading to complete epithelialization of the wound after 4 wk^[29]. In a similar situation, Devries *et al*^[31] were unsuccessful in treating soft tissue infection on the leg of a KT recipient, that culminated in amputation. As the patient was on sirolimus, wound healing could have been compromised by the same drug^[5,31,39].

Lymphocele following KT can cause significant morbidity due to infection and compression of ureter and blood vessels. The reported incidence of lymphocele ranges between 0.6% to 49%^[40]. Franchin *et al*^[35] have described successful management of a large deep-seated lymphocele infected with *Staphylococcus haemolyticus*, *Escherichia coli* and *Enterococcus faecalis*, with the application of NPWT. Following surgical drainage, the wound had completely dehisced and transplanted kidney exposed. The cavity was packed with foam dressing and device was applied. A negative pressure of 80 mmHg was maintained. The dressing was changed every 5 d. After 45 d, the lymphocele had sealed and skin closed^[35].

In a prospective study reported by Shrestha *et al*^[30], 9 KT patients had developed wound infection with cavitation and wound dehiscence. This was associated with significant amount of discharge from the wound, which failed to respond to standard method of treatment. Treatment with NPWT for a median of 9 (range 3-30) d led to cessation of discharge from the wound. Of the 9 patients, 4 patients were managed on an outpatient with portable NPWT device, where the treatment was discontinued after a median of 5.5 (range 3-7) d. The median hospital stay since the employment of NPWT was significantly shorter (5, range 2-12 d) compared to the standard method of treatment prior to application of NPWT (11 d, range, 5-20 d; $P = 0.003$). The wound healed completely in all 9 cases after the therapy^[30].

Recently, Bozkurt *et al*^[33], for the first time, employed Prevena incision management system (Kinetic Concept Inc. San Antonio, Texas, United States) to the clean closed surgical wound for 5 d after a KT and observed complete healing of the wound with no skin or device-

related problems.

DISCUSSION

All infected wounds with associated collections require surgical drainage for early healing. Fleischmann *et al*^[41] from Germany, in 1993, for the first time described the benefit of exposing wounds to sub-atmospheric pressure, which promoted wound debridement and healing. He applied this method in 15 patients with compound fractures and observed enhanced proliferation of the granulation tissue with no associated bone infection leading to complete healing of fractures^[41]. In 1997, Louis Argenta and Michael Morykwas introduced NPWT therapy, for the first time, in the treatment of bed sores and slow healing wounds. Since then, NPWT has been extended to treat various types of wounds resulting from surgery, trauma, infection, congenital deformities and tumours^[42-44]. The experience of NPWT gained over the past two decades has encouraged clinicians to treat patients globally in both hospital and domiciliary environments^[44-46].

This systematic review has confirmed the available evidence on the safety and efficacy of the application of NPWT in KT recipients limited to case reports. On the other hand, the reported experiences do support NPWT in the management of complex wounds following KT, including urine leak from KT in ileal conduits and lymphoceles. The theoretical risk of haemorrhage and urine leak from transmission of suction pressure on the vascular and ureteric anastomoses cannot be ignored. Prolonged urine leak had occurred in two reported cases after KT where NPWT was applied. Discontinuation of NPWT had led to resolution of urine leak. In author's single KT patient with a urine leak from the ureterovesical junction, treatment with NPWT led to persistence of urine leak for 1 wk. Resolution of the urine leak occurred 2 d after discontinuation of NPWT therapy. Successful outcomes of NPWT in the management of wound infections in cardiac and liver transplant recipients have been described previously^[47,48].

Development of enterocutaneous fistula during the course of NPWT is always a concern, which is particularly

applicable in deep wounds after KT, where thin layer of peritoneum lies between the bowel and the foam dressing. Occurrence of enterocutaneous fistula has been observed after NPWT in open abdominal wound. However, the evidence in support of the occurrence of this complication after NPWT is weak^[49-51].

Shrestha *et al*^[30], in their largest reported series of 9 patients, observed benefit of NPWT on wound healing, reduced hospital stays and convenience of wound management. The management of 4 patients on an outpatient basis with the NPWT device *in situ*, was convenient to the patient and saved hospital cost significantly^[30].

Comparison of NPWT with standard treatment modalities

There is no data available comparing the safety and efficacy of NPWT over conventional methods of wound management in KT recipients. However, there are several randomised trials and meta-analyses, which have assessed the effectiveness of NPWT for skin grafts and surgical wound healing by primary and secondary intentions and in chronic wounds compared with several conventional treatment methods. With regards to healing of surgical wound by primary intention, the evidence for the effect of NPWT for reducing surgical site infections, time to complete healing and wound dehiscence remains unclear^[52]. A Cochrane Database Systematic Review assessed the effect of NPWT for the treatment of chronic wounds in comparison with five different comparators, which did not show that NPWT significantly increased the healing rate. The trials did have methodical flaws, therefore need for better quality research was recommended^[53]. Similarly, a recent Cochrane review did not show clinical effectiveness of NPWT over alginate dressings in the treatment of open infected groin wounds and a silicone dressing in the treatment of excised pilonidal sinus when they were allowed to heal by secondary intention^[54].

NPWT with instillation

NPWT with instillation (NPWTi) is a recent advancement, which is being assessed in the management of complicated surgical wounds. The wound is covered with normal saline (0.9%) and left for 10-20 min for diffusion to take place. Then, 2-4 h of negative pressure at -125 mmHg is applied. A panel of experts in the first International Consensus Guidelines for NPWTi have recommended its use in high risk patients with multiple comorbidities including diabetes, contaminated traumatic wounds, and wounds complicated by invasive infection or extensive biofilm. Available evidence suggest achievement of better outcomes with the addition of NPWTi to standard of care in properly selected cases, compared to standard care alone^[55,56]. As majority of KT recipients often have associated co-morbidities, NPWTi may be an option in this group of patients.

Negative pressure incision management

Colli *et al*^[57] employed the negative pressure incision

management system in clean closed incisions, for the first time, in 10 patients after cardiac surgery and observed normal wound healing in patients where complications were expected after surgery. Bozkurt *et al*^[33], have reported their experience of using Prevena incision management device in a KT recipient. A recent meta-analysis of NPWT for closed surgical incisions, (including 10 studies, 1311 incisions in 1089 patients) showed significant reduction in wound infection (RR = 0.54) and seroma formation (RR = 0.48), when NPWT was compared with standard care. The reduction in wound dehiscence was not significant. The numbers needed to treat were 3 (seroma), 17 (dehiscence) and 25 (infection). Due to heterogeneity between the included studies, no general recommendations could be made yet^[58]. However, this device has a potential for its use in immunosuppressed and obese patients undergoing KT.

Endoscopic vacuum-assisted closure system

Endoscopic vacuum-assisted closure system (E-VAC) has developed as an important alternative in patients with upper gastrointestinal leaks not responding to standard endoscopic or surgical treatment procedures. Leak from oesophageal and gastric anastomosis sites and perforations resulting from endoscopic procedures were successfully closed using the E-VAC therapy^[59,60]. Application of this device in KT recipients remains to be explored.

This systematic review has shown successful healing of wounds, leg ulcer, lymphocele and urine leak from ileal conduit following application of NPWT in KT recipients and there was no report of complications associated with NPWT. However, there is lack of robust evidence on safety and efficacy of NPWT in KT patients. Based on available evidence on the application of NPWT in KT recipients, NPWT can be considered as a valuable adjunct in the management of infected and dehiscent wounds following KT. The safety and efficacy of NPWT, negative pressure incision management system, NPWT with instillation and E-VAC system, and efficacy of NPWT in comparison with standard methods of wound management, need to be examined prospectively by including large number patients in multicentre studies.

COMMENTS

Background

Negative pressure wound therapy (NPWT) is a useful adjunct to the conventional methods of management of infected wounds with deep cavitation in the kidney transplant (KT) recipients. A systematic review was performed to assess the safety and efficacy of NPWT in KT recipients, which showed 11 case reports including 22 KT recipients who were treated with NPWT showing beneficial outcomes.

Research frontiers

There are no randomised trials comparing the safety and efficacy of NPWT with alternative modalities of wound management, hence multicentre prospective study by including large number of patients is recommended.

Innovations and breakthroughs

The negative pressure incision management, NPWT with instillation, and

endoscopic vacuum-assisted closure system are the new developments in this field, which need to be applied and examined in the RT recipients.

Applications

NPWT has been applied in the treatment of abdominal wounds, leg ulcers, lymphoceles and urine leak from ileal conduit in RT recipients successfully.

Terminology

Negative pressure wound therapy (NPWT), also referred to as, vacuum-assisted closure therapy (VACT), topical negative pressure therapy (TNPT) or microdeformational wound therapy.

Peer-review

The authors made a comprehensive review on NPWT on KTx recipients. It provides useful information for clinicians.

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Outcomes in randomized controlled trials of exercise interventions in solid organ transplant

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Abstract

AIM

To identify the outcome measures that have been used in randomized controlled trials (RCTs) of exercise training in solid organ transplant (SOT) recipients and to link these outcomes to the International Classification of Functioning, Disability and Health (ICF) framework.

METHODS

Electronic literature searches of MEDLINE, EMBASE, CINAHL, Cochrane, Scopus, and Web of Science were performed. We sought RCTs that investigated the effect of exercise training in SOT recipients. Reference lists of all eligible publications were searched for other appropriate studies not identified by the electronic search. A complete list of outcome measures used in the RCTs was generated and each of these was linked to an ICF category.

RESULTS

Four hundred and thirteen articles were retrieved, of which 35 met our inclusion criteria. The studies included were designed to compare the effects of exercise training programs to usual care or to another exercise training program and reported on recipients of heart ($n = 21$), kidney ($n = 9$), lung ($n = 3$) or liver ($n = 2$) transplant. Of the 126 outcome measures identified, 62 were used as primary outcome measures. The most commonly occurring primary outcomes were aerobic capacity using the peak VO_2 ($n = 13$), quality of life using the short-form-36 ($n = 8$), and muscle strength ($n = 7$). These

outcome measures were linked to 113 ICF categories and the majority of outcomes fall into the body function domain ($n = 93$).

CONCLUSION

There is little standardization in outcome measures used in RCTs of exercise interventions in SOT recipients. The ICF framework can be used to select a core set of outcomes that cross all domains of ICF and that would be appropriate to all SOT recipients.

Key words: Solid organ transplantation; Systematic review; Rehabilitation; Exercise; Outcome measures; International Classification of Functioning, Disability and Health

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Core tip: Over 30 randomized controlled trials (RCTs) have been conducted to examine the effectiveness of exercise training on outcomes in solid organ transplant recipients. However, the synthesis of findings across studies has been limited by the lack of similar outcomes. We identified 126 unique outcomes used in RCTs of exercise training and categorized them according to the International Classification of Functioning, Disability and Health framework. Most commonly, outcomes fell into the domains of body structure and body function, whereas there were a limited number of outcomes examining activities and participation. This review highlights the need for a core set of outcomes for RCTs in exercise training for this population.

Janaudis-Ferreira T, Mathur S, Konidis S, Tansey CM, Beaufort C. Outcomes in randomized controlled trials of exercise interventions in solid organ transplant. *World J Transplant* 2016; 6(4): 774-789 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/774.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.774>

INTRODUCTION

As the acute morbidity and mortality associated with solid organ transplantation continues to improve, interventions that improve quality of life and long-term health outcomes are needed. Exercise training has several important health benefits for solid organ transplant (SOT) recipients, such as improving maximal aerobic capacity (VO_2 peak), body composition and quality of life^[1]. Exercise and physical activity also have potential effects for mitigating long-term complications post-transplant and side-effects of immunosuppressant medication such as reducing blood pressure, controlling blood glucose^[2], managing weight gain^[3], improving muscle^[4] and bone strength^[5], and reducing fatigue^[6-8]. A limitation of the current literature on exercise for SOT is the inability to combine outcomes from studies due to the wide range of reported outcomes. In a

systematic review of exercise training in SOT recipients conducted in 2012 by Didsbury *et al.*^[1], the authors included 15 randomized controlled trials (RCTs) with 28 unique outcomes. The majority of outcomes were related to cardiovascular parameters (VO_2 peak, blood pressure, cholesterol), with fewer studies examining body composition, frailty indicators or quality of life. The authors were therefore hampered in their ability to conduct meta-analyses, which limited the conclusions of their comprehensive review.

The inability to synthesize data from studies in the field of SOT is of particular concern, as this is a small population and studies on exercise training are often conducted at single transplant centres with relatively small sample sizes. In order to gain greater statistical power to draw conclusions, studies need to be combined using knowledge synthesis approaches, which require common outcomes. Inconsistencies in the reporting of outcomes can affect the conclusions of systematic reviews and may contribute to reporting bias^[9]. Therefore, in order to facilitate standard reporting of key outcomes across studies, the development of core outcomes sets for clinical trials is gaining more attention^[10,11].

The International Classification of Functioning, Disability and Health (ICF) is an established framework developed by the World Health Organization and is commonly used in rehabilitation. The ICF is designed to describe health and health-related status from biological, personal and societal perspectives^[12]. The framework classifies human function into four domains: Body functions; body structures; activities and participation; and environmental factors^[12]. These domains match well with the goals of exercise training and physical rehabilitation programs; specifically to identify, measure and treat physical impairments (body function and structure); to reverse or normalize activity limitations; and to enhance participation in all settings^[13]. Using the ICF to map the outcomes of the current literature on exercise training in SOT recipients will assist in classifying the breadth of outcomes that have been used in the studies to date and also in identifying any domains that are understudied in this population. This information can provide a starting point for developing a core set of standard outcomes^[10] for clinical trials of exercise and physical rehabilitation in SOT recipients.

The objectives of this systematic review were to identify the outcome measures that have been used in RCTs of exercise training in SOT recipients and to link these outcomes to the ICF framework.

MATERIALS AND METHODS

Data sources and search strategy

This systematic review is in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement^[14]. A librarian designed and performed electronic literature searches of Medline from inception until May 2016. The search was then adapted for EMBASE, CINAHL, Cochrane, Scopus, and Web of Science and run on these databases.

Table 1 Electronic search strategy used in MEDLINE

Search #	Keywords and number of records identified
Search #1	Organ transplantation (110179)
Search #2	Transplantation conditioning (7738)
Search #3	Transplant recipients (195)
Search #4	"Transplant recipients" (27594)
Search #5	1 or 2 or 3 or 4 (122169)
Search #6	Exercise/or Exercise Therapy/or exercise\$ (192344)
Search #7	Rehab\$/or rehabilitation (151761)
Search #8	Resistance training/or "physical education and training"/or training (181282)
Search #9	"Physical activity" (47446)
Search #10	Physical exertion (11451)
Search #11	6 or 7 or 8 or 9 or 10 (474657)
Search #12	5 and 11 (2399)
Search #13	Heart or lung or kidney or pancreas or liver (1433618)
Search #14	12 and 13 (2200)
Search #15	Limit 14 to humans (2156)
Search #16	Limit 14 to animals (76)
Search #17	15 not 16 (2121)
Search #18	Limit 17 to randomized controlled trial (60)

Search terms included organ transplantation, transplant recipients, graft recipient, heart, lung, kidney, pancreas, liver, exercise, exercise therapy, rehab, rehabilitation, resistance training, physical education, training, physical activity, and physical exertion (Table 1). The searches were limited to RCTs, published in English, and in humans. One investigator (Stacey Konidis) also conducted hand searches of the reference lists of all the studies that met the inclusion criteria to identify additional relevant articles.

Criteria for including studies in the review

We selected all RCTs that investigated the effect of exercise training in SOT recipients. We included trials that compared the effects of exercise training programs to standard care as well as trials that compared two or more different exercise training programs in SOT recipients. In the case of multiple publications of the same study, we considered all of them if the outcomes measures were different. We excluded studies that did not have an isolated exercise intervention group (*i.e.*, those that examined the effect of a drug combined with exercise). We also excluded non-English articles and conference abstracts. One investigator (Stacey Konidis) reviewed the study titles and abstracts to determine potential study eligibility. When this investigator was uncertain, a second reviewer (Tania Janaudis-Ferreira) was consulted. Two investigators independently reviewed the full texts of the articles to determine eligibility (Stacey Konidis and Tania Janaudis-Ferreira).

Data extraction and synthesis

Two reviewers (Stacey Konidis and Cecile Beaurepaire) performed the data extraction and tabulation. A third reviewer (Tania Janaudis-Ferreira) double-checked the extracted data. Outcome measures were abstracted using a standard form and imported into a spreadsheet, sorted into primary and secondary outcomes and

classified according to four domains of the ICF (body functions, body structures, activities and participation, and environmental factors). Information about the exercise interventions and patient populations were also retrieved. Considering the purpose of this review, study quality or risk of bias assessments of the included studies were not deemed to be necessary.

RESULTS

Literature search

The electronic and hand searches led to the identification of 522 articles. After excluding 109 duplicates, there were 413 articles left for title and abstract screening. Following the study title and abstract screening, 366 were considered to be unrelated to the objectives of the review. Of the 47 articles that remained for full-text analysis, 12 were excluded. This left a total of 35^[2-5,15-45] articles for inclusion in this review. The study flow and reasons for exclusion are shown in Figure 1.

Review of studies and outcome domains assessed

The studies included were designed to compare the effects of exercise training programs to usual care or to another exercise training program and reported on transplantation of heart ($n = 21$), kidney ($n = 9$), lung ($n = 3$), and liver ($n = 2$). A total of 1313 patients were randomized in the 35 studies. Description of the exercise programs and other details about the studies is presented in Table 2.

Table 3 outlines the outcome measures that were used in each study. In total, there were 126 outcome measures. Of the 126 outcome measures, 62 were used as primary outcome measures in at least one study. The most commonly occurring primary outcomes were peak VO_2 ($n = 13$), SF-36 ($n = 8$), and muscle strength ($n = 7$).

Each outcome measure was linked to an ICF

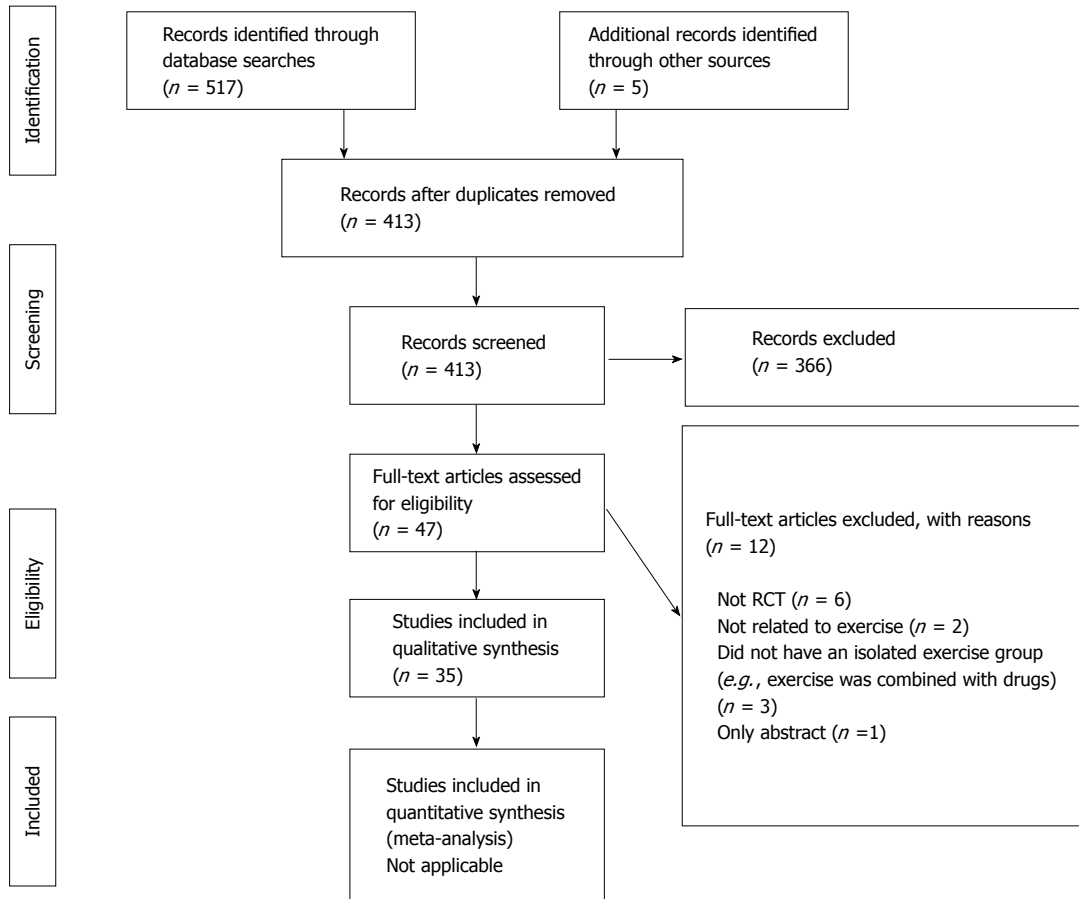


Figure 1 PRISMA 2009 flow diagram. From: Moher *et al.*^[14]. For more information, visit www.prisma-statement.org.

Table 2 Description of studies

Ref.	Country	Year	Organ	Time-post transplant (wk)	Treatment duration (wk)	Randomized patients ¹	Exercise intervention	Comparison
Braith <i>et al.</i> ^[5]	United States	1996	Heart	> 8	24	16	Lumbar extension 1 d/wk; variable resistance exercises 2 d/wk	Usual care
Braith <i>et al.</i> ^[4]	United States	1998	Heart	> 8	24	16 ²	Lumbar extension 1 d/wk; variable resistance exercises 2 d/wk	Usual care
Kobashigawa <i>et al.</i> ^[15]	United States	1999	Heart	> 2	26	27	Individualized cardiac rehabilitation (strengthening, flexibility, and moderate aerobic exercises) 1-3 d/wk	Usual care (unstructured therapy at home)
Painter <i>et al.</i> ^[16]	United States	2002	Kidney	4-8	48	167	Independent home-based exercise 4 d/wk	Usual care
Mitchell <i>et al.</i> ^[17]	United States	2003	Lung	> 8	26	16	Lumbar extension resistance exercise 1 d/wk and walking program	Usual care (walking program)
Painter <i>et al.</i> ^[18]	United States	2003	Kidney	> 4	48	96	Independent home-based exercise 4 d/wk	Usual care
Braith <i>et al.</i> ^[19]	United States	2005	Heart	> 8	24	15	Variable resistance exercises 2 d/wk	Usual care
Juskowa <i>et al.</i> ^[20]	Poland	2006	Kidney	> 0.5	4-5	69	Strength exercise training 7 d/wk	Usual care
Krasnoff <i>et al.</i> ^[3]	United States	2006	Liver	> 8	40	151	Cardiovascular exercise training 3 d/wk	Usual care
Bernardi <i>et al.</i> ^[21]	Italy	2007	Heart	> 24	24	26	Stationary bicycle; 30 min/5 d per week	Usual care
Karapolat <i>et al.</i> ^[22]	Turkey	2007	Heart	Mean 14-17	8	38	Hospital-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk	Home-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk
Braith <i>et al.</i> ^[23]	United States	2008	Heart	> 8	12	20	Aerobic treadmill exercise	Usual care

Karopola <i>et al</i> ^[24]	Turkey	2008	Heart	Mean 14-17	8	38 ³	Hospital-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk	Home-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk
Pierce <i>et al</i> ^[25]	United States	2008	Heart	> 8	12	20	Aerobic exercise training	Usual care
Wu <i>et al</i> ^[26]	Taiwan	2008	Heart	> 52	8	37	Resistance and aerobic training 3 d/wk	Usual care
Haykowsky <i>et al</i> ^[27]	Canada	2009	Heart	> 26	12	23	Aerobic 5 d/wk and strength training 2 d/wk	Usual care
Mandel <i>et al</i> ^[28]	United States	2009	Liver	6-12	12	50	Targeted lower body resistance strengthening exercise 3-4 d/wk	Usual care (walking program)
Hermann <i>et al</i> ^[29]	Denmark	2011	Heart	> 52	8	27	Aerobic interval training program 3 d/wk	Usual care
Ihle <i>et al</i> ^[30]	Germany	2011	Lung	> 52	4	60	Inpatient rehabilitation (exercise training 4 d/wk and aerobic session 5 d/wk)	Outpatient physiotherapy
Christensen <i>et al</i> ^[31]	Denmark	2012	Heart	Mean 84	8	⁴	High-intensity aerobic interval training 3 d/wk	Usual care
Langer <i>et al</i> ^[32]	Belgium	2012	Lung	1-6	12	40	Aerobic and resistance training 3 d/wk	Usual care
Nytrøen <i>et al</i> ^[32]	Norway	2012	Heart	52-416	52	52	High-intensity aerobic interval training 3 d/wk	Usual care
Rustad <i>et al</i> ^[33]	Norway	2012	Heart	52-416	12	52	High-intensity aerobic interval training 3 d/wk	Usual care
Kawauchi <i>et al</i> ^[34]	Brazil	2013	Heart	< 1	to hospital discharge	22	10-phase incremental exercise program (breathing, active resistance exercises, aerobic exercises, stretching)	Institution exercise routine (breathing, stretching walking) 5 d/wk
Kouidi <i>et al</i> ^[35]	Greece	2013	Kidney	> 52	26	24	Aerobic exercise and strength training 4 d/wk	Usual care
Nytrøen <i>et al</i> ^[36]	Norway	2013	Heart	52-416	52	52 ⁵	High-intensity aerobic interval training 3 d/wk	Usual care
Dall <i>et al</i> ^[37]	Denmark	2014	Heart	> 52	12 (5 mo washout)	17	High-intensity aerobic interval training 3 d/wk	Moderate biking exercise 3 d/wk
Monk-Hansen <i>et al</i> ^[38]	Denmark	2014	Heart	> 52	8	30	High intensity training 3 d/wk	Usual care
Pascoalino <i>et al</i> ^[39]	Brazil	2015	Heart	> 52	12	42	Endurance exercise training 3 d/wk	Usual care
Pooranfar <i>et al</i> ^[40]	Iran	2013	Kidney	104-156	10	44	Aerobic and resistance training 3 d/wk	Usual care
Riess <i>et al</i> ^[41]	Canada	2013	Kidney	> 26	12	31	Endurance and strength training 2 d/wk	Usual care
Tzvetanov <i>et al</i> ^[42]	United States	2014	Kidney	> 4	52	17	Resistance exercise training 2 d/wk (as well as behaviour and nutrition)	Usual care
Dall <i>et al</i> ^[43]	Denmark	2015	Heart	> 52	12 (5 mo washout)	17 ⁶	High-intensity aerobic interval training 3 d/wk	Moderate biking exercise 3 d/wk
Greenwood <i>et al</i> ^[44]	England	2015	Kidney	< 52	12	60	Home-based aerobic training and resistance training 3 d/wk	Usual care
Karelis <i>et al</i> ^[45]	Canada	2015	Kidney	6-8	16	24	Resistance training 3 d/wk (once a week in hospital and 2 × /week at home)	Usual care (no exercise)

¹Does not add to 1313 since some patients included in more than one study; ²Same patients as Braith 1996; ³Same patients as Karolopat 2007; ⁴Same patients as Hermann 2011; ⁵Same patients as Nytrøen 2012; ⁶Same patients as Dall 2014. BMD: Bone mineral density; HR: Heart rate; BP: Blood pressure; HRQOL: Health-related quality-of-life; CVD: Cardiovascular disease; BMI: Body mass index; METs: Metabolic unit of task; HRR: Heart rate reserve; HRR1: Heart rate recovery; CRI: Chronotropic response index; CRP: C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; sICAM-1: Intercellular adhesion molecule-1; 6MWD: 6 minute walk distance; FVC: Forced vital capacity; HRV: Heart rate variability; BRS: Baroreflex sensitivity.

domain and the list is shown in Table 4. The majority of outcomes fell into the body function domain ($n = 93$). Fourteen outcome measures were linked to the activities and participation, 5 to body structures, 2 to environmental factors and 2 described outcomes were unclassified in the ICF. Frailty indicators such as grip strength ($n = 1$), fatigue ($n = 0$) or gait speed (6-minute-walk) ($n = 3$) were rarely used. Ten multi-dimensional questionnaires were used in the studies

reviewed.

DISCUSSION

Physical rehabilitation in SOT patients strives to minimize the impairments associated with prolonged chronic illness, allowing individuals to improve their ability to carry out daily tasks and activities and to participate in life roles. When selecting outcome measures to use in clinical trials

Table 3 List of outcome measures by study

Ref.	Year	Organ group	Primary outcome measures	Secondary outcome measures
Braith <i>et al</i> ^[5]	1996	Heart	Bone mineral density (body and regional: Femur neck, lumbar vertebra)	Bone mineral content Total bone calcium Acute rejection episodes Percent body fat Acute rejection episodes
Braith <i>et al</i> ^[4]	1998	Heart	Body mass Fat-free mass Fat mass Muscle strength (upper and lower body)	
Kobashigawa <i>et al</i> ^[15]	1999	Heart	Blood pressure (peak and resting) Heart rate (peak and resting) Anaerobic threshold Exercise duration (to exhaustion) Peak ventilation Peak VO ₂ Peak workload Ventilatory equivalent for carbon dioxide and oxygen	Muscle strength (lower limb)
Painter <i>et al</i> ^[16]	2002	Kidney	Body mass index Body weight Fat mass/body fat Lean tissue mass Percent body fat Blood pressure (peak) Muscle strength (quadriceps) Peak ventilation Peak VO ₂ SF-36	Self-reported activity level (frequency, type, length, and intensity of exercise) Blood creatinine Blood urea nitrogen levels Hematocrit Hemoglobin Bone mineral density Peak workload Rating of perceived exertion (Borg) Peak respiratory exchange ratio Immunosuppression use (type, dose) Acute rejection episodes Muscle strength (lumbar extensor)
Mitchell <i>et al</i> ^[17]	2003	Lung	Bone mineral density (lumbar spine)	Blood lipids Incidence of diabetes Smoking status
Painter <i>et al</i> ^[18]	2003	Kidney	Cholesterol (TC, HDL) Body mass index Total CVD risk (Framingham) Blood pressure Peak workload (METs)	
Braith <i>et al</i> ^[19]	2005	Heart	Muscle composition (fiber types) Muscle metabolic enzyme activity	Muscle strength (upper and lower body)
Juskowa <i>et al</i> ^[20]	2006	Kidney	Blood lipids Cholesterol (TC, HDL, LDL) Body mass index	Blood calcium level Blood creatinine Blood electrolytes Blood glucose Blood phosphorus Blood protein levels (albumin, fibrinogen, total protein level) Enzyme levels (alanine transferase, alkaline phosphatase, aspartate aminotransferase) Folate concentrations Hemoglobin Interleukin-18 Total-homocysteine Vitamin B12 Blood pressure Muscle strength (upper limbs) Peak expiratory flow Rating of perceived exertion (Borg)
Krasnoff <i>et al</i> ^[3]	2006	Liver	Body mass index Body weight Bone mineral content Bone mineral density Fat mass/body fat Lean tissue mass Percent body fat Muscle strength (quadriceps) Peak VO ₂ SF-36 Peak respiratory exchange ratio Nutritional intake (Block-95 - calories/day; protein, carb and fat calories)	

Bernardi <i>et al</i> ^[21]	2007	Heart	Baroreceptor control of blood pressure Baroreceptor control of heart rate	Blood pressure; Heart rate Neck pressure RR interval Anaerobic threshold CO ₂ production Exercise duration (to exhaustion) Peak ventilation Peak VO ₂ ; Peak workload Ventilatory equivalent for CO ₂ and oxygen
Karapolat <i>et al</i> ^[22]	2007	Heart	Peak VO ₂ Beck depression inventory SF-36 State-trait anxiety inventory	
Braith <i>et al</i> ^[23]	2008	Heart	Endothelial function (flow-mediated dilation)	Blood glucose Blood lipids Cholesterol (TC, HDL, LDL) Oxidative stress-induced lipid peroxidation Plasma norepinephrine Serum metabolic and hematologic indicators Body mass Acute rejection episodes Blood pressure (resting and peak) Brachial artery diameter Exercise duration (to exhaustion) Peak VO ₂ Duke Treadmill Score
Karapolat <i>et al</i> ^[24]	2008	Heart	Chronotropic response index Heart rate recovery Heart rate reserve Peak VO ₂	
Pierce <i>et al</i> ^[25]	2008	Heart	C-reactive protein Interleukin-6 Serum metabolic profile Soluble cell adhesion molecules (sICAM-1) Tumour necrosis factor-alpha Muscle vasodilation (forearm and calf)	Blood glucose Cholesterol (TC, HDL, LDL) Cytomegalovirus IgG status White blood cell levels Acute rejection episodes Blood pressure (resting) Heart rate (peak and resting) Exercise duration (to exhaustion) Rating of perceived exertion (Borg) Peak respiratory exchange ratio Daily physical activity Blood pressure Heart rate (resting and peak) Nutritional intake (caloric intake questionnaire) Peak ventilation Peak workload Rating of perceived exertion (Borg)
Wu <i>et al</i> ^[26]	2008	Heart	Muscle endurance (quadriceps) Muscle strength (quadriceps) Peak VO ₂ World Health Organization Questionnaire on Quality of Life - BREF	Lean tissue mass (total and leg) Blood pressure (peak) Endothelial function (endothelial-dependent vasodilation, endothelial-independent vasodilation, reactive hyperemia index) Heart rate (peak) Left ventricular systolic function Muscle strength (upper and lower body) Peak power output Peak respiratory exchange ratio
Haykowsky <i>et al</i> ^[27]	2009	Heart	Peak VO ₂	
Mandel <i>et al</i> ^[28]	2009	Liver	6MWD Muscle strength (lower body) Chronic liver disease questionnaire (CLDQ) SF-36 (physical function/limitations)	
Hermann <i>et al</i> ^[29]	2011	Heart	Peak VO ₂	Blood creatinine Blood glucose; Blood lipids Blood protein levels (adiponectin, MR-proANP, NT-proBNP, proasopressin/copeptin) Cholesterol Hemoglobin High sensitive C-reactive protein Interleukin-6 Serum insulin Tumour necrosis factor-alpha Body mass index; Body weight

				Hip-waist ratio Blood pressure (resting) Brachial artery diameter Endothelial function (flow-mediated vasodilation, nitroglycerin-induced vasodilation) Heart rate (resting) Peak power output Heart rate (peak and resting) Anaerobic threshold Oxygen uptake at anaerobic threshold Peak workload Peak respiratory exchange ratio Ventilatory reserve and capacity Peak VO ₂
Ihle <i>et al</i> ^[30]	2011	Lung	6MWD Peak VO ₂ SF-36 St. George's Respiratory Questionnaire	
Christensen <i>et al</i> ^[31]	2012	Heart	Hospital Anxiety and Depression Scale	
Langer <i>et al</i> ^[2]	2012	Lung	SF-36 Daily walking time (time spend in different postures: sedentary, standing, walking)	Daily steps Movement intensity Time spent in moderate intense activities Blood lipids Body weight Bone mineral density Blood pressure 6MWD Muscle strength (quadriceps and handgrip) Peak workload Mood status SF-36 Forced expiratory volume Respiratory muscle force Incidence of morbidity (diabetes, hyperlipidemia, hypertension, osteoporosis) Blood lipids Blood protein levels (NT-proBNP) C-reactive protein Interleukin-6, 8 and 10 levels Body mass index; Body weight; % body fat Chronotropic response index Glycemic control parameters Blood pressure (peak and resting) Heart rate (peak and resting) Heart rate recovery and reserve Stroke volume (O ₂ pulse; resting and peak) Anaerobic threshold Exercise duration (to exhaustion) Muscle strength (quadriceps and hamstrings) Peak ventilation Rating of perceived exertion (Borg) SF-36 Visual Analog Scale (subjective difference in HRQoL) Peak respiratory exchange ratio
Nytrøen <i>et al</i> ^[32]	2012	Heart	Peak VO ₂	Biochemical parameters Blood pressure Cardiac allograft vasculopathy (coronary angiography) Cardiac output Heart rate (resting and peak) Stroke volume Peak workload Peak respiratory exchange ratio Muscle strength (upper and lower limbs) Maximum expiratory/inspiratory pressure
Rustad <i>et al</i> ^[33]	2012	Heart	Echocardiographic parameters (rest and during exercise; systolic and diastolic parameters) Peak VO ₂	Baroreflex effectiveness index Blood pressure (peak and resting) Heart rate (peak and resting) Exercise duration (to exhaustion) Peak ventilation Peak VO ₂
Kawauchi <i>et al</i> ^[34]	2013	Heart	6MWD Forced vital capacity Respiratory muscle force/strength	Blood creatinine Blood glucose Blood lipids
Kouidi <i>et al</i> ^[35]	2013	Kidney	Baroreflex sensitivity Heart rate variability parameters (SDNN, rMSSD, pNN50, LF, HF, LF/HF)	
Nytrøen <i>et al</i> ^[36]	2013	Heart	Cardiac allograft vasculopathy (intravascular ultrasound and virtual histology)	

				C-reactive protein Cholesterol (TC, HDL, LDL) Hemoglobin Interleukin-6, 8 and 10 levels Body mass index Body water (total) Body weight Bone mass Lean tissue mass Percent body fat Visceral fat scale Basal metabolic rate Glycemic control parameters Metabolic age Muscle strength (quadriceps and hamstrings) Peak VO ₂ Body weight Blood pressure Heart rate (peak and resting) Heart rate recovery Heart rate reserve CO ₂ production Peak ventilation Peak workload Peak respiratory exchange ratio
Dall <i>et al</i> ^[37]	2014	Heart	Peak VO ₂	Body mass index Blood pressure Heart rate (peak and resting) Heart rate recovery Heart rate reserve CO ₂ production Peak ventilation Peak workload Peak respiratory exchange ratio
Monk-Hansen <i>et al</i> ^[38]	2014	Heart	Echocardiography parameters (systolic and diastolic function)	Body mass index Blood pressure Heart rate (peak and resting) Peak VO ₂ Peak workload Plasma norepinephrine Heart rate (peak and resting) Anaerobic threshold CO ₂ production Exercise duration (to exhaustion) Peak VO ₂ Peak respiratory exchange ratio Respiratory compensation point
Pascoalino <i>et al</i> ^[39]	2015	Heart	Arterial stiffness (carotid-femoral pulse wave velocity) Blood pressure (ambulatory; peak and resting)	Peak workload Plasma norepinephrine Heart rate (peak and resting) Anaerobic threshold CO ₂ production Exercise duration (to exhaustion) Peak VO ₂ Peak respiratory exchange ratio Respiratory compensation point
Pooranfar <i>et al</i> ^[40]	2013	Kidney	Blood lipids Cholesterol (TC, HDL, LDL) Sleep quality and quantity questionnaire (self-report; Pittsburgh Sleep Quality Index)	
Riess <i>et al</i> ^[41]	2013	Kidney	Peak VO ₂	Cholesterol (TC, HDL) Lean tissue mass Total CVD risk (Framingham) Arterial pressure (mean) Arterial stiffness (pulse wave velocity) Arteriovenous oxygen difference (a-vO ₂) Blood pressure (ambulatory; peak and resting) Cardiac output Heart rate (peak); Stroke volume Systemic vascular endurance Muscle strength (lower body) Peak workload SF-36 Peak respiratory exchange ratio
Tzvetanov <i>et al</i> ^[42]	2014	Kidney	Glomerular filtration rate SF-36 Adherence to training and follow-up Employment status	Blood creatinine; Blood glucose; Blood lipids Cholesterol (TC, HDL, LDL) Hemoglobin Body mass index Body weight Bone mineral content Lean tissue mass Percent body fat Arterial stiffness (carotid-femoral pulse wave velocity) Blood pressure Carotid intima-media thickness Muscle strength

Dall <i>et al</i> ^[43]	2015	Heart	Blood glucose Blood protein levels (adiponectin, orosomucoid, YLK 40) Interleukin-6 Serum insulin Tumour necrosis factor-alpha Arterial stiffness (augmentation index) Endothelial function (reactive hyperemia index) Hospital Anxiety and Depression Scale SF-36	Body weight Homeostasis model assessment Heart rate (peak) Peak VO ₂ Peak respiratory exchange ratio
Greenwood <i>et al</i> ^[44]	2015	Kidney	Muscle strength (quadriceps)	Arterial stiffness (pulse wave velocity) Blood pressure (peak and resting) Heart rate (peak and resting) STS-60 Peak VO ₂ Body mass index; Body weight Waist girth Glomerular filtration rate high-sensitivity C-reactive protein interleukin-6 Fetuin A Tumor necrosis factor-alpha tumor necrosis factor receptors 1 and 2 SF-36 Duke Activity Status Index
Karelis <i>et al</i> ^[45]	2015	Kidney	World Health Organization-5 Well-Being Index Muscle strength index Adherence to training and follow-up (feasibility)	Body weight Body height Body mass index Waist girth Hip girth Fat mass/body fat Lean tissue mass Cholesterol (TC, HDL, LDL) Blood glucose Blood pressure Peak VO ₂

SF-36: Short-form 36; TC: Total cholesterol; HDL: High-density lipoprotein fraction of cholesterol; LDL: Low-density lipoprotein fraction of cholesterol; RR-interval: Inter-beat interval (heart rate); BREF: A shorter version of the original; rMSSD: Root-mean-square of successive NN interval differences; pNN50: Percentage value of NN50 count; LF: Low-frequency components; HF: High-frequency components; CVD: Cardio-vascular disease; STS-60: Sit-to-stand 60.

Table 4 International Classification of Functioning, Disability and Health outcome classifications

ICF component	Domain	Category	Outcome measures	Count primary ¹	Organ group
Body Function	Global mental functions	b134	Sleep quality and quantity	1	Kidney
		b152	Mood status	0	Lung
	Functions of the cardiovascular system (heart functions)	b410	Cardiac output	0	Heart, kidney
		b410	Carotid intima-media thickness	0	Kidney
		b410	Echocardiographic parameters	2	Heart
		b410	Endothelial function	2	Heart
		b410	Left ventricular systolic function	0	Heart
		b410	RR interval	0	Heart
		b410	Stroke volume	0	Heart, kidney
		b410	Systemic vascular endurance	0	Kidney
	Functions of the cardiovascular system (heart rate)	b4100	Heart rate	1	Heart, kidney, lung
		b4100	Heart rate recovery	1	Heart
		b4100	Heart rate reserve	1	Heart
		b4100	Heart rate variability	1	Kidney
	Functions of the cardiovascular system	b410-429	Baroreceptor control of blood pressure	1	Heart
		b410-429	Baroreceptor control of heart rate	1	Heart
		b410-429	Baroflex effectiveness index	0	Kidney
		b410-429	Baroflex sensitivity	1	Kidney
		b410-429	Chronotropic response index	1	Heart
		b410-429	Total CVD risk	1	Kidney
		b410-429	Cardiac allograft vasculopathy	1	Heart
	Functions of the cardiovascular system (blood vessel functions)	b415	Arterial stiffness	3	Heart, kidney
		b415	Brachial artery diameter	0	Heart
	Functions of the cardiovascular system (blood pressure functions)	b420	Arterial pressure	0	Kidney
		b420	Blood pressure	4	Heart, kidney, lung

Functions of the cardiovascular system (oxygen-carrying functions of the blood)	b420	Neck pressure	0	Heart
	b4301	Arteriovenous oxygen difference	0	Kidney
Functions of the hematological and immunological systems	b430-439	Biochemical parameters	0	Heart
	b430-439	Blood calcium level	0	Kidney
	b430-439	Blood creatinine	0	Heart, kidney
	b430-439	Blood electrolytes	0	Kidney
	b430-439	Blood glucose	1	Heart, kidney
	b430-439	Blood lipids	2	Heart, kidney, lung
	b430-439	Blood phosphorus	0	Kidney
	b430-439	Blood protein levels	1	Heart, kidney
	b430-439	Blood urea nitrogen levels	0	Kidney
	b430-439	C-reactive protein	1	Heart
	b430-439	Cholesterol	3	Heart, kidney
	b430-439	Folate concentrations	0	Kidney
	b430-439	Hematocrit	0	Kidney
	b430-439	Hemoglobin	0	Heart, kidney
	b430-439	High sensitive C-reactive protein	0	Heart
	b430-439	Interleukin levels	2	Heart, kidney
	b430-439	Plasma norepinephrine	0	Heart
	b430-439	Soluble cell adhesion molecules	1	Heart
	b430-439	Total-homocysteine	0	Kidney
	b430-439	Tumour necrosis factor-alpha	2	Heart
	B430-439	Tumor necrosis factor receptor	0	Kidney
	b435	Cytomegalovirus IgG status	0	Heart
	b435	White blood cell levels	0	Heart
	b435	Acute rejection episodes	0	Heart, lung
Functions of the respiratory system (respiration functions)	b440	Forced expiratory volume	0	Lung
	b440	Forced vital capacity	1	Heart
	b440	Maximum expiratory/inspiratory pressure	0	Heart
	b440	Peak expiratory flow	0	Kidney
	b440	Peak respiratory exchange ratio	1	Heart, kidney, liver, lung
	b440	Respiratory compensation point	0	Heart
	b440	Ventilatory reserve and capacity	0	Lung
Functions of the respiratory system (respiration rate)	b4400	CO ₂ production	0	Heart
	b4400	Oxygen uptake at anaerobic threshold	0	Lung
	b4400	Peak ventilation	2	Heart, kidney
	b4400	Peak VO ₂	13	Heart, kidney, liver, lung
	b4400	Ventilatory equivalent for carbon dioxide and oxygen	1	Heart
Functions of the respiratory system (respiratory muscle functions)	b445	Respiratory muscle force/strength	1	Heart, lung
Functions of the cardiovascular system (general physical endurance)	b4550	Rating of perceived exertion	0	Heart, kidney, liver
Functions related to the digestive, metabolism and the endocrine system	b530	Body mass index	4	Heart, kidney, liver
	b530	Body weight/mass	3	Heart, kidney, liver, lung
	b530	Fat mass/body fat	3	Heart, kidney, liver
	b530	Fat-free mass	1	Heart
	b530	Hip girth	0	Kidney
	b530	Hip-waist ratio	0	Heart
	b530	Lean tissue mass	2	Heart, kidney, liver
	b530	Percent body fat	2	Heart, kidney, liver
	b530	Visceral fat scale	0	Heart
	b530	Waist girth	0	Kidney
General metabolic functions, unspecified	b5400	Basal metabolic rate	0	Heart
	b5400	Metabolic age	0	Heart
General metabolic functions, other, specified	B5408	Maximal metabolic units	1	Kidney
Functions related to metabolism and the endocrine system	b540-559	Enzyme levels	0	Kidney
	b540-559	Fetuin A	0	Kidney
	b540-559	Oxidative stress-induced lipid peroxidation	0	Heart
	b540-559	Serum insulin	1	Heart

		b540-559	Serum metabolic and/or hematologic profile	1	Heart
		b540-559	Vitamin B ₁₂	0	Kidney
		b540-559	Glycemic control parameters	0	Heart, kidney
		b540-559	Muscle metabolic enzyme activity	1	Heart
		b545	Body water	0	Heart
		b545	Homeostasis model assessment	0	Heart
	Functions of the genitourinary and reproductive functions (urinary functions)	b610-639	Glomerular filtration rate	1	Kidney
	Neuromusculoskeletal and movement-related functions (muscle power functions)	b730	Peak workload/power output	1	Heart, kidney, lung
		b730	Muscle strength	7	Heart, kidney, liver, lung
		b730-b749	Muscle vasodilation	1	Heart
		b740	Muscle endurance	1	Heart
Body structure	Structures related to movement - additional musculoskeletal structures related to movement (bones)	s7700	Bone mass	0	Heart
		s7700	Bone mineral content	1	Heart, kidney, liver
		s7700	Bone mineral density	3	Heart, kidney, liver, lung
		s7700	Total bone calcium	0	Heart
		s7702	Muscle composition (fibre types)	1	Heart
Activities and participation	Mobility - walking and moving	d410	STS-60	0	Kidney
participation	Mobility - walking and moving (walking)	d450	Daily steps	0	Lung
		d450	Daily walking time	1	Lung
		d450	6 Minute Walk Distance	3	Heart, liver, lung
		d450	Anaerobic threshold	1	Heart, lung
	Mobility - walking and moving	d450-469	Daily physical activity	0	Heart
		d450-469	Movement intensity	0	Lung
		d450-469	Self-reported activity level	0	Kidney
		d450-469	Time spent in moderate intense activities	0	Lung
		d450-469	Duke Treadmill Score	0	Heart
		d450-469	Exercise duration	1	Heart, kidney
	Managing diet and fitness	d5701	Caloric intake	0	Heart
		d5701	Nutritional intake	1	Liver
	Major life areas (work and employment)	d840-859	Employment status	1	Kidney
Environmental factors	Products or substances for personal consumption, other specified	e1108	Smoking status	0	Kidney
Questionnaires	Drugs	e1101	Immunosuppression use	0	Kidney
			DASI	0	Kidney
			Quality of Life Profile for Chronic Diseases Questionnaire	1	Lung
			SF-36	8	Heart, kidney, liver, lung
			St. George's Respiratory Questionnaire	1	Lung
			State-Trait Anxiety Inventory	1	Heart
			Beck Depression Inventory	1	Heart
			Hospital Anxiety and Depression Scale	2	Heart
			Visual Analog Scale (change in HRQoL)	0	Heart
			WHOQOL-BREF	2	Heart, kidney
Not covered by ICF			Chronic Liver Disease Questionnaire	1	Liver
			Incidence of morbidity	0	Kidney, lung
			Adherence to training and follow-up	2	Kidney

¹Count Primary: Count of studies that used this measure as a primary measure. RR-interval: Inter-beat interval (heart rate); CVD: Cardio-vascular disease; STS-60: Sit-to-stand 60; SF-36: Short-form 36; HRQoL: Health-related quality of life; WHOQOL-BREF: A shorter version of the original World Health Organization Quality of Life Questionnaire; DASI: Duke Activity Status Index.

of SOT recipients, it is important to capture changes across all domains that are relevant to the primary goals of the physical rehabilitation intervention. We have used the ICF categories to classify the outcome measures used in RCTs of exercise interventions after SOT. From this systematic review, we have learned that the outcome measures used in these RCTs vary widely. This finding is in line with the results of similar systematic reviews conducted in

other populations (e.g., individuals with critical illness, post-surgery and stroke)^[11] Some of the studies focused on multiple primary outcomes and others used just two or three. In total, 62 different primary outcomes were used with the most common being peak VO₂ ($n = 13$) and the SF-36 ($n = 8$). Most of the outcomes used fell into the body functions domain ($n = 93$) with very few in the activities and participation domain ($n = 14$). Few

studies included outcomes that are also considered frailty indicators. These are important outcomes as frailty is present in many SOT recipients and can have a negative impact on transplant outcomes^[6-8].

As we did, Disbury *et al.*^[1] found that the most commonly used outcome measure was VO₂ peak. However, this is an expensive test that requires complex equipment as well as expertise from a professional to interpret the results. Functional exercise capacity tests that are more relevant to patients' activities and participation in daily life and less costly to administer should be considered.

Disbury *et al.*^[1] were unable to merge data on health-related quality-of-life (HRQoL) measures since so many different questionnaires were used. We found that 11 of the RCTs analyzed used multi-dimensional questionnaires as an outcome measure with several using more than one. These questionnaires each cover many different ICF categories. For instance, Cieza and Stucki^[46] have linked individual questions from the short-form-36 (SF-36) questionnaire to ICF domains and found that this questionnaire incorporates at least 21 ICF codes. Linking individual items on HRQoL questionnaires could help researchers select a questionnaire that covers many ICF codes and that would be most suited to be part of the core set of outcome measures recommended, thus making it possible to meaningfully merge data from multiple studies.

A core set of outcome measures to be used in all of these populations would be helpful to minimize and standardize the number of outcomes used in this patient group. While it is important to conduct a comprehensive assessment, the use of a large number of outcome measures can be burdensome for both patients and evaluators. Ideally, the core set of variables should cover all four domains of the ICF, *i.e.*, they need to cover all aspects of the health condition. Furthermore, the core set of variables needs to include outcomes that are common to all organ groups. Many of the issues that affect physical function and exercise capacity are common across the transplant types despite each SOT having its own unique characteristics and challenges^[47]. Some of the pre-transplant issues that limit physical function are specific to the failing organ, but the physiological changes associated with severe chronic disease, deconditioning and nutritional depletion are common to all groups^[48]. Post-transplant issues that limit physical function vary depending on the phase of recovery, but include things such as extended hospital and intensive care stay, prolonged sedentary time, immunosuppressant medications and episodes of organ rejection^[48]. Outcome measures that relating to these commonalities and to increasing physical function would be suitable for inclusion in the core set of variables. However, there are some organ specific issues that may be important to address differently among the groups (*e.g.*, the effects of exercise in the denervation of the heart after transplant or the effects of exercise on early onset of diabetes after

kidney transplant) and researchers should be encouraged to include secondary outcomes to address them.

The selection of outcome measures should reflect the length of time since the transplant and whether the course of recovery has been complicated. For example, the main goal of physical rehabilitation for acute phase post-transplant is usually to improve basic mobility and activities of daily living while rehabilitation for long-term recipients is generally focused on improving their exercise capacity and levels of physical activity to prevent cardiovascular complications. When considering appropriate outcomes, is also important to take into account their psychometric properties^[49]. Knowing the validity of the outcomes in the transplant population can help researchers with sample size calculations for interventional studies and justify the use of the selected primary outcomes.

None of the studies reviewed included an economic evaluation of the exercise programs and the potential cost savings if SOT recipients experience less long-term cardiovascular disease and fewer hospital readmission related to frailty and physical disability. Although robust economic studies can be challenging, they may be important to convince healthcare funders that exercise programs can be cost-effective and have a positive impact on transplant outcomes and survival. Exercise programs also need to be more readily available for transplant recipients as lack of availability of post-transplant exercise programs has been identified for example in Canada^[50].

Limitations

A limitation of this systematic review is the inclusion of only RCTs. There are other studies on exercise training in SOT recipients that use different research designs, especially observational studies using pre-post designs that were not included. We chose this strategy because RCTs are of the highest quality of study design. We assumed that investigators conducting RCTs have chosen their outcomes carefully and that this group of studies is representative of all rehabilitation trials in transplant recipients. We have also limited our search to studies published in English, which may have reduced our sample size.

There is little standardization in outcome measures used in RCTs of exercise interventions in SOT recipients. Outcome measures for clinical trials should also be selected based on their psychometric properties, stage post transplantation and severity of impairments of the patient population. Further research is needed to develop consensus on a standardized core set of outcomes to measure the effectiveness of such interventions. The ICF framework can be used to select appropriate outcomes that cross all domains and that would be appropriate to all SOT recipients.

COMMENTS

Background

Over 30 randomized controlled trials (RCTs) have been conducted to examine

the effectiveness of exercise training on outcomes in solid organ transplant (SOT) recipients. However, the synthesis of findings across studies has been limited by the lack of similar outcomes across studies. The objectives of this systematic review were to identify the outcome measures that have been used in RCTs of exercise training in SOT recipients and to link these outcomes to the International Classification of Functioning, Disability and Health (ICF) framework.

Research frontiers

Between 1996 and 2015 more than 30 RCTs were published on the effects of exercise training in SOT recipients. Taken together, the results of these RCTs show that exercise training improves maximal aerobic capacity, muscle strength, body composition, cardiopulmonary variables and quality of life. There is little evidence for the effect of exercise in physical activity and participation in SOT recipients. In a systematic review of exercise training in SOT recipients conducted in 2012 by Didsbury *et al.*, the authors included 15 RCTs with 28 unique outcomes. The majority of outcomes were related to cardiovascular parameters (VO₂ peak, blood pressure, cholesterol), with fewer studies examining body composition, frailty indicators or quality of life. The authors were therefore hampered in their ability to conduct meta-analyses, which limited the conclusions of their comprehensive review.

Innovations and breakthroughs

There are numerous studies examining the role of exercise training to improve outcomes following SOT. Exercise training has several important health benefits for SOT recipients, such as improving maximal aerobic capacity (VO₂ peak), body composition and quality of life. A limitation of the current literature on exercise for SOT is the inability to combine outcomes from studies due to the wide range of reported outcomes.

Applications

This systematic review suggests that there is a need to develop consensus on a standardized core set of outcomes to measure the effectiveness of exercise interventions in SOT. A standardized core set of outcomes would facilitate standard reporting of key outcomes across studies.

Terminology

The ICF is an established framework developed by the World Health Organization and is commonly used in rehabilitation. The ICF is designed to describe health and health-related status from biological, personal and societal perspectives. The framework classifies human function into four domains: body functions; body structures; activities and participation; and environmental factors. These domains match well with the goals of exercise training and physical rehabilitation programs; specifically to identify, measure and treat physical impairments (body function and structure); to reverse or normalize activity limitations; and to enhance participation in all settings.

Peer-review

It is a well written review concerning several domains to assess the function outcome of patients with organ transplants subjected to exercise training. It is very helpful for the readers.

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Incidence of kidney stones in kidney transplant recipients: A systematic review and meta-analysis

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Abstract

AIM

To evaluate the incidence and characteristics of kidney stones in kidney transplant recipients.

METHODS

A literature search was performed using MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews from the inception of the databases through March 2016. Studies assessing the incidence of kidney stones in kidney transplant recipients were included. We applied a random-effects model to estimate the incidence of kidney stones.

RESULTS

Twenty one studies with 64416 kidney transplant patients were included in the analyses to assess the incidence of kidney stones after kidney transplantation. The estimated incidence of kidney stones was 1.0% (95%CI: 0.6%-1.4%). The mean duration to diagnosis of kidney stones after kidney transplantation was 28 ± 22 mo. The mean age of patients with kidney stones was 42 ± 7 years. Within reported studies, approximately 50% of kidney transplant recipients with kidney stones were males. 67% of kidney stones were calcium-based stones (30% mixed CaOx/CaP, 27%CaOx and 10%CaP), followed by struvite stones (20%) and uric acid stones (13%).

CONCLUSION

The estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Although calcium based stones are the most common kidney stones after

transplantation, struvite stones (also known as "infection stones") are not uncommon in kidney transplant recipients. These findings may impact the prevention and clinical management of kidney stones after kidney transplantation.

Key words: Nephrolithiasis; Incidence; Kidney stones; Kidney transplantation; Transplantation

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Core tip: The authors performed this meta-analysis to assess the incidence and characteristics of kidney stones in kidney transplant recipients. The estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Calcium based stones (CaOx and CaP) are the most common kidney stones after transplantation following by struvite stones and uric acid stones. The findings from this study may impact the management of kidney stone prevention after kidney transplantation.

Cheungpasitporn W, Thongprayoon C, Mao MA, Kittanamongkolchai W, Jaffer Sathick IJ, Dhondup T, Erickson SB. Incidence of kidney stones in kidney transplant recipients: A systematic review and meta-analysis. *World J Transplant* 2016; 6(4): 790-797 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v6/i4/790.htm> DOI: <http://dx.doi.org/10.5500/wjt.v6.i4.790>

INTRODUCTION

Kidney stones are one of the most common metabolic disorders and urological problems with a prevalence of 7.2%-7.7% in the adult population, and a ten-year recurrence rate of $\geq 30\%$ ^[1-4]. The incidence of kidney stones is increasing especially in industrialized countries with an estimated global prevalence between 10%-15%^[5-8]. Approximately 13% of men and 7% women will have a kidney stone during their lifetime^[5,8].

Previous studies have shown that stone recurrence rates may be lower, when glomerular filtration rate (GFR) reduced^[9,10]. Thus, patients with advanced chronic kidney disease (CKD) or end-stage kidney disease (ESRD) may encounter less stone disease^[10], reported being as low as 0.68%^[11]. After successful kidney transplantation, ESRD patients subsequently have significant improvement in renal function resulting in urinary excretion of metabolites that increases risk of stone disease. Studies have identified kidney stones in allograft kidney as one of the serious problems in kidney transplant recipients^[12-40]. However, unlike the general population, the incidence and characteristics of kidney stones in kidney transplant recipients are not well studied. The aim of this meta-analysis was to appraise the incidence and types of kidney stones after kidney transplantation.

MATERIALS AND METHODS

Cheungpasitporn W and Thongprayoon C individually

examined published studies and conference abstracts indexed in MEDLINE, EMBASE, and Cochrane Database from the inception of the databases through March 2016. The search strategy used is detailed in the supplementary material (Item 1). Further pertinent studies were retrieved by conducting a manual search using references from the articles that were reclaimed from the search strategy noted above. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews and meta-analyses^[41] and previously published guidelines^[42,43].

The inclusion criteria were as follows: (1) randomized controlled trials or observational studies (case-control, cross-sectional, cohort studies, or case series); (2) patient population age > 18 years old; and (3) data on kidney stones in kidney transplant recipients were provided. The search was limited to English-language studies. Both published studies and conference abstracts were incorporated. Study eligibility was independently determined by the two investigators mentioned earlier. Differing decisions were settled by joint agreement.

A standardized information collection form was applied to derive the following data: The first author of each study, study design, year of publication, country where the study was conducted, number of kidney transplant recipients studied, number of patients with kidney stone, age and gender of patients with kidney stones, time of diagnosis after kidney transplantation, type of donor (Live or deceased donor), type and location of kidney stones, and period of follow-up.

Statistical analysis

MetaXL software (EpiGear International Pty Ltd)^[44] was utilized for data analysis. The incidence rates (IRs) and 95%CIs of adverse effects were reported using a DerSimonian-Laird random-effects model^[45]. A random-effects model was implemented due to the high likelihood of inter-study variances. The Cochran Q test was completed to assess statistical heterogeneity. The I^2 statistic was added to evaluate the degree of variation across studies related to heterogeneity instead of chance. An I^2 of 0%-25% represents insignificant heterogeneity, 26%-50% low heterogeneity, 51%-75% moderate heterogeneity and > 75% high heterogeneity^[46]. To assess for publication bias funnel plots were used^[47].

RESULTS

Our search strategy yielded 1554 articles. Of these, 1397 articles were excluded following the review of their title and abstract based on their relevance and the eligibility criteria. The remaining 157 articles underwent full-length review, and an additional 136 were excluded for failing to meet the criteria. Twenty one articles^[12-29,36,38,40] met all inclusion criteria and were identified for the meta-analysis of kidney stones in kidney transplant recipients (Table 1). Supplementary Item 2 outlines our search methodology

Table 1 Main characteristics of the studies included in this meta-analysis

Ref.	Country	Year	Total number	No. of patients with kidney stone	Time of diagnosis	Sex of patients with stone	Age of patients with stone	Donors	Stone location	Stone composition	Mean follow-up time
Cho <i>et al</i> ^[12]	United States	1988	544	9	Mean 14.7 mo, Median 7 mo (range 3-42 mo)	6 male, 3 female	Mean 30 yr (range 8-65 yr)	6 living, 3 cadaveric	4 bladder, 3 kidney, 2 unknown	4 calcium oxalate/ calcium phosphate, 2 ammonium magnesium phosphate and carbonate appetite, 1 uric acid, 2 not studied	5 (range 1.5-15.5) yr
Hayes <i>et al</i> ^[13]	United States	1989	892	10	Mean 13 mo (range 4-49 mo)	7 male, 3 female	Mean 29 yr (range 17-53 yr)	3 living, 7 cadaveric	NR	NR	NR
Harper <i>et al</i> ^[38]	United Kingdom	1994	178	6	NR	4 male, 1 female	NR	4 living, 1 cadaveric	NR	1 uric acid, 2 calcium phosphate, 1 calcium oxalate, 1 Magnesium ammonium phosphate	NR
Shoskes <i>et al</i> ^[14]	United Kingdom	1995	812	2	Mean 3.5 yr (range 2-5 yr)	NR	Mean 40 yr	NR	2 ureter	NR	At least 1 yr
Benoit <i>et al</i> ^[36]	France	1996	1500	12	NR	7 male, 5 female	Mean 36 yr	2 living, 10 cadaveric	5 calyces, 6 ureter, 1 pyeloureteral junction	4 calcium oxalate and phosphate, 2 struvite	NR
Del Pizzo <i>et al</i> ^[15]	United States	1998	540 (445 renal transplant, 95 pancreas/renal transplant)	4	NR	NR	NR	NR	NR	NR	NR
Rhee <i>et al</i> ^[16]	United States	1999	1813 (1730 renal transplant, 83 pancreas/renal transplant)	8	NR	4 male, 4 female	Mean 51 yr (range 34-60 yr)	2 living, 1 cadaveric, 5 pancreas/renal	3 kidney, 1 ureter, 4 bladder	1 uric acid, 1 calcium oxalate, 1 calcium phosphate, 1 calcium phosphate, 1 struvite stone, 3 unknown	Mean 68.6 mo (range 27-98 mo)
El-Mekresh <i>et al</i> ^[17]	Egypt	2001	1200	11	NR	NR	NR	NR	3 kidney, 4 ureter, 4 bladder	NR	NR
Kim <i>et al</i> ^[18]	United States	2001	849	15	Mean 17.8 mo (range 3-109 mo)	10 male, 5 female	Mean 41.5 yr (range 28-67 yr)	8 living, 7 cadaveric	11 bladder, 3 kidney, 1 multiple sites	5 mixed form of calcium oxalate and calcium phosphate, 1 calcium oxalate, 3 predominant calcium phosphate, 2 struvite, 2 mixed form of struvite and calcium phosphate, 2 not studied	Mean 58 mo (range 11-149 mo)

Klinger <i>et al</i> ^[19]	Austria	2002	1027	19 (4 diagnosis during transplant, 5 perioperative, 10 <i>de novo</i>)	For <i>de novo</i> : Mean 27.7 mo (range 13 to 48 mo)	8 male, 11 female	Mean 48.1 yr (range 26-72 yr)	1 living, 18 cadaveric	14 kidney, 3 infundibulum, 1 distal ureter, 1 staghorn	11 calcium oxalate, 2 uric acid, 1 calcium phosphate, 5 not studied	Mean 29 mo (range 14-48 mo)
Doehn <i>et al</i> ^[20]	Germany	2002	1500	11	NR	5 male, 6 female	Median 50 yr	11 cadaveric	NR	3 uric acid, 3 calcium oxalate, 2 magnesium ammonium stone, 3 not studied	Median 4 yr
Streeter <i>et al</i> ^[21]	United Kingdom	2002	1535	12	For renal calculi: Median 150 d (range 56-1280 d); For bladder calculi: Range 8 mo - 4 yr	NR	NR	NR	9 ureter, 3 bladder	NR	NR
Abbott <i>et al</i> ^[22]	United States	2003	42906	52	NR	NR	NR		35 kidney, 17 ureter	NR	1.89 ± 1.15 yr
Lipke <i>et al</i> ^[23]	United States	2004	500	7	9 mo (range 1.5-26 mo)	7 female	Mean 50 yr (range 8-73 yr)	4 living, 3 cadaveric	7 bladder	7 mixed between calcium oxalate and calcium phosphate	NR
Yigit <i>et al</i> ^[24]	Turkey	2004	125	5 (2 preoperative, 1 early posttransplant, 2 <i>de novo</i>)	For <i>de novo</i> : Mean 6.5 mo (range 6-7 mo)	3 male, 2 female	Mean 35.2 yr	NR	NR	2 calcium oxalate, 1 uric acid, 2 infectious	Mean 32.4 mo
Challacombe <i>et al</i> ^[25]	United Kingdom	2005	2085	21	3.7 (0.17-18) yr	8 male, 13 female	Mean 41 yr (range 15-64 yr)	3 living, 18 cadaveric	13 kidney, 7 ureter, 1 bladder	NR	NR
Ferreira <i>et al</i> ^[26]	Brazil	2012	1313	12 <i>de novo</i>	Range 6 mo to 13 yr	8 males, 9 females	Mean 45.6 yr (range 32-63 yr)	2 living, 15 cadaveric	6 calyces, 3 renal pelvis, 3 ureter	NR	NR
Stravodimos <i>et al</i> ^[27]	Greece	2012	1525	7	Mean 3.2 (2-7) yr	NR	NR	NR	5 kidney, 2 ureter	NR	Mean 8 yr
Cicerello <i>et al</i> ^[40]	Italy	2014	953	10	NR	4 male, 6 female	Mean 43 yr	NR	7 kidney, 3 ureter	NR	NR
Mamarelis <i>et al</i> ^[28]	Greece	2014	2045	9	Mean 3.1 yr (range 1-7 yr)	NR	NR	NR	6 kidney, 3 ureter	NR	6.6 yr (range 1-15 yr)
Rezaee-Zavereh <i>et al</i> ^[29]	Iran	2015	574	25	NR	NR	NR	NR	NR	NR	55 ± 53 mo

CaOx: Calcium oxalate; CaP: Calcium phosphate; NR: Not reported.

and selection process.

Incidence of kidney stones in kidney transplant recipients

The incidence of kidney stones after kidney transplantation within the 21 individual study ranged between 0.2% to 4.4% with an overall meta-analytical incidence of 1.0% (95%CI: 0.6%-1.4%) with evidence of a high level of heterogeneity ($I^2 = 93\%$, $P < 0.001$; Figure 1).

We performed a sensitivity analysis limited only to the studies that provided data on time of kidney stone diagnosis after kidney transplantation; the estimated incidence of kidney stones was 0.9% (95%CI: 0.7%-1.2%), and there was evidence of a high level

of heterogeneity ($I^2 = 60\%$, $P < 0.001$; Figure 2). The mean duration to diagnosis of kidney stones after kidney transplantation was 28 ± 22 mo.

Subgroup analyses by geographic information were also performed. The estimated incidences of kidney stones were 0.9% (95%CI: 0.3%-1.7%; $I^2 = 94\%$) and 0.7% (95%CI: 0.5%-0.9%; $I^2 = 40\%$) in the United States and Europe, respectively. Data on the incidence of kidney stones in kidney transplant recipients in other geographical area were limited as shown in Table 1.

Characteristics of kidney transplant recipients with kidney stones

The mean age of patients with kidney stones was 42 ± 7

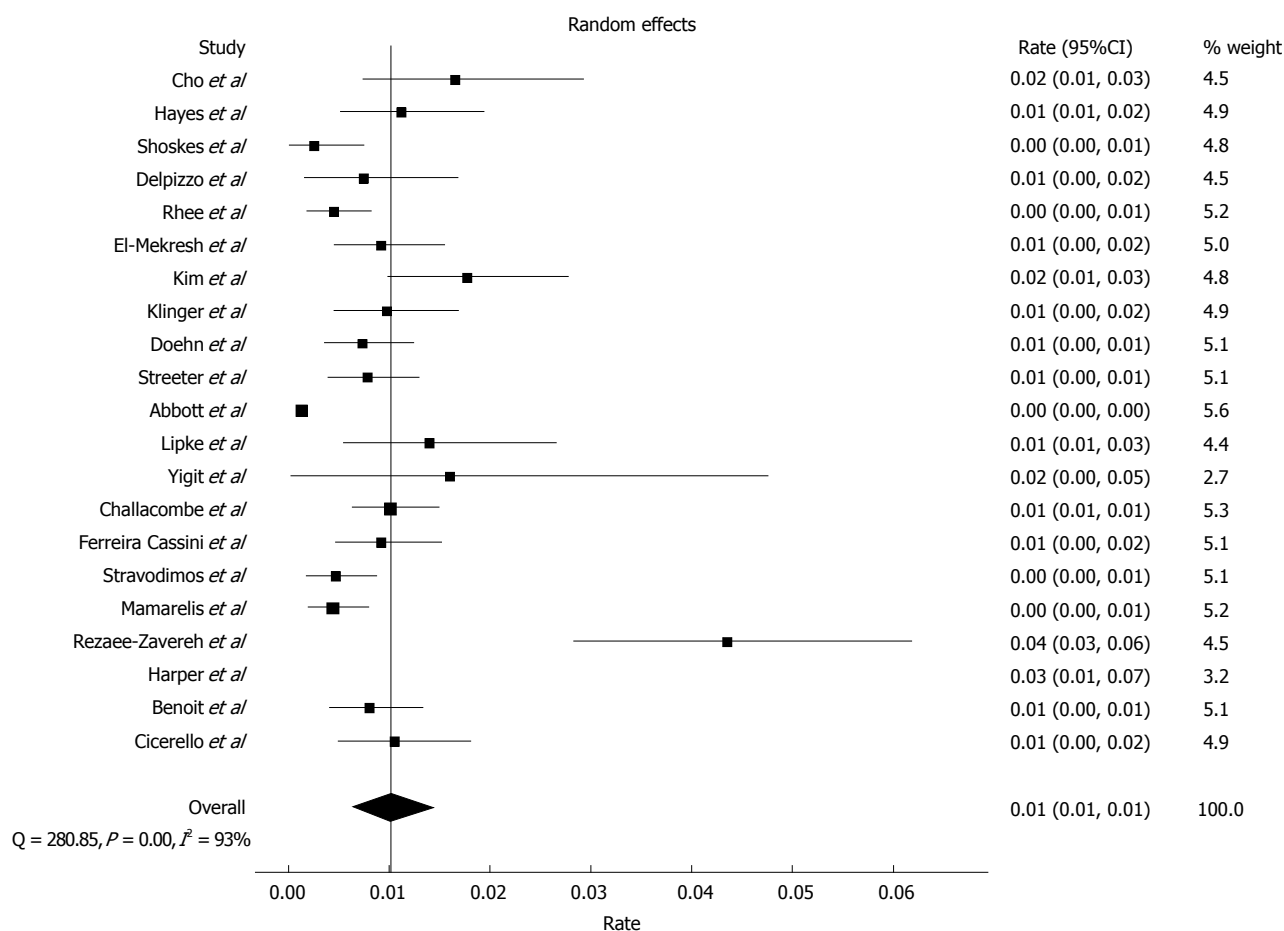


Figure 1 Forest plot of incidence of kidney stones in kidney transplant populations.

years. Within reported studies (Table 1), approximately 50% of kidney transplant recipients with kidney stones were males.

Types of kidney stones in kidney transplant recipients

Sixty-seven percent of kidney stones were calcium-based stones (30% mixed CaOx/CaP, 27%CaOx and 10%CaP), followed by struvite stones (20%) and uric acid stones (13%) as shown in Table 1.

Risk factors for kidney stones in kidney transplant recipients

Despite limited data on urinary supersaturation and risk factors for kidney stones, studies reported increased risk of kidney stones in kidney transplant recipients with hyperparathyroidism, hypercalciuria, hypocitraturia, hypophosphatemia, and urinary tract infection^[28,38]. Harper *et al*^[38] found that urinary excretion of magnesium and phosphate was at the lower range for all kidney transplant recipients with kidney stones. Uncommonly, urinary outflow obstruction and foreign bodies were also found as risk factors for kidney stones in kidney transplant patients^[28,48].

Allograft failure in kidney transplant recipients with kidney stones

As in general patient populations, kidney stones can

also cause acute kidney injury in kidney transplant recipients^[49-52]. Since kidney transplant recipients can have obstructed kidney stones without any symptom of pain^[26,28], prompt diagnosis and the removal of obstructed stones are the keys to preventing renal allograft failure^[18]. Rezaee-Zavareh *et al*^[29] reported no significant association between kidney stones after transplantation and graft survival (OR = 1.04; CI: 0.71-1.54). With the prompt removal of stones, Kim *et al*^[18] found no significant changes in renal allograft function at diagnosis and after removal of kidney stones.

Evaluation for publication bias

Funnel plot evaluating publication bias for the incidence of kidney stones in kidney transplant recipients demonstrated slight asymmetry of the graph and thus suggested the presence of publication for positive studies regarding the incidence of kidney stones.

DISCUSSION

In this study, we demonstrated that an overall incidence of kidney stones in kidney transplant recipients was 1.0%. The mean age of recipients with kidney stones was 42, and half of stone formers were males. Calcium based (CaOx and CaP) stones were the most common types of kidney stones after kidney transplantation,

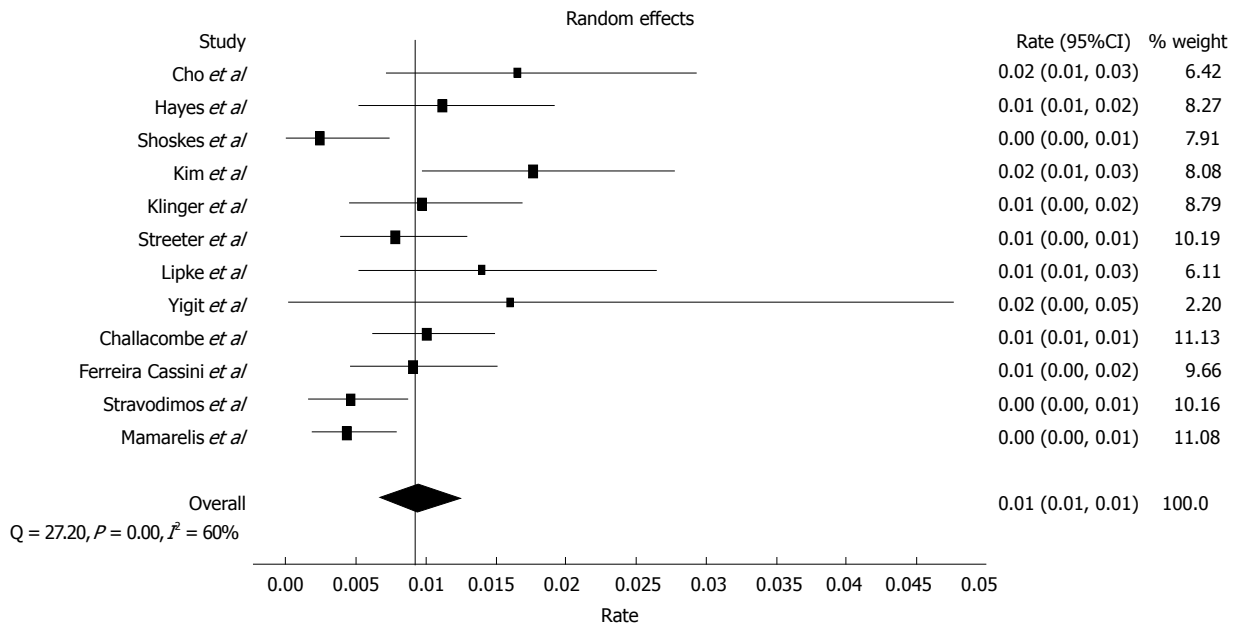


Figure 2 Forest plot of incidence of kidney stones in kidney transplant populations limited only to the studies that provided data on the time of kidney stone diagnosis after kidney transplantation.

followed by struvite stones and then uric acid stones.

The incidence of kidney stones after kidney transplantation from our meta-analysis is much lower than reported in the general adult populations^[5-8]. Although the mechanisms behind the lower incidence of kidney stones after kidney transplantation, when compared with the general population, are only speculative, there are several plausible explanations. First, with the observation that new kidney stones are usually formed in transplanted allograft kidney but not in native non-functioning kidneys, kidney transplant recipients have significantly improved but still lower GFRs than those in healthy general populations, which may be "protective" for stone disease^[9,10]. Second, transplanted kidneys are from healthy donors ideally without tubulointerstitial defects, one not uncommon cause of kidney stones. Third, it is possible that kidney stones after kidney transplantation are underdiagnosed since recipients may spontaneously pass them from the transplanted kidney/ureter without pain or awareness.

Calcium based (CaOx and CaP) stones are the most common types of kidney stones in the general population as well as after kidney transplantation as demonstrated in our meta-analysis. Interestingly, struvite stones (ammonium magnesium phosphate) or "infection stones" is more common in kidney transplant recipients (20%) than in the general population (10%-15%)^[53]. Since struvite stones are associated with infection with urea-splitting bacteria and the principles of treating struvite stones are different than other stones types, including removal of all stone fragments and use of antibiotics^[53], this information is important for future studies targeting prevention and management of kidney stones after kidney transplantation.

There are several limitations to our study. First, there were statistical heterogeneities in the analysis of the incidence of kidney stones. The potential sources of this heterogeneity included differences in diagnostic methodology of kidney stones and follow-up duration. However, a sensitivity analysis that limited studies to those that only provided data on time of kidney stone diagnosis still showed a similar incidence rate of kidney stones, consistent with the finding of our primary analysis. Second, most included studies were conducted in developed Western countries with the majority of the subjects being Caucasian. Thus, our findings may not represent renal transplant populations from other parts of the world. Lastly, the data on urinary supersaturation and risk factors for kidney stones were limited. Although struvite stones represent an association with urinary tract infection, it is still unclear the risk factors for other stone types after kidney transplantation, and future studies are needed.

Our meta-analysis demonstrates that the estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Although calcium based stones are the most common kidney stones after transplantation, struvite stones are the second common type. These findings may impact clinical prevention and management of kidney stones in kidney transplant recipients.

COMMENTS

Background

Renal stones are one of the most prevalent metabolic disorders and urological problems. However, with reduced kidney functions, patients with advanced chronic kidney disease (CKD) or end-stage kidney disease (ESRD) may encounter less stone disease. After successful kidney transplantation, ESRD patients have significant improvement in kidney functions and may develop

kidney stones in their allograft kidney.

Research frontiers

The incidence and characteristics of kidney stones in kidney transplant recipients are not well studied. It is thus necessary to assess the incidence and types of kidney stones after kidney transplantation.

Innovations and breakthroughs

In this study, the authors demonstrated that an overall incidence of kidney stones in kidney transplant recipients was 1.0%. The mean age of recipients with kidney stones was 42, and half of stone formers were males. Calcium based (CaOx and CaP) stones were the most common types of kidney stones after kidney transplantation, followed by struvite stones and then uric acid stones.

Applications

The data in this study demonstrates an estimated incidence of kidney stones in patients after kidney transplantation of 1.0%. Calcium based stones and struvite stones are common types of kidney stones after transplantation. These findings may impact the clinical management of kidney stones prevention in kidney transplant recipients.

Terminology

CaOx: Calcium oxalate; CaP: Calcium phosphate; CKD: Chronic kidney disease; GFR: Glomerular filtration rate; NR: Not reported.

Peer-review

This is a reasonable first meta-analysis of incidence of kidney stones in kidney transplant recipients. The results have potential clinical applications.

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