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CASE REPORT

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REVIEW

Old game, new players: Linking classical theories to new trends in transplant immunology

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Abstract

The evolutionary emergence of an efficient immune system has a fundamental role in our survival against pathogenic attacks. Nevertheless, this same protective mechanism may also establish a negative consequence in the setting of disorders such as autoimmunity and transplant rejection. In light of the latter, although research has long uncovered main concepts of allogeneic recognition, immune rejection is still the main obstacle to long-term graft survival. Therefore, in order to define effective therapies that prolong graft viability, it is essential that we understand the underlying mediators and mechanisms that participate in transplant rejection. This multifaceted process is characterized by diverse cellular and humoral participants with innate and adaptive functions that can determine the type of rejection or promote graft acceptance. Although a number of mediators of graft recognition have been described in traditional immunology, recent studies indicate that defining rigid roles for certain immune cells and factors may be more complicated than originally conceived. Current research has also targeted specific cells and drugs that regulate immune activation and induce tolerance. This review will give a broad view of the most recent understanding of the allogeneic inflammatory/tolerogenic response and current insights into cellular and drug therapies that modulate immune activation that may prove to be useful in the induction of tolerance in the clinical setting.

Key words: Transplant immunology; Immune rejection; Inflammation; Adaptive immunity; Innate immunity; Graft

tolerance

Core tip: Although the basic mechanisms of transplant allorecognition have been the object of intense study for the last 80 years, graft rejection is still an important obstacle in clinical practice. This review focuses on the principal concepts of transplant immunology and how they apply to the most recent discoveries in the field. It also reviews current treatments used to prolong graft survival and recent approach trends toward tolerance induction in the translational setting.

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INTRODUCTION

Although the first attempts at organ and tissue transplantation date to many centuries ago, knowledge of the underlying principles that orchestrate the immune response to this surgical procedure only began to be understood in the mid-twentieth century. Initial studies by Medawar and Gibson in the 1940's showed that allogeneic skin rejection resulted from a response of the recipient to the graft[1,2], and years later, further studies demonstrated the characteristics mediated by cells in this response[3,4]. Since then, great advances have surged as further studies determined the role of different components of the immune system, such as antibodies, antigen-presenting cells (APCs) and T lymphocyte subpopulations, in allograft rejection and tolerance. Nevertheless, rejection is still the main barrier to the success of transplantation, and the development of agents that interfere with the alloimmune response and graft rejection has played a crucial role in the success of organ transplantation. This review will discuss the basic mediators that determine graft rejection and focus on the current immunobiology underlying transplantation research in this area.

ALLOANTIGENS

Major histocompatibility complex/human leukocyte antigens and non-human leukocyte antigens

Classically, transplantation is classified into four categories according to the origin of material to be grafted: Autologous, syngeneic, allogeneic or xenogeneic. Autologous transplantation occurs when cells, tissues or organs originate from the same individual, or in other words, a patient's own tissue or organ is transferred. Syngeneic transplantation, in turn, occurs between two syngeneic or genetically identical individuals. A third type, which is the most common in the clinical setting, is allogeneic transplantation, which is performed between individuals of the same species that are genetically different, while xenogeneic transplantation occurs when the donor graft originates from a different species of the recipient.

The immune system has the intrinsic ability to distinguish between self and foreign (non-self) antigens, which allow it to develop a response against foreign organisms in order to destroy them. Specifically, in the context of transplants, this capacity is termed allorecognition and refers to the phenomenon by which the recipient's immune system recognizes and reacts against donor antigens[5-7]. Thus, the transplantation of tissues or cells between genetically different individuals invariably triggers an immune response that may manifest itself as rejection depending on the magnitude of this response[8-10].

The success of solid organ transplants depends fundamentally on the control of the immune response to foreign molecules that differ among the same species, better known as alloantigens. Currently, a variety of relevant antigens have been described in the context of transplantation, including major histocompatibility complex (MHC) molecules, minor histocompatibility antigens (mHAgs), ABO antigens and endothelial/monocytic cell antigens.

In 1950, Snell[11] and Gorer[7] characterized and determined various antigens responsible for rejection not only in allogeneic tumors but also in healthy allogeneic tissue. Because they were the first antigens discovered regarding the rejection process, these were termed the MHC and are currently known to be the main targets of immune recognition of the surface of donor cells.

This group of genes is common among all vertebrates, and it has an important role in the immune system, mainly in determining the biological identity of individuals. In humans, it is termed human leukocyte antigen (HLA), and it is contained in the short arm of chromosome 6, which is a large chromosomal region with more than 200 coding loci. Based on structural and functional differences as well as on tissue distribution, the HLA products have been divided into three classes (Ⅰ, Ⅱ and Ⅲ), with only classes Ⅰ and Ⅱ encoding HLA surface antigens, whereas class Ⅲ encodes the components C2, C4 and factor B of the complement system[12-14]. These antigens are encoded by different genes inherited from both parents, which are expressed in a codominant fashion[15]. In addition to this, HLA surface antigens are extremely polymorphic[14], which contributes to numerous possible combinations and explains the difficulty in finding close compatibility between individuals. These codominant polymorphic genes influence, among other things, how the immune system responds to the graft recipient. Considering the differential immunogenicity of HLA mismatches observed in epidemiological studies[16], there are some acceptable mismatches, in which the recipient immune system could only weakly react to the donor, enabling longer graft survival. A greater impact of

HLA-DR, HLA-A and HLA-B antigens has been observed in renal graft rejection[17], with a much larger effect of DR matching than the others[18,19]. Retrospective analysis of graft survival data also showed that certain HLA mismatch combinations are linked to increased allograft rejection[16,20].

MHC molecules play a critical role in the immune system, which corresponds to the presentation of peptides in a form that allows them to be recognized by T cells. Their highly polymorphic genes encode for cell surface receptors that have a central role in the control of immune recognition of self and non-self as well as subsequent tissue rejection, autoimmunity and immune responses to infectious diseases. Among all genes included in this region, two highly variable groups (MHC class Ⅰ and class Ⅱ) with differences in structure and presentation function are central in allorecognition.

In humans, MHC class Ⅰ molecules have three loci (HLA-A, HLA-B and HLA-C) and their products result in the classical class Ⅰ molecules, which are expressed codominantly on all nucleated cells. Structurally, these molecules are formed by a heavy α chain (domains α1, α2 and α3), which is non-covalently associated with a light chain (β2-microglobulin) encoded by a gene located on chromosome 15[12]. These molecules have a groove formed by domains α1 and α2, to which endogenous peptides with length of 8 to 11 amino acids from the cytosol, intracellular parasites or tumors are attached, allowing their presentation on the cell surface of MHC class Ⅰ-expressing cells, especially to cytotoxic $CD8^+$ T cells[21-23] (Figure 1).

MHC class Ⅱ molecules, which are encoded by three polymorphic genes (HLA-DR, HLA-DQ and HLA-DP), are constitutively expressed only on APCs, such as macrophages, dendritic cells (DCs), B cells and also thymic epithelial cells, although they may also be induced in other cells such as fibroblasts and endothelial cells under specific stimuli[12]. These molecules consist of a non-covalent association of the α and β polypeptide heterodimer chains, which are encoded by genes of the HLA-D region. Moreover, on class Ⅱ molecules, the groove region consists of the α1 and β1 domains, and it is slightly larger than in class Ⅰ molecules, allowing the binding of peptides between 13 and 18 amino acids. These molecules present exogenous peptides (*via* the endosome) on the surface of APCs[24], especially to helper $CD4^+$ T cells[21-23] (Figure 1).

The MHC is the densest region of the human genome, and it is also one of the most variable, contributing to differences among individuals in immune responsiveness. It is well-known that MHC variants confer susceptibility to many chronic inflammatory and autoimmune conditions, including multiple sclerosis, type Ⅰ diabetes and Crohn's disease, as well as infectious diseases such as malaria and HIV[25-27]. Analysis of MHC variants has facilitated the localization of susceptibility loci for autoimmune diseases; however, for most genetic diseases, the specific loci involved remain undefined, and the mechanisms underlying the association of the MHC in autoimmune diseases remains poorly understood.

In 1994, a new group of polymorphic genes located near the HLA-B locus on chromosome 6, termed MHC class Ⅰ chain-related genes (*MIC* genes), was described[28]. Only two members of the *MIC* gene family encode functional proteins, MHC class Ⅰ chain-related protein A (MICA) and B (MICB), which are highly polymorphic[29]. The expression of these genes are induced by stress, encoding cell-surface glycoproteins that do not associate with β-2 microglobulin and are unable to bind peptides for presentation to T cells[30,31], in contrast to MHC class Ⅰ molecules. MIC antigens bind to the NKG2D receptor present on NK cells, γδ and CD8 T lymphocytes[29,30], resulting in a cytotoxic response against cells expressing these MIC genes[32]. Moreover, the expression of the *MIC* gene family in an allograft can generate anti-MIC antibodies, which can lead to cell destruction and progressively to graft failure, as observed in renal allografts[33-35].

Several molecules encoded outside the MHC loci, such as the CD1 family, are structurally and functionally similar to classical MHC molecules and are therefore termed MHC-like molecules. The CD1 family consists of five glycoproteins coding for MHC-like molecules that associate with β2-microglobulin but have a deeper groove that is more hydrophobic than classical MHC molecules; this hydrophobic groove binds to lipid fragments and glycolipid antigens[36,37]. These molecules can present endogenous or exogenous lipid antigens to natural killer T (NKT) cells *via* the CD1d isoform. NKT cells are essential for cornea allograft survival because they are required for the induction of allospecific T regulatory cells[38]. Furthermore, human CD1d has been identified as a transplantation antigen that mediates a transplantation rejection response in a skin graft mouse model[39].

Acute and hyperacute rejection[40-42] may also occur in the absence of detectable HLA antibodies, suggesting that non-HLA molecules also play roles in rejection. One of these are mHAgs[43], which are peptides presented by MHC class Ⅰ and Ⅱ molecules with discrete polymorphisms and considerable allogeneic properties[44]. These antigens were initially characterized to possess a weaker potential to induce rejection in comparison to MHC antigens, although it has been shown that in MHC-compatible transplanted tissues, recognition of mHAgs[43] may also lead to early rejection. This may result from the principle that any polymorphic protein within a species can become a mHAg, thus expanding the possible number of mHAgs between non-identical individuals with compatible MHC. Nevertheless, mHAg-related rejection appears to be restricted to only some immunodominant epitopes[44,45]. Although the molecular basis of this phenomenon is not completely understood[46], these antigens may be encoded by sex chromosomes (the most widely studied are present in the Y chromosome), autosomal chromosomes (with various origins, such as myosin and the *BCL2A1*

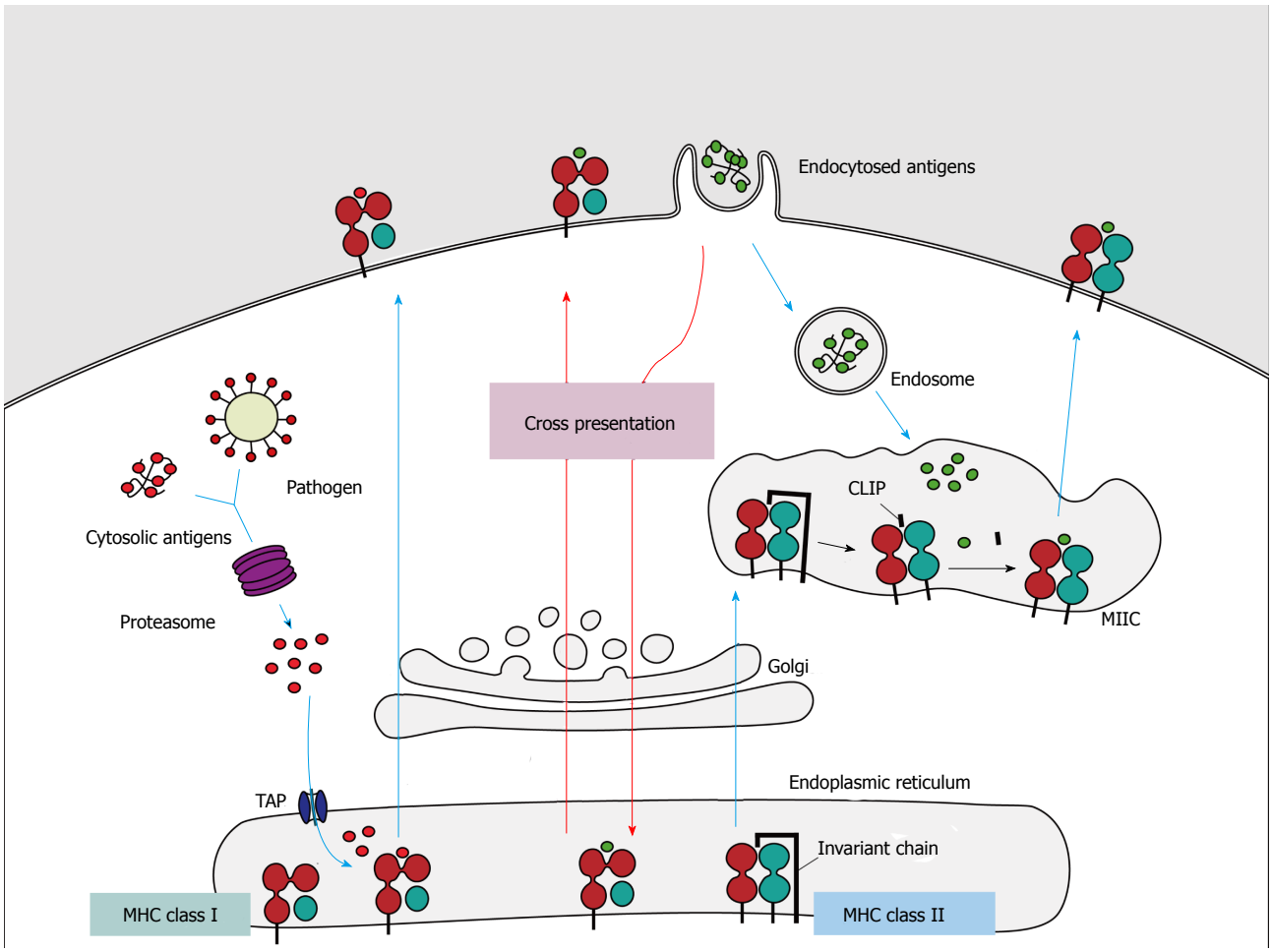

Figure 1 Major histocompatibility complex class Ⅰ and Ⅱ pathways. (1) MHC class Ⅰ molecules present peptides derived from proteins presented in the cytosol of endogenous or pathogen origin. The proteasome breaks down these proteins into peptides, which are then translocated to ER by the transporter associated with antigen processing (TAP) to access the MHC class Ⅰ molecules. In absence of peptides, MHC class Ⅰ molecule is stabilized by ER chaperones (calreticulin, PDIA3, PDI and tapasin), but when peptides with sufficient affinity bind to class Ⅰ molecules, these chaperones are released and the peptide: MHC complex leaves the ER for presentation on cell surface of $CD8^+$ T cells; (2) MHC class Ⅱ molecules present peptides derived from proteins that enter the cell through endocytosis. The chains α and β are assembled in the endoplasmatic reticulum associated with the invariant-chain (Ii) to prevent binding of endogenous proteins. This complex (MHC:Ii) is translocated to MHC class Ⅱ compartment (MIIC) where Ii is degraded to class Ⅱ-associated invariant chain (CLIP). In the MIIC the MHC class Ⅱ molecules acquire HLA-DM to facilitate the exchange of CLIP to specific antigen derived from degraded protein on the endosomal pathway, thus the complexes are transported to the plasma membrane to present the peptide to $CD4^+$ T cells; (3) Cross presentation involves dendritic cells with the unique ability to present exogenous antigens *via* MHC class Ⅰ (by a mechanism not completely understood). MHC: Major histocompatibility complex.

and *LBC* oncogenes), and ultimately, mitochondrial DNA[47-50]. Additionally, immunity against these antigens is a significant clinical problem, as evidenced by the need for immunosuppression, even in the setting of HLA-identical transplantation, and the incidence of graft-*vs*-host disease (GVHD) following HLA-identical stem cell transplantation[51].

In addition, there are many other non-HLA antigenic determinants that are expressed on endothelial cells and monocytes that may also be potential targets in allorecognition[33], and non-HLA antibodies reactive with these cells appear to have a deleterious effect in several transplant models[46,52-54]. Moreover, ABO incompatibility arising from differences between the antigens of the ABO system, in turn, has less relevance in graft survival, but may also result in the hyperacute rejection of vascularized grafts such as kidney and heart grafts[55,56].

ANTIGEN PRESENTATION IN TRANSPLANTATION

Antigen presentation is the primary component linking the innate and adaptive immune systems. It does so by permitting lymphocytes to establish effective immune surveillance of their environment through APCs and consequently mounting strong cellular and humoral responses. Nevertheless, this same process, which is essential for the detection of pathogens and potential tumor cells, is also responsible for the recognition of allogeneic antigens in a transplant setting. Thus, the allospecific immune response is mediated mainly by recipient lymphoid cell adaptive responses, which are orchestrated by T and B cells specific for MHC

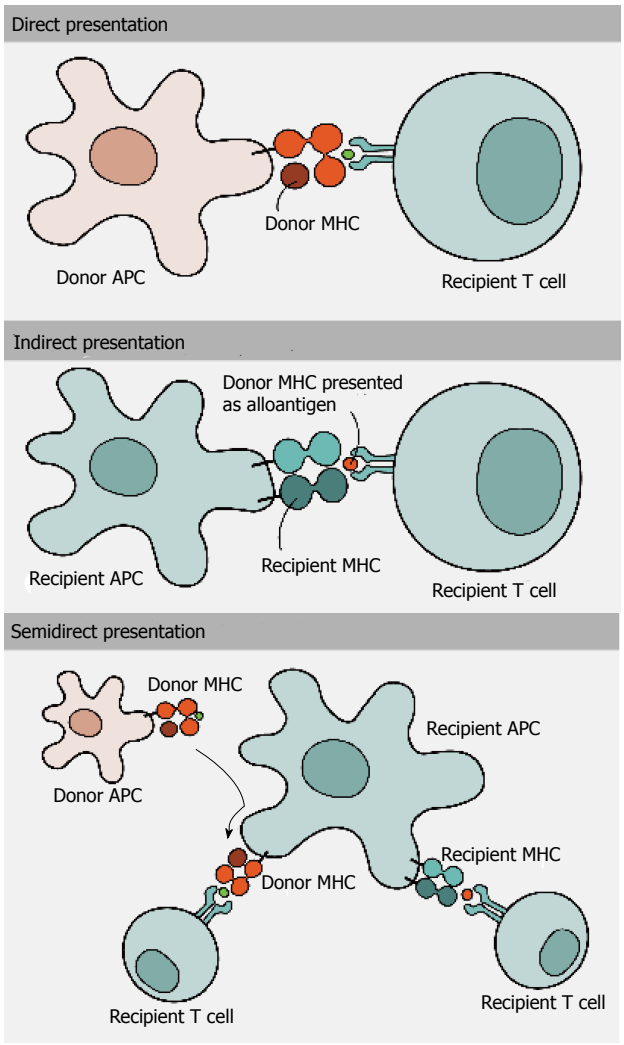

Figure 2 Antigen presentation and allorecognition. T cells can recognize alloantigens by three different pathways of allorecognition: (1) Direct pathway involves the recognition of intact donor MHC molecules on the cell surface of donor APCs by recipient T cells; (2) In contrast, in indirect pathway donor MHC molecules are processed and presented as peptides by recipient MHC molecules to recipient T cells; and (3) Semi-direct pathway, in turn, involves the transfer of intact donor MHC molecules to recipient APCs, being presented to recipient T cells. MHC: Major histocompatibility complex; APCs: Antigen-presenting cells.

alloantigens expressed by the donor.

To achieve appropriate naïve T cell activation responses, a series of sequential signals are required consisting of: (1) T cell receptor (TCR) recognition; (2) costimulatory molecule signaling; and (3) cytokine activation. Each T lymphocyte has a unique and highly specific TCR on its surface that binds to the peptide-MHC complex on APCs, allowing their recognition as self or non-self. In the context of transplant rejection, this occurs as T cells specific for MHC antigens recognize foreign MHC-peptide complexes, which elicit a highly efficient response. Indeed, it is estimated that the frequency of alloreactive precursor T cells may be up to one thousand times greater than that of common antigens, demonstrating the efficiency of allogeneic immune responses[57]. If a lymphocyte recognizes the complex as non-self, it then becomes activated and begins to proliferate, adopting effector and memory functions that contribute to the response against the graft, which are detailed further in later sections.

B cells also play a major role in adaptive responses by producing antibodies directed against the graft. In this case, antigen presentation occurs when B-cell antigen receptors (BCRs), which consist of cell-surface immunoglobulins, recognize antigens either directly or through MHC presentation. Importantly, in the first setting, direct recognition induces antigen internalization and consequent MHC class Ⅱ-peptide presentation to T cells, which in turn, along with co-stimulatory activation, drives B cell differentiation into antibody-producing plasma cells and memory B cells[58-60].

Allogeneic MHC molecules may be presented for recognition by TCRs *via* four fundamentally different, though not exclusive, pathways and thus may be involved in mediating allograft rejection simultaneously or in different contexts[51] (Figures 1 and 2). With direct presentation, recipient alloreactive T cells are directly activated after the recognition of allogeneic/non-self intact MHC class Ⅰ and Ⅱ molecules on the surface of donor APCs[5,61-63]. The presence of APCs in transplanted donor tissue dictates a strong anti-donor response early after engraftment, which decreases over time due the eventual death and removal of these donor APCs[64]. Indirect presentation, on the other hand, involves the capture and processing of allogeneic MHC class Ⅰ and Ⅱ donor molecules by recipient APCs[65,66], generating small peptides that are later presented by MHC class Ⅱ molecules. This presentation results in alloresponses led by $CD4^+$ T cells[67,68] and corresponds to slower responses than those generated *via* the direct route. The lower frequency of T cells with indirect allospecificity (compared to direct) in the normal repertoire suggests that the direct response dominates the early post-transplant period, while the indirect response develops a role in long-term alloantigen presentation, when donor APCs are already dead[69-71]. Semi-direct presentation, in turn, comprises the interaction between the recipient T cells and APCs, involving the exchange of intact peptide: MHC complexes by direct cell-to-cell contact[72-74] or by the release of small vesicles called exosomes[75,76]. Thus, the recipient APCs are able to present alloantigens directly to recipient T cells, allowing donor MHC and self MHC with donor peptide to be presented on the surface of the same cell. Even so, the precise role of this type of allorecognition in transplant rejection and tolerance remains to be fully elucidated[10].

The fourth type of presentation, cross-presentation, results from the ability of certain APCs to carry peptides that are derived from exogenous antigens on MHC class Ⅰ molecules, an atypical characteristic, as endogenous antigens are commonly expressed on class Ⅰ molecules and exogenous are expressed on class Ⅱ. This type of presentation allows responses to pathogens that do not infect directly or replicate little within the APC[77]; however, this mechanism is not

exclusive of infectious diseases, and the efficient priming of $CD8^+$ T cells can occur after allogeneic transplantation as a consequence of cross-presentation of proteins derived from the donor by the recipient DCs[78].

ALLOGENEIC REJECTION: THE CLASSICAL VIEW

Rejection can be divided into three main types: Hyperacute, acute or chronic, according to the cells and mechanisms involved in tissue damage and the consequent time course of graft loss.

Hyperacute rejection occurs due to the presence of preexisting antibodies towards graft antigens, caused by previous sensitization, which occurs in blood transfusions, organ transplant or even pregnancies. This recognition usually happens as soon as the organ is perfused, and widespread vascular injury associated with thrombosis prevents blood flow, leading to tissue necrosis and consequent graft loss within minutes to hours after the transplant. Nevertheless, this type of rejection is rarely observed in modern medicine due to pre-transplant CDC crossmatch exams that preemptively detect receptor reactivity to donor antigens.

Acute and chronic rejection are more difficult to prevent and less predictable. Acute rejection happens in the first weeks after transplant and is mainly associated with direct antigen presentation pathways, which activate $CD4^+$ T lymphocytes to produce cytokines that amplify inflammation, and $CD8^+$ T lymphocytes, which differentiate into cytotoxic cells upon activation and mediate direct graft cell destruction. These, in turn, also promote monocyte activation at graft sites, which also mediates the balance between tissue damage and repair[79-81].

Moreover, as donor APCs disappear with time, chronic rejection is mainly driven by indirect antigen presentation, where graft antigens are presented by recipient APCs[82,83]. In parallel, various studies also indicate that initial ischemia/reperfusion injury plays an important part in chronic graft rejection, and with time, together these factors ultimately culminate in a particular type of immune activation that causes progressive arterial damage and tissue fibrosis[84,85].

All these types of rejection simply establish a didactic form of characterizing the complex and often concomitant forms of graft rejection. The following portion of the review will approach the main cells mediating the sensitization and effector phases of graft rejection, focusing on the most recent data in literature.

ALLOGENEIC REJECTION: AN UPDATED VIEW

Innate immunity in graft rejection

Since the beginning of transplant immunology, scientists have always focused on the adaptive mechanisms responsible for graft rejection and immunological memory, and until recently, little emphasis has been placed on the role of innate cells in allogeneic transplantation. Nonetheless, more recent research has noted that innate immune cells have a crucial role in triggering initial signals in transplant rejection and play an active role in establishing tolerance in transplantation (Figure 3).

The first immunological trigger to unfold during transplantation is almost always of innate origin due to the inevitable physical and ischemia-reperfusion (I/R) injury to solid organs during transplantation in addition to common conditioning regimens, such as chemotherapy, before bone marrow transplantation (BMT). This is particularly important, as it is responsible for the initial activation of innate cells and maturation of APCs to efficiently present antigens to T cells. These signals are expressed as damage-associated molecular patterns (DAMPS), such as heat shock proteins, heparin sulfate and reactive oxygen species (ROS), and activate pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), leading to innate cell activation. These cells, in turn, secrete cytokines and chemokines such as TNF-α and IL-6, which give way to a cascade of events that amplify inflammation and attract further immune cell infiltration. Moreover, some reports have even suggested that innate cells may be able to distinguish allogeneic antigens, putting into question the lasting paradigms that divide the innate and adaptive responses[86,87]. This idea is defended by reports showing differential, memory-like recognition of alloantigens in $RAG^{-/-}$ mice. Some of these reports suggest that NK cells may participate in this phenomenon, showing that these cells develop a stronger IFN-γ response to a secondary stimulus[88]. NK cell-independent recall responses have also been shown in these mice, suggesting that other innate immune cells may also play a bigger role in adaptive immunity than first imagined. Nevertheless, recent research has also suggested that this recognition alone is insufficient to initiate alloimmunity, indicating that effective rejection can take place even in the absence of an innate response[89,90].

As cited previously, lymphocyte activation depends not only on an appropriate peptide presentation to antigen-specific T lymphocytes but also on the presence of efficient co-stimulatory signals. Therefore, there are two main signals needed for T cell activation: A first signal, involving antigen-specific MHC-peptide complex interaction to TCR molecules present in T cells, and a second signal, which consists of antigen-non-specific co-stimulation receptors on APCs and T cells that in turn drive intracellular activation signals with IL-2 production, T cell differentiation and survival. The basic literature usually describes main APC co-receptors such as B7 (CD80 and CD86), which interact with CD28 on T cells. However, a diverse number of other co-receptors are also known to have positive and negative effects on T cell activation (Figure 4), acting simultaneously at the immune synapse to effect cell activation or inhibition. The majority of known receptors belong to the immunoglobulin superfamily (IgSF) or the tumor necrosis factor receptor superfamily (TNFRSF), including OX40, CD40 and 4-1BB. Without the appropriate stimuli, T cells become anergic or enter apoptosis, and thus, these molecules are important targets

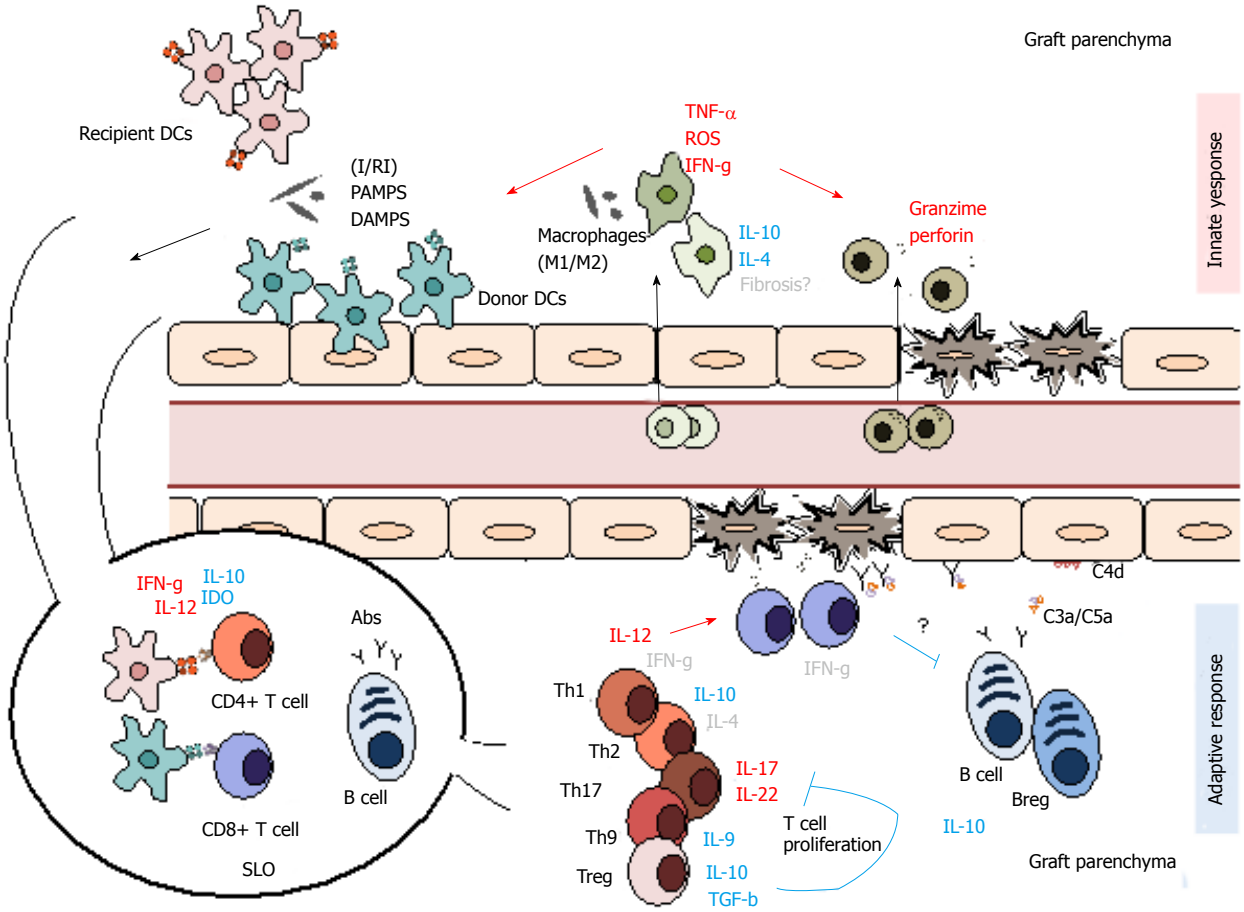

Figure 3 Summary of the main innate and adaptive mediators of graft rejection. Alloimmune rejection is a multifaceted process that involves both innate and adaptive mediators. Initial tissue damage is mostly mediated by innate participants as macrophages and NK cells along with dendritic cells, which link the both innate and adaptive responses. With time, these gradually give way to more adaptive mediators as T and B lymphocytes and antibody production. I/RI: Isquemia/reperfusion injury; PAMPs: Pathogen-associated molecular patterns; DAMPS: Danger-associated molecular patterns; IDO: Indoleamine 2,3-dioxygenase; Abs: Antibodies; DC: Dendritic cells; SLO: Secondary lymphoid organ; ROS: Reactive oxygen species; TNF: Tumor necrosis factor. Mediator roles are represented in red (pro-inflammatory), blue (regulatory) and grey (indetermined).

for immunosuppression and cancer therapy, which will be detailed further on. Moreover, many different cells, such as DCs, macrophages and even B-lymphocytes, serve as APCs, as they all express both MHC and co-stimulatory molecules. These cells are considered professional APCs, and each have important roles in different contexts of graft allorecognition. It is also important to highlight that non-APCs also regulate lymphocyte activation, as is the case for apoptotic cells that express phosphatidylserine[91-94].

Macrophages

Macrophages are also important mediators of graft rejection, playing a part in antigen presentation and tissue inflammation and damage. These cells have been suggested as predictors of graft failure and are considered by some researchers to be even more reliable predictors than T cell infiltrates[95,96]. Macrophages originate from circulating monocytes, which infiltrate the graft due to multiple chemotactic factors and receptors, such as monocyte chemoattractant protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF)[97-100], and CX3C chemokine receptor 1 (CX3CR1). Some of these molecules have also been linked to kidney graft infiltration[101,102], differentiating into active mature cells that promote tissue injury. Accordingly, some studies even suggest a central role for CD68 monocytes in allograft dysfunction[103]. Studies assessing the preoperative Campath-1H (Alemtuzumab) treatment of renal recipients demonstrate the effects of monocytes in mediating acute rejection. Because Campath-1H depletes more T lymphocytes that monocytes, this study showed that CD68 monocytes were a dominant population in acute rejection[79,104].

In addition, mature monocytes are especially responsive to I/R injury and are activated soon after DAMP and PAMP stimuli, thereby secreting a range of cytokines that further activate other innate immune cells and also promote lymphocyte activation[105]. Macrophages are also prominent producers of ROS and eicosanoids that induce tissue damage and amplify the inflammatory cascade after tissue engraftment[106]. There are numerous subtypes of macrophages, ranging from inflammatory M1 cells, which produce increased amounts of TNF-α and IFN-γ, to more tolerogenic M2 macrophages, which secrete

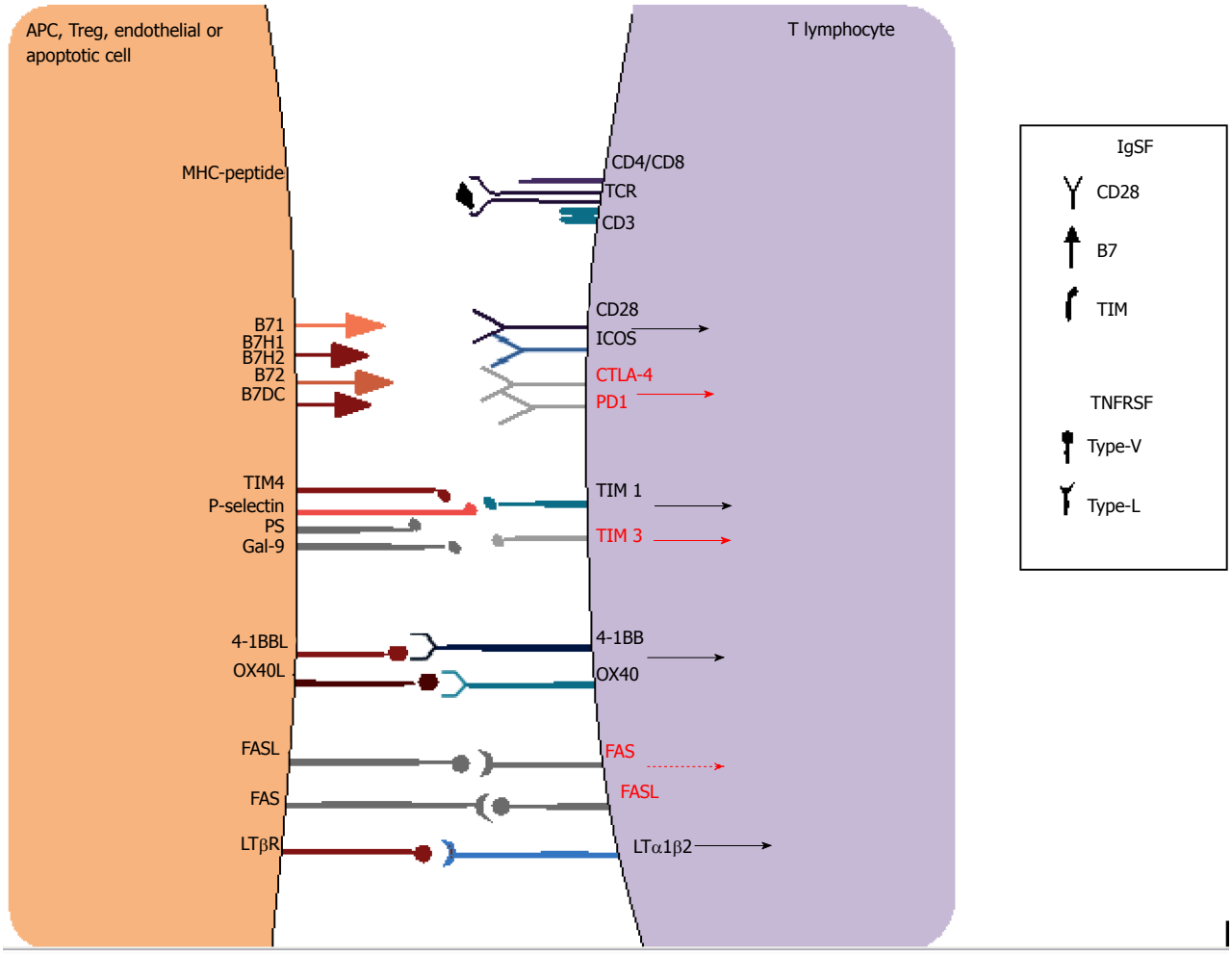

Figure 4 Examples of co-stimulatory receptors in immune synapse interactions. T cell activation depends not only on the antigen-specific signals provided by MHC-TCR signaling but also on a complex balance of co-stimulatory signals that may enhance or inhibit activation. These receptors are mainly classified into the immunoglobulin superfamily (IgSF) or the tumor necrosis factor receptor superfamily (TNFRSF) and may provide positive (black arrows) or negative signals (red arrows), or even apoptotic signals (red dashed arrow), which in turn decide cell fate. MHC: Major histocompatibility complex; TCR: T cell receptor.

cytokines such as IL-4 and IL-10 and are associated with wound-healing and regulatory properties[107]. One study has indicated that the transfer of human regulatory macrophages ($CD14^{-/low}HLA\text{-}DR^{+}CD80^{-/low}CD86^{+}CD16^{-}CD64^{+}TLR2^{-}$ and $CD163^{-/low}$) induces protection after renal transplant[108]. However, some studies have also associated M2 macrophages with increased allograft fibrosis[109-111]. However, this might depend on the time course of cell activation and the type of macrophage present, as DAMP, PAMP and dead cell clearance also reduces cell stimulation and innate immune activation.

NK cells

Classically, NK cells are lymphocytes that respond to signals provided by tumor cells or virally infected cells. However, other stress-related signals can activate NK cells[112] through an unbalanced positive signal *via* membrane receptors. Although these cells share many characteristics with classical lymphoid cells, their activation takes shape through antigen-independent signals and does not produce immunological memory, falling therefore into the category of innate immunity. These cells recognize activating and inhibitory cell surface receptors that indicate cell stress, such as TLRs, class Ⅰ MHC binding inhibitor receptors (*e.g.*, Ly49), MHC class Ⅰ -related binding activating receptors (*e.g.*, NKG2D) and Fc receptors (*e.g.*, CD16)[113,114]. In addition, NK cells are also activated by cytokines, such as IL-2, IL-15, IL-12 and IL-18[115]. Moreover, after activation, NK cells go on to perform effector functions such as cytotoxicity (perforin and granzymes) and cytokine production (IFN-γ, TNF-α, IL-22)[116]. Because NK cell class Ⅰ MHC inhibitory receptors are polymorphic and recognize self-MHC, these cells are readily capable of responding to allogeneic graft cells due to the "missing-self" principle, leading researchers to investigate these cells' role in graft rejection, especially after BMT. However, literature pertaining to NK cells in allorecognition is contradictory. Some authors demonstrated that these cells are important mediators in GVL (graft *vs* leukemia) effects, although they may accelerate graft failure due to an attack on donor cells[116,117]. On the other hand, recent research has also indicated that donor cells may evade allorecognition by acquiring host MHC class Ⅰ molecules

through the transfer of surface proteins from receptor cells, therefore inhibiting NK responses[118]. Nonetheless, most articles have shown that NK cells may also facilitate bone marrow engraftment and regulate graft-*vs*-host disease by suppressing donor and host T cells[119-122].

NKT cells

NKT cells are a heterogeneous population of T cells that express TCRs and NK markers and have properties of both T and NK cells. These cells recognize glycolipid antigens presented by CD1d on APCs instead of MHC molecules. They can be divided into two main subtypes depending on the TCR subchain expressed. Invariant or type Ⅰ NKT cells express an invariant TCR β-chain (Vα14-Jα18 - mouse or Vα24-Jα18 - human) that is paired with a semi-invariant TCR β-chain (Vb11 - humans or Vβ2, Vβ7 or Vβ8.2 - mice), while type Ⅱ NKT cells include all other CD1d-dependent T cells[123], with a very small frequency in the peripheral blood. After TCR activation, these cells can modulate the immune system by producing significant amount of Th1, Th2 and Th17 profile cytokines[124-126] and by increasing the expression of co-stimulatory molecules[127].

Recent studies indicate that these cells have tolerogenic effects and are crucial for the induction of peripheral tolerance. NKT cells induced transplantation tolerance towards allogeneic and xenogeneic islet cells transplanted into the liver and towards cardiac allografts[128-130]. The presence of these cells suppresses GvHD and solid organ rejection, which seems to be mediated by the production of IL-4 and IL-10 and by Treg activation[131-133].

DCs

DCs are the most prominent APCs involved in antigen presentation, mainly due to their particular ability to capture, process and express peptides *via* the MHC and their ability to migrate to T cell zones in lymph nodes, expressing high levels of co-stimulatory molecules along with peptides to T lymphocytes. These cells comprise an expressively diverse population that, after differentiating from the common DC precursor (CDP) or monocytes, when activated by danger signals as described above, transition from an immature state (iDCs) with low costimulatory receptor and MHC expression to a mature state (mDC), expressing high levels of costimulatory and MHC molecules.

DCs are classified into various subsets depending on their origin and the way they are activated, with the main types being plasmacytoid DCs (pDCs), conventional or classical DCs (cDCs) and inflammatory monocyte-derived dendritic cells (moDCs). The first population produces significant amounts of Type Ⅰ and Ⅲ IFN and diverse chemokines including CXCL1, CXCL3 and others[134,135]. However, they are considered poor APCs and are considered important in the induction of tolerance to grafts, which will be detailed further on.

In contrast, cDCs are efficient APCs that, when mature, produce various cytokines, such as IFN-γ, IL-12 and IL-10, which can direct T-cell activation towards an immunogenic or tolerogenic profile. Research suggests that cDCs are the main APCs responsible for alloantigen presentation during GvHD early after BMT[136]. cDCs are divided into $CD8^+$ or $CD8^-$ cells, and there are many different reports on their effects on graft rejection. CD8+ DCs are only expressed in mice (not in humans), but some reports suggest that they have a regulatory role in BMT and solid organ transplantation, where they suppress the activation of other inflammatory DCs by producing indoleamine 2,3-dioxygenase (IDO) and increase Treg numbers and Treg production of IL-10[137-140].

Finally, moDCs possess strong inflammatory properties, differing from cDCs in that they originate from a monocyte precursor and express Gr-1/Ly6C. Although there are almost no *in vivo* data on the role of this specific population against other cells in graft rejection, some studies indicate that these cells have intense antigen-presenting functions, maybe even more than cDCs[86,87,141,142]. Other studies have also shown that these cells can effectively activate NK cells[143], which are discussed later. Future research shall elucidate the role of these cells in a transplantation setting.

ADAPTIVE IMMUNITY

The adaptive immune system has been recognized to have a critical response to organ transplantation. The rejection process is characterized by a highly complex series of cellular and humoral interactions in which T and B lymphocytes as well as DCs exhibit central and essential roles. Nevertheless, the immune response underlying allograft rejection is an ongoing dialogue between the innate and adaptive immune system, whereby innate immune cells modulate and direct the development of adaptive responses through pattern recognition receptor signaling (Figure 3).

T cells

To reduce transplant rejection, the biggest challenge faced is overcoming or suppressing adaptive immunity. T cells have a central role in adaptive effector responses due to their cytokine production and cytotoxic functions. After $CD4^+$ T cell activation, the cells differentiate into subtypes, mainly including Th1, Th2, Treg, Th17 and Th9 cells, according to their signature cytokine production. Nevertheless, although these are some of the most studied mediators in transplantation, little consensus exists on their effects on graft rejection, with most of these cell types displaying dual roles in immune activation in transplantation.

In immunology, Th1 cells are considered classic pro-inflammatory actors. These cells are characterized by the expression of the T-bet transcription factor, along with the secretion of IFN-γ, TNF-α/β and IL-2, which in turn stimulate macrophages and lymphocytes towards enhanced effector functions associated with intracellular immunity. Specifically, IL-2 is essential to promoting T cell proliferation, while IFN-γ expression increases

$CD8^+$ T cell activation[144]. Many studies correlate IFN-γ expression to kidney graft rejection[145,146]. However, there are also data that showing that IFN-γ may prolong survival by reducing tissue necrosis and local granzyme-perforin secretion[147,148]. This has also been described in GvHD, whereas it prevented early onset of rejection[149], although this effect may depend on conditioning regimens[150]. In addition, IFN-γ expression by Tregs may also be important in reducing GvHD[151].

In contrast to Th1 cells, Th2 cells are traditionally considered immunomodulatory cells associated with extracellular immunity. They express the Gata-3 transcription factor and secrete IL-4, IL-5, IL-10 and IL-13. However, in a transplantation context, some studies demonstrate that Th2 cells have limited immunomodulatory properties[152-154]. Most recent data suggest that Th2 responses may have a negative role in transplant rejection[155]. In addition, some reports also suggest that IL-4 production by Th2 cells may accelerate cardiac and kidney rejection[156,157].

Th17 cells have also an important role in graft rejection. These cells express the transcription factor RORγT and are characterized by IL-17 and IL-22 production. Studies show that the absence of Th17 cells leads to prolonged renal graft survival with reduced IFN-γ and enhanced Treg function[158]. In addition, IL-17/IL-22 levels correlate with acute liver, kidney, islet and lung rejection in addition to GvHD[159-164]. However, the exact role of Th17 cells in transplant rejection may be more complex, as some studies have suggested that Th17 cells are more important for chronic rejection[165].

Finally, there are little data on the role of the recently discovered Th9 cells, which express increased levels of IL-9, in allograft rejection. Two articles suggest that $CD4^+$ T cells that were co-stimulated and polarized with TGF-β and IL-4 in the presence or absence of rapamycin yielded effector cells of the Th9 phenotype that secreted increased IL-9 and expressed a transcription factor profile characteristic of both Th9 and Th2 cells (high GATA-3/low T-bet). Another transcription factor that promotes Th9 is PU.1. Its epigenetic modifications are important for Th9 immunity regulation[166]. These cells may have regulatory functions similar to Th2 cells by reducing IFN-γ alloreactivity and $CD4^+$ and $CD8^+$ T cell engraftment in BMT but also by inhibiting GVHD while increasing GVL[167,168].

Cytotoxic T cells

$CD8^+$ T cells have an important role in cell-mediated transplant rejection, with distinct cytotoxic effector functions, and were able to be activated even in the absence of $CD4^+$ T cells[169], promoting cellular damage through the secretion of granules containing perforin, granzyme and granulysin. While perforin polymerizes, forming transmembrane pores on target cells, granzymes consist of a class of proteases that cleave substrates in the cytoplasm of target cells, triggering rapid apoptosis. Moreover, granulysin also mediates cell death, inducing ionic unbalance and mitochondria-mediated cell apoptosis in addition to facilitating intracellular bacterial killing[170]. In addition, $CD8^+$ T cells can also express FasL, which binds to Fas receptors on target cells, causing caspase activation and consequently also leading to cell apoptosis. It has also been reported that APO2L/TRAIL constitute an additional pathway of T cell-mediated cytotoxicity[171,172], inducing apoptosis in a FasL- and perforin-independent manner.

In practice, there is no consensus on the specific importance of these cells in the context of allogeneic activation. Although $CD8^+$ T cells may not be essential for some types of allograft rejection[173], others correlate their presence with graft cytotoxicity[174,175]. Recent data have shown that these cells may also inhibit alloantibody production by promoting alloprimed IgG1 (+), resulting in B cell death through FasL- and perforin-mediated apoptosis[176]. Moreover, these cells can also secrete a range of cytokines and are divided into two subclasses, Tc1 or Tc2. Type 1 $CD8^+$ T cells (Tc1) cells mainly secrete IFN-γ, which was recently shown to promote hematopoiesis *via* increased myeloid differentiation in order to reinforce target cell clearance[177], and on the other hand also reduce IL-4-dependent IgG1 alloantibody production. In parallel, Tc2 cells mainly secrete IL-4 and IL-5 and have been shown to reduce GvHD[178-180].

Memory T cells

Memory T cells represent a major challenge in the context of transplantation. Although they have an important role in defense against pathogens, especially in immunocompromised patients, they are also important in transplant rejection. These are very heterogeneous cells, both functionally and phenotypically, expressing different surface markers and residing in lymphoid and non-lymphoid tissues, such as the lung and liver[181,182]. Memory T cells are different from naive T cells because they are long-lasting cells, are antigen-independent persistent, and are capable of self-renewal[183]. Furthermore, they are able to be activated more easily than naïve T cells because they are less dependent on TCR stimulation and on co-stimulatory molecules[184]. These cells can be $CD4^+$ or $CD8^+$ cells, with the $CD8^+$ subtype much more frequent and commonly studied[185]. They are dependent on sensitization, are linked to adaptive immune responses, and are responsible for the recall response[183]. These cells are also derived in an IL-7 dependent manner from effector T cells resistant to apoptosis[186,187]. Memory T cells also have greater and faster responsiveness to antigens than naive T cells because they are derived from effector T cells[188] and are more effective in the immediate response against antigens[189,190].

These cells are also expressly involved in transplant rejection[191-194]. Analysis in patients showed that higher frequencies of memory T cells pre-transplantation are related to higher post-transplantation complications[195,196]. Treatment with immunosuppressive drugs that reduce alloreactive T cells also favors the generation of memory

T cells because this generate homeostatic proliferation without antigen stimulation[197], which causes naïve T cells to be converted into effector memory T cells[198,199]. Memory T cells are also involved in heterologous immunity, a process whereby cells activated by pathogens cross-react against alloantigens[200,201].

Memory T cells are also involved in tolerance resistance, mainly because they are highly reactive to donor antigens[191,202,203]. These cells have the ability to break Treg-induced suppression[193,204], constituting a barrier to treatments that aim to induce tolerance in transplantation. To circumvent this, studies have demonstrated that the depletion of memory T cells along with mixed chimerism through BMT after renal transplantation successfully induced a state of delayed tolerance[205].

A recent study has demonstrated that the level of CD38 on $CD8^+$ memory T cells in the peripheral blood can predict the occurrence of GVHD[206]. Thus, the observation of T cell memory and its frequency in recipients may permit the establishment of a relative risk assessment of rejection mediated by these cells, or conversely, the possibility of establishing tolerance and the reduced probability of rejection.

B cells

B lymphoid cells are one of the main players in transplant rejection, and along with their antibody-producing properties, they also play an important part in allogeneic responses as APCs and cytokine producers. During B cell ontogeny, these cells go through different maturation stages, starting at the immature B cell stage and roaming to the spleen to complete their maturation. There, the majority of B cells become mature follicular B cells, which circulate between secondary lymphoid organs until they are activated, or marginal zone B cells, which continue in the spleen. Some articles have reported that B cells increase acute GvHD by accentuating T cell activation[207,208]. Chronic GvHD has also been linked to B cell responses *via* a positive correlation with high levels of autoantibodies[209,210]. Likewise, sex-mismatched BMT has also been associated with H-Y antibodies derived from donor B cells[211]. In addition, B cells also promote T cell activation as a result of antigen presentation and are able to induce graft rejection, even in an antibody-independent manner[212]. However, extensive literature has indicated that B cells may also have important tolerogenic properties in a transplantation setting, mainly *via* the suppression of T cells and DCs through cytokine production, which will be discussed in detail later in the review.

Antibody-mediated rejection and complement

Antibodies are one of the most important mediators in transplant rejection and play a key role in both acute and chronic rejection. They are produced by transient plasmablasts and long-lived memory plasma B cells resident in secondary lymphoid organs and bone marrow. After transplantation, patients may display pre-existing or *de novo* donor-specific antibodies (DSAs) that target both HLA and non-HLA molecules. Data suggest that 20% of transplant patients will develop DSA within the first 5 years, and there are substantial data showing that these are responsible for accelerating graft rejection[213,214]. In summary, antigen recognition by antibodies results in the formation of antigen-antibody complexes, which recruit inflammatory cells through Fc receptor recognition and activate the classical pathway of complement activation. This, in turn, leads to the formation of active soluble byproducts that activate inflammatory cells and also leads to the formation of the membrane attack complex (MAC), leading to pore formation and consequent allogeneic cell death. Many studies have demonstrated the important role of complement activation in graft rejection, and many of its byproducts correlate with graft rejection. Both CD3a and C5a have been shown to induce APC and T cell activation, with increased expression of IL-6, costimulatory molecules and MHC Ⅱ along with reduced $FOXP3^+$ Treg formation[215-217]. In addition, C1q has also been shown to activate DCs, increasing TNF-α production and leading to a Th1 response[218]. Due to the vast formation of byproducts of complement activation, many researchers have also aimed to use these as biomarkers of antibody-mediated rejection. Among these, C4d, which is a product of C4d breakdown and easily localizes to endothelial cells and the basement membrane, has been shown to be of great value[219], although C4d-negative antibody-mediated rejection also exists.

IMMUNOSUPRESSIVE DRUGS AND TOLERANCE

Immunosuppression

The use of immunosuppressive drugs is essential in cases of solid organ transplantation because it can avoid the immune response against the graft or delay the appearance of *de novo* baseline disease. Thus, the most frequently used drugs act on pathways that inhibit the proliferation and activation of T cells, the main mechanisms involved in rejection[220]. Commonly, these drugs are used in combination, which can vary according to the patient, the type of transplant and also with the transplant center.

Azathioprine is the oldest immunosuppressive drug to be used in the prevention of rejection, and it was used with the first successful deceased kidney transplantation in 1962[221]. Although currently, it has not been commonly used in transplants, it is still an important treatment for autoimmune and inflammatory diseases[222-224].

Calcineurin inhibitors (CNIs), such as cyclosporin A and tacrolimus, are the most commonly used treatments. Cyclosporin A emerged as an alternative to azathioprine and triggered an important advance in medical transplants[225,226]. Tacrolimus has been the first choice of treatment in most transplant centers in Europe and the United States[223]. These drugs inhibit the calcineurin

pathway, avoiding the dephosphorylation of NFAT (nuclear factor of activated T lymphocytes) and its translocation to the nucleus, ultimately blocking the activation of genes involved in T cell activation and, consequently, the propagation of the immune response[226,227]. However, the use of these drugs may induce nephrotoxicity[228,229] and can cause diabetes, dyslipidemia, hypertension, cardiovascular and kidney disease[230,231].

Everolimus and Sirolimus belong to another class of immunosuppressive drugs widely used in kidney transplantation in combination with other drugs. They inhibit mTOR (mammalian target of rapamycin), a kinase protein involved in the activation and proliferation of lymphocytes and tumor growth, among other functions[232], that is also related to the expansion of Treg cells[233,234].

Mycophenolate mofetil has been increasingly used as an initial immunosuppressive drug in recent years[222]. After it is metabolized, it generates mycophenolic acid, which inhibits inosine-5-monophosphate dehydrogenase (IMPDH), an important enzyme involved in purine synthesis. By inhibiting this enzyme, the drug can reduce T and B cell proliferation, in addition to decreasing the recruitment of lymphocytes to sites of inflammation and inducing necrosis in activated lymphocytes[235].

A more recent therapeutic option is Belatacept, a fusion receptor protein that blocks the CD80/CD86-CD28 co-stimulatory pathway, selectively inhibiting T cell activation[236]. Clinical studies have demonstrated that continuous treatment with Belatacept was associated with a consistent improvement in renal function post-transplantation[237-239].

Other treatment alternatives have also been tested. Studies have shown that the use of anti-CD40 can be effective[240] at preventing acute renal transplant rejection[241]. Clinical trials with a JAK3 (Janus kinase) inhibitor, Tofacitinib, in kidney transplantation showed low rates of rejection and a high graft survival, similar to cyclosporin, which was used as a control[242,243]. Phase Ⅱ studies with Sotrastaurin have also been carried out. This molecule selectively inhibits protein kinase C, blocking T cell activation, although contradictory results regarding its efficacy in preventing rejection have been obtained[244,245].

Moreover, in some, cases, pre-treatment using monoclonal antibodies, such as Alemtuzumab, or polyclonal antibodies, such as anti-thymocyte globulin, can be used as induction therapy at the time of transplantation. This treatment depletes peripheral blood leukocytes, inducing lymphopenia[190], and can stimulate Treg cells[246,247] and regulatory B cells[248], enabling a reduction in the use of other immunosuppressive drugs.

Another class of drugs, proteasome inhibitors, can act directly on T and DC cells. The proteasome is essential for the maintenance and regulation of basic cellular processes, including cell signaling and survival pathways. The inhibition of proteasomal proteolytic activity by proteasome inhibitors suppresses essential immune functions. They can inhibit the activation of nuclear factor (NF)-κB and the transcriptional regulation of pro-inflammatory cytokine release and/or induce the apoptosis of activated immune cells. They can affect T cell activation, function, proliferation, and viability and suppress DC maturation and inhibit DC function. For this reason, they have already been tested in diverse autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis[249-252].

Tolerance

The use of immunosuppressive drugs has been the main option for transplant patients and has provided improvements in graft survival rates. However, many of these drugs present medical complications such as infections, nephrotoxicity, cardiovascular problems and cancer[228,253,254]. Furthermore, the treatment is not able to prevent chronic rejection, and the rates of chronic allograft dysfunction are still very high[255,256]. Additionally, the prolonged use and the high cost of immunosuppressors can lead to non-adherence to treatment[257]. Therefore, alternative therapies are needed, and the induction of tolerance would be an ideal substitute for the use of immunosuppressive drugs[258].

Immunological tolerance is an important mechanism to prevent anti-self immune responses and autoimmune diseases. In central tolerance, which occurs in the fetus thymus before T cell maturation, cells that react against self-antigens are deleted and regulatory T cells are expanded. On the other hand, peripheral tolerance, a secondary process of immunological tolerance, occurs in peripheral lymphoid organs, where there is induction of anergy and deletion of T cells that self-react against antigens that did not exist in the thymus or somehow escaped central deletion[259].

In the context of transplantation, true tolerance is by definition a permanent state of acceptance of alloantigens without the use of immunosuppressive drugs[260], given that in experimental models, animals must retain the ability to reject a third donor organ[261].

The induction of chimeras, or mixed chimerism, is a situation in which donor cells and recipient cells co-exist in the immune system[262], and it is an important technique to induce immunological tolerance. In chimera induction, hematopoietic cells from the donor are transferred to the recipient, and the recipient cells are retained, being only partially replaced by the donor[260]. In parallel, host bone marrow and donor thymus cells cause the central deletion of donor alloreactive T and B cells[260,263], allowing a new concept of what is self.

In experimental models, this method induces donor-specific tolerance and enables prolonged graft acceptance[264]. In humans, Alexander *et al*[265] demonstrated in a patient who received liver transplantation that the induction of mixed chimerism promoted tolerance and prevented GVHD occurrence. This method has already been used in transplants with good results[266] and is an important way to induce tolerance and prevent rejection or GVHD, allowing the long-term withdrawal of immunosuppressive drugs[267-269].

Currently, the existence of cells capable of regulating the immune response, leading to a more tolerogenic and less inflammatory profile, and restoring the balance of the immune system is well established. In the transplantation context, these cells are responsible for the balance between the survival and rejection of the graft[270]. Regulatory cells of the immune system, such as Tregs[271], tolerogenic DCs[272], and Bregs[273,274], have been detected in recipients that have developed operational tolerance. Therefore, the direct use of these cells, or of elements that stimulate these cells, may be important tools for tolerance induction because they are able to prevent or minimize the use of immunosuppressive drugs and their adverse effects[270,275-278].

Regulatory T cells

Regulatory T cells play an important role in regulating the immune response and are responsible for the balance between the inhibition of autoimmunity (acting in tolerance against self antigens) and preventing tissue damage (acting on innate and adaptive response against non-self antigens)[259]. Two major subtypes of Tregs have been described. Naturally occurring Tregs are generated in the thymus from T-cell precursors expressing CD4, CD25 and the transcription factor Foxp3 and play an important role in maintaining tolerance to self-antigens or other antigens present in the thymus[279,280]. Moreover, induced or adaptive Tregs (iTregs), which are induced in the periphery in various tissues[281,282], express CD4 and Foxp3 and are responsible for the response against antigens not found in the thymus[283]. Thus, both subtypes may be responsible for the recognition of donor alloantigens and for the immune tolerogenic response against them[284].

Treg cells act through different mechanisms that can direct or indirectly inhibit T cell activation and proliferation. These cells can transmit inhibitory signals *via* cell-cell contact or secrete regulatory cytokines such as TGF-β, IL-10 or IL-35. In addition, they can also limit the availability of trophic factors, such as IL-2, to effector T cells, generate direct toxicity against target cells, or modulate APC functions. Moreover, these cells also act on other immune cells, such as B cells, NK, NKT and mast cells[259,279,283,285].

The induction of operational tolerance to transplantation is strongly associated with Tregs[270,283]. Therefore, the use of these cells has been tested in several ways. The use of these cells as a conditioning therapy before transplantation was able to induce tolerance[286], as was the use of Tregs for the generation of mixed chimerism, where donor Tregs were essential for the suppression of immune response[287,288]. The use of drugs or cytokines that induce Tregs *in vivo* also improve graft survival[289-291] along with donor alloantigen inoculation pre-transplantation[292], which promotes the expansion and proliferation of Tregs *in vivo*. Direct inoculation of Tregs or inoculation after *ex vivo* expansion was also effective in reducing rejection[293-295] and in the prevention of GVHD[296,297]. In humans, clinical trials have also shown that the infusion of Tregs is able to reduce GVHD[298,299]. Importantly, the immunosuppression generated is not global, as the injected Tregs retained the ability to respond to infections[296,298], which was an important advantage in comparison to immunosuppressive drugs.

DCs

As described previously, DCs are APCs that participate in T cell activation and are crucial for the activation of the immune response, including the response against alloantigens. When they become mature, they express some co-stimulatory surface markers, such as CD80, CD86, CD40, and MHC Ⅱ[300]. Immature DCs have decreased expression of MHC Ⅱ, CD86 and CD40, generating a more tolerogenic profile. Tolerogenic DCs have reduced production of cytokines such as IL-6 and IL-12 and increased IL-10 secretion[301]. Thus, they are capable of inducing clonal deletion, inhibiting memory T cells and inducing or expanding Tregs[277,302].

New therapies based on the transfer of tolerogenic DCs have been tested, especially for autoimmune diseases[278]. Blockade of DC-T cell interactions *via* co-stimulatory receptors and T cell surface molecules impairs T cell proliferation, preventing an exacerbated immune response[303]. Additionally, immature DCs are also able to promote tolerance in animal models of solid organ and BMT. Treatment with donor immature DCs[304-306] or regulatory DCs[307] in transplantation also prolongs graft survival and the development of GVHD. Moreover, DCs can also be conditioned to become tolerogenic through the use of cytokines, growth factors and drugs[308], and the use of TGF-β[309], and rapamycin[310], for example, were observed to prolong graft survival.

Regulatory B cells

The role of B cells has always been related to the activation of the immune response and transplant rejection, especially through the production of antibodies. However, some B cell subtypes with regulatory functions are also observed to produce regulatory cytokines[311]. Many regulatory B cell subtypes (Bregs) have already been described, including the transitional cell (T1B and T2B), the marginal zone (MZ) B cell, the transitional 2 marginal zone precursor B cell (T2-MZP)[312] and a rarer $CD1d^{hi}CD5^{+}$ subtype, known as the B10 cell, that has received the most attention[313,314]. MZ B lymphocytes have been shown to produce high levels of IL-10 after the anti-CD40-mediated induction of tolerance[314]. In addition, B10 cells are found mainly in the spleen and also exert their actions exclusively *via* the production of IL-10, which regulates T-cell activation and inflammatory responses[315]. In an EAE model, Matsushita *et al*[316] demonstrated that regulatory B cells (B10) exert their function by altering IFN-γ and TNF-α secretion and suppressing T cell proliferation and acting on DCs, downregulating their antigen-presenting ability. Furthermore, another study has also demonstrated that Breg cells play an important role in

the induction of Treg cells, maintaining high Treg levels in comparison to Th1 and Th17 cells[317].

B cells are strongly related to operational tolerance. Studies involving transplant patients show an increased percentage of B cells in the blood of tolerant patients compared to patients treated with immunosuppressive drugs or those who have suffered rejection[274,318,319]. B-cell-related genes are also differentially expressed in tolerant patients[273,319]. In addition, when evaluated *in vitro*, B cells from tolerant patients produced a higher amount of IL10 compared to those from non-tolerant patients[273]. Another study also showed that B cells from patients with chronic rejection do not inhibit autologous T cell proliferation, whereas B cells from healthy patients do[320], confirming the involvement of Breg cells in the tolerance induction process.

Thus, research in recent years has also aimed towards the use of B cells as a cellular therapy to induce tolerance. To this end, Breg cells were shown to induce chimerism and tolerance to donor antigens[321]. Likewise, studies in transplantation models indicated that Breg inoculation is effective towards prolonged graft acceptance[322,323] and the suppression of T cell activation[324], promoting the development of Treg cells, possibly *via* TGF-β production[325].

Mesenchymal stromal cells

Mesenchymal stromal cells have known immunosuppressive properties and are capable of inhibiting T cell function and proliferation, inducing T cell apoptosis and inducing regulatory T cells[326]. The use of MSCs in solid organ transplantation has had important results. MSCs attenuate ischemia-reperfusion injury[327] and prevent graft rejection[328,329]. These cells are able to inhibit the T cell response[330,331] and inhibit the migration of activated T cells into the graft[332,333] in addition to expanding Treg cells[334-336] and tolerogenic DCs[337-339], generating a state of tolerance[326].

Based on evidence in experimental models that MSCs favor the development of tolerance and have demonstrated efficacy and safety, some clinical trials are in development[340]. The infusion of these cells was able to maintain stable graft function *via* Treg expansion and the reduction of memory T cells[341] and decrease the incidence of acute rejection[342].

Fetal tolerance

Finally, the induction of tolerance is also essential to the fetus, which must tolerate maternal antigens, preventing an immune response against the mother. The immune environment of the developing fetus is specially prepared to generate immune tolerance, especially to non-inherited maternal antigens (NIMAs), protein products derived from polymorphic genes expressed by the mother. Fetal $CD4^+$ T cells have a strong predisposition to differentiate into Tregs after activation by maternal antigens, which actively promotes tolerance to maternal cells residing in fetal tissues[343]. Afterwards, shortly before birth, the fetal cells transition to a more defensive adult-type response, with the ability to combat pathogens[344]. Maternal cells also play an important role in fetal protection during pregnancy. Maternal Treg cells are involved in this process, as they are enriched in the decidua and return to normal levels after birth[345], which does not occur in cases of miscarriage[346,347].

The establishment of microchimerism is the primary factor responsible for the generation of Tregs because fetal cells also have access to the mother. This chimerism occurs both in the maternal tissues and in the fetal tissues, and maternal cells are often found in fetal tissues[343,348], remaining for a long period after birth[349]. Even after development, the ability to generate tolerance to antigens that have been in contact with the fetus is not lost, consisting in a postnatal tolerance. This fact was confirmed in a study by Burlingham *et al*[350], who showed that patients who received HLA-haploidentical sibling renal transplantation of which the mismatch corresponded to a NIMA had a significant increase in graft survival compared to those in which the mismatch was a non-inherited paternal antigen (NIPA), suggesting a relationship with the exposure to antigens during the fetal period. Other studies using a heart transplantation model also demonstrated that allografts expressing NIMAs were protected from rejection when implanted in offspring mice that had come into contact with the same NIMAs during pregnancy, therefore creating a predisposition to transplantation tolerance in mice as an adult[351], mainly through the induction of NIMA-specific Treg cells[352].

CONCLUSION

Although the basic mechanisms of transplant allorecognition have been the object of intense study for the last 80 years, graft rejection is still an important obstacle in clinical practice. Allorecognition is an unfortunate disadvantage to the evolution of more effective immunological surveillance and is therefore especially complex to surpass. Nonetheless, current advances have shed light on important mediators that fuel graft rejection, making the search for new therapies possible. In addition, promising discoveries have been made in the search for effective immunosuppressive regimens and, more importantly, the achievement of functional tolerance.

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MINIREVIEWS

Influence of tacrolimus metabolism rate on renal function after solid organ transplantation

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Abstract

The calcineurin inhibitor (CNI) tacrolimus (TAC) is an integral part of the immunosuppressive regimen after solid organ transplantation. Although TAC is very effective in prevention of acute rejection episodes, its highly variable pharmacokinetic and narrow therapeutic window require frequent monitoring of drug levels and dose adjustments. TAC can cause CNI nephrotoxicity even at low blood trough levels (4-6 ng/mL). Thus, other factors besides the TAC trough level might contribute to CNI-related kidney injury. Unfortunately, TAC pharmacokinetic is determined by a whole bunch of parameters. However, for daily clinical routine a simple application strategy is needed. To address this problem, we and others have evaluated a simple calculation method in which the TAC blood trough concentration (C) is divided by the daily dose (D). Fast TAC metabolism (C/D ratio < 1.05) was identified as a potential risk factor for an inferior kidney function after transplantation. In this regard, we recently showed a strong association between fast TAC metabolism and CNI nephrotoxicity as well as BKV infection. Therefore, the TAC C/D ratio may assist transplant clinicians in a simple way to individualize the immunosuppressive regimen.

Key words: Tacrolimus; Liver; Metabolism; Transplantation; Kidney

Core tip: The calcineurin inhibitor tacrolimus (TAC) is the mainstay of the immunosuppressive regimen after solid organ transplantation. Nevertheless, TAC can cause nephrotoxicity even at low blood trough levels. Thus, other factors than the TAC trough level might be responsible for kidney injury. Recently published studies showed a strong association between fast TAC metabolism and nephrotoxicity as well as BK virus infection. The TAC

metabolism rate defined as the TAC concentration/dose ratio is a cost neutral tool to identify patients at risk for TAC-associated decline in renal function after transplantation.

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INTRODUCTION

The calcineurin inhibitor (CNI) tacrolimus (TAC) is a cornerstone of the immunosuppressive regimen after solid organ transplantation. Nevertheless, its highly variable pharmacokinetics and narrow therapeutic window require frequent therapeutic drug monitoring (TDM) and the (nephrotoxic) side effects of TAC might limit its application[1]. In particular dose adjustment after TAC prescription is difficult as many patients often show troughs above or below their target range despite TDM. In order to overcome these limitations, new TAC formulations with different galenics have been developed and different protocols with TAC dose reduction, switch, elimination and combination of reduced TAC and mechanistic target of rapamycin (mTOR) inhibitors have been studied[2-5]. *E.g.*, the recent ATHENA trial evaluates a *de novo* everolimus (EVR)-based regimen in combination with reduced cyclosporine A (CSA) or TAC *vs* a standard regimen in patients that underwent renal transplantion (RTx)[6]. Results of this trial are expected soon.

After RTx, low dosed TAC regimens showed superiority regarding the prevention of biopsy-proven acute rejection (BPAR) and preserving the kidney function compared to the CNI CSA and the mTOR inhibitor sirolimus (SRL)[7,8]. Consistently, the present KDIGO guideline recommends TAC-based immunosuppression after RTx[9].

TAC has also become a first choice immunosuppressive drug after liver transplantation (LTx)[10]. Compared to CSA, TAC-treated patients - though experiencing a higher rate of posttransplant diabetes mellitus - showed a significantly reduced mortality at 1- and 3-years post-transplant; rates of graft loss and (steroid-resistant) rejection were lower in these patients[11,12]. In order to avoid CNI nephrotoxicity in LTx patients, several studies have been conducted to evaluate treatment strategies in which standard dosed TAC was either replaced by low dose TAC and mTOR inhibitor or CNI were even completely eliminated from the regimen. In a study with 78 LTx patients renal function recovered slightly after conversion from TAC to an mTOR inhibitor-based regimen[13]. Immunosuppression was switched 31 mo (median) after LTx. Additionally, Fischer *et al*[14] showed in a prospective, multicenter, open-label study with *de novo* LTx patients that patients who were randomized to regimen with reduced TAC dose and EVR 30 d after LTx developed lower rates of BPAR and had an improved renal function from randomization to month 36 compared to patients with standard TAC doses. Of note, randomization to the TAC elimination arm in this study was stopped prematurely due to significant higher BPAR rates[15].

In pancreas, heart, lung, or combined organ transplantation, TAC also constitutes an integral part of the immunosuppressive regimen[16-20]. CNI-sparing or -free regimens in these patients are currently investigated but safety of these concepts is still under debate. Notably, none of these CNI-free regimens has yet been shown to provide an immunosuppressive efficacy that equals those of CNIs[21-23].

After pancreas transplantation TAC and mycophenolate mofetil (MMF) maintenance therapy seems to be the most effective immunosuppressive regimen with regard to long term survival and prevention of acute rejection[16,24]. However, occurrence of TAC-related side effects like posttransplant diabetes mellitus or nephrotoxicity has led to increasing efforts to minimize CNI in this cohort. *E.g.*, in one study pancreas transplanted patients were switched from standard immunosuppression with TAC and MMF to low dose TAC and SRL[25]. From the authors view, the low dose TAC and SRL regimen was safe and did not worsen proteinuria and renal function when compared with TAC and MMF. In simultaneous pancreas and kidney transplantation Sageshima *et al*[17] evaluated the efficacy and safety of TAC and EVR compared to TAC and MMF in a retrospective study. Unfortunately, both studies failed to show relevant advantages of the combined TAC/mTOR regimen.

The introduction of EVR in the maintenance therapy of heart transplant recipients, with reduced CNI, has been shown to significantly improve the renal function during an observational period of at least 5 years[18]. An early renal benefit in lung transplant recipients was lost over the time but long-term immunosuppressive efficacy was maintained[18].

Despite all efforts to minimize TAC exposure and its side effects even in low dose regimens (4-6 ng/mL)[26], TAC, however, remains the mainstay of the immunosuppressive regimen after solid organ transplantation[2,14]. Therefore, transplant physicians need an approach to identify patients at risk to develop TAC-related side effects in clinical routine. We and others proposed that the patient's individual TAC metabolism type can be used for adaption of the immunosuppressive regimen. We believe that the TAC metabolism rate defined as the TAC blood trough concentration (C) divided by the daily dose (D) is such a convenient predictor for TAC metabolism estimation. Perspectively, C/D tests could probably detect patients at high risk of developing TAC-related complications even before their transplantation.

Due to missing data on the TAC metabolism rate and its value in recipients of other organ transplants than kidney and liver, we herein focus on the impact of the C/D ratio in the latter.

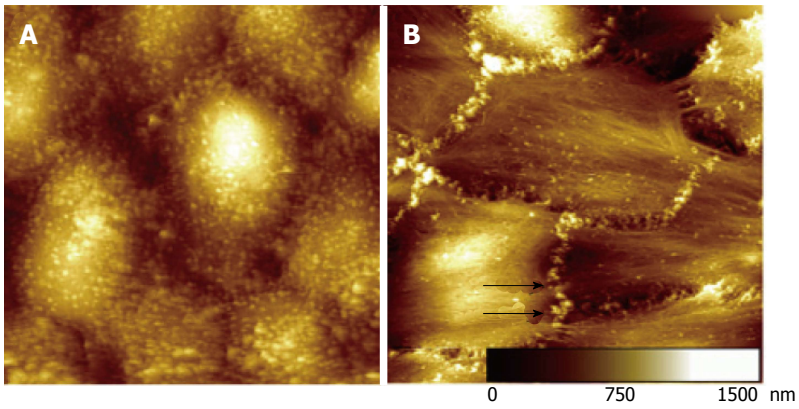

Figure 1 Morphological changes of cells undergoing epithelial to mesenchymal transformation. Images from atomic force microscopy of glutardialdehyde fixed cells in fluid (highest sample areas are represented in white). A: Typically, epithelial tubular cells (NRK-52E), (50 μm)^2, appear with numerous microvillus compatible structures on the cellular surface; B: Tubular cells after six days of TGF-β1 treatment (50 μm)^2. Cells show a fibrillary surface structure with rarefied microvilli. Nodular protrusions developed at the cell borders (black arrows)[35]. ©IOP Publishing. Reproduced with permission. All rights reserved. TGF: Transforming growth factor.

HISTOLOGICAL MANIFESTATION OF CNI-NEPHROTOXICITY

TAC has a narrow therapeutic window and can cause acute and chronic nephrotoxicity. However, some authors even question the concept of a "harmless" therapeutic window. They state that yet to be effective, CNI must operate within their nephrotoxic therapeutic range as can be seen when CNI withdrawal leads to an immediate increase in estimated glomerular filtration rate (eGFR)[1]. Activation of the renin-angiotensin system and increased sympathetic nerve activity causing vasospasm of renal arteries might be involved in this context[27,28]. An imbalance of vasodilatory factors like nitric oxide[29,30] and prostaglandins[30] and vasoconstrictive variables like thromboxane[31] and endothelin[32] is discussed to promote further renal damage.

Acute CNI nephrotoxicity typically appears early after RTx correlating to the period of high CNI exposure. It presents, *e.g.*, with acute arteriolopathy, tubular vacuolization and swelling of endothelial cells and death of myocytes of the tunica media[1]. The prevalence of CNI-associated chronic lesions increases by time[1].

Tubule-interstitial fibrosis/tubular atrophy (IF/TA) is a typical histological finding in chronic CNI-related allograft injury. Tubular microcalcifications, glomerulosclerosis and arteriolar hyalinosis are further chronic manifestations. In contrast to some acute CNI-related kidney injuries which can be resolved within the first months after RTx, chronic CNI-nephrotoxicity observed after the third month after RTx is usually progressive[1].

For example, TAC exposure induces epithelial-mesenchymal transition (EMT) by activation of the profibrotic cytokine transforming growth factor-β1 (TGF-β1) pathway in renal tubular cells[33]. TGF-β1 in turn induces cell growth, increases the production of smooth-muscle actin and stress fiber formation in epithelial cells[33,34]. This results in a decrease of cellular surface microvilli and increases stiffness of tubular epithelial cells[35]. During this conversion process, tubular cells loose epithelial characteristics and appear in a mesenchymal shape (Figure 1[35]) (EMT). However, these effects seem to be cell specific. While some cells have the ability to proliferate, others are decomposed by autophagy[33,36].

Early withdrawal or dose reduction of TAC/CNI and introduction of an mTOR inhibitor might stabilize fibrosis[37]. However, the adequate time point for TAC/CNI withdrawal or dose reduction is still elusive and the group of patients who might benefit from this intervention remains yet to be clearly identified[2,3,13,14,38].

INFLUENCES ON TACROLIMUS METABOLISM

TAC metabolism underlies several individual, genetic and clinical, as well as pharmacokinetic factors. Recipient age, gender, body mass index, delayed graft function, hematocrit, serum albumin and absorption have been proposed to be relevant determinants[39-42]. However, some of these factors are still a matter of debate.

Drug interactions interfering with TAC metabolism are of high clinical relevance for physicians. Changes of the TAC pharmacokinetic by other immunosuppressive drugs, such as EVR, SRL and corticosteroids have to be considered in daily routine. Especially, induction of TAC metabolism by high doses of corticosteroids has to be taken into account early after transplantation[40,41,43]. Whether these interactions are of clinical relevance or not remains largely unknown[44].

TAC metabolism is influenced by cytochrome-P450 enzymes *CYP3A* expression variants, *e.g.*, in the intestine[45,46]. This genetic expression variant determines the first-pass effect of orally administered TAC. This is important, because Sato *et al*[47] showed that recipients taking their usual dose of TAC in case of diarrhea had significant elevated trough levels and a prolongation of maximum concentrations when compared to the regular situation. It is supposed, that this phenomenon is caused by a shift of the main intestinal areas of TAC metabolism (duodenum and jejunum) to the lower intestine[47,48].

The *CYP3A4* and *CYP3A5* variants in the liver lead to significant differences in TAC pharmacokinetics[39,41,49]. Predominantly but not exclusively, *CYP3A5*1*-expressors have been characterized as fast TAC metabolizers, while slow metabolizers mostly express *CYP3A5*3*[45,50-52]. Early after transplantation, it has been shown that a rapid decline in TAC metabolism is only present in *CYP3A5*3/*3* patients while the decline is absent in *CYP3A5*1* allele

carriers[53,54]. This finding might be explained by high steroid doses and a gradual rise in hematocrit that affect *CYP3A5*3/*3* and *CYP3A4* activity. In comparison, CYP 3A5 carriers (*CYP3A5*1*) receive higher TAC doses (fast metabolizers) early after transplantation and continue with a higher or even increased exposure as time after transplantation elapses[41]. In a meta-analysis, Shi *et al*[55] showed that especially *CYP3A4*1B* genetic polymorphism may affect TAC metabolism. If the presence of *CYP3A5* in the kidney, *i.e.*, in the renal apical tubular plasma membrane impacts, *e.g.*, on the degree of CNI nephrotoxicity is still a matter of debate[56,57].

Unfortunately, the dosage needed to achieve the target TAC level varies in patients with known *CYP3A* polymorphisms over time[58]. Therefore, genetic testing does not solve the dosing problem and we still have to rely on trough level testing. To end this, genotyping of patients is still far from being a routine test and at present of questionable relevance in the daily transplant setting.

CLINICAL IMPACT OF TAC METABOLISM RATE

The TAC concentration/dose ratio (C/D ratio) is an established equation to describe the TAC metabolism rate[59-61]: C/D ratio (ng/mL * 1/mg) = [Blood TAC trough concentration (ng/mL)]/[Daily TAC dose (mg)].

We intended to keep the approach very simple and tested if body weight (which was suggested to be included into the equation by others) can be removed from the equation[59,60,62]. Our approach was supported by Kim *et al*[58] who showed that TAC adverse events in a 5-year follow-up of RTx patients were independent from body weight.

The presented equation provides a simple, cost neutral clinical tool which can be applied without performing additional tests. Standard trough levels from regular therapeutic TAC drug monitoring can be used for C/D ratio calculation of in- as well as outpatients.

We analyzed TAC metabolism using the C/D ratio in a study of 248 RTx patients at our center. Analyzing the outcomes and distribution of recipients' C/D ratios in our cohort, we calculated a cut off for the TAC C/D ratio of 1.05 for definition of fast metabolizers. After a 24 mo follow-up, patients with a C/D ratio < 1.05 had a lower eGFR, needed more indication biopsies and showed more often biopsy proven CNI nephrotoxicity compared to intermediate and slow TAC metabolizers[60]. In accordance with our data, Kuypers *et al*[63] showed that *CYP3A5*1* genotype carriers (mainly fast metabolizers) had a significantly increased risk for biopsy-proven TAC-induced nephrotoxicity [HR: 2.38 (1.15-4.92), P = 0.01] at 3 mo post-transplant. These results were confirmed by Genvigir *et al*[64] who also reported that carriage of two or more fast metabolization *CYP3A5* alleles is associated with lower eGFR values ninety days after RTx. Rojas *et al*[65] showed that the weight adjusted C/D ratio in RTx recipients among *CYP3A5*1* allele carriers compared with carriers of the *CYP3A5*3/*3* genotype was lower and demonstrated that the expresser genotype was associated with a higher risk of acute rejection and chronic nephrotoxicity. Nevertheless, further studies on similar and different ethnical cohorts showed partly contradictory results[66-68]. Thus, until now, the prediction of renal function by *CYP3A* genotyping still remains ambiguous.

We confirmed our findings in a cohort of LTx patients. During a 36 mo follow-up renal function was lower in fast TAC metabolizers (defined by C/D ratio) than in slow TAC metabolizers (Figure 2)[59]. In this study, fast metabolizers had more TAC side effects like higher rates of assumed CNI nephrotoxicity and had been more often switched from TAC to other immunosuppressive drugs.

It is well known that higher TAC trough levels are more toxic and increase the risk of side effects[69]. Jacobson *et al* for example calculated a HR of 1.22 for a 1 ng/mL increase of the TAC trough level to develop acute CNI nephrotoxicity after RTx. It is important to note that our RTx patients in the fast metabolizer cohort had lower TAC trough levels at 1, 3 and 6 mo after transplantation compared to slower metabolizers (Table 1[60]). This was confirmed in a cohort of LTx patients[59]. Kuypers *et al*[53] identified nephrotoxicity to be dependent on the TAC dose. In accordance to these results, in our studies fast metabolizers received higher TAC doses than slow metabolizers 1, 3 and 6 mo after transplantation[59,60]. These findings led us to the hypothesis that CNI nephropathy predominantly seen in fast metabolizers might be related to TAC overexposure during the first hours after TAC intake.

This hypothesis is supported by the finding that besides increased rates of CNI nephrotoxicity, a higher incidence of BKV nephropathy (BKN) is observed in fast TAC metabolizers[60]. This was confirmed in a second study involving 192 RTx patients (96 BKV positive and 96 BKV negative controls). Patients with BKV infection showed lower Tac C/D ratios at 1, 3 and 6 mo after RTx and were mainly classified as fast TAC metabolizers[62]. Therefore, fast TAC metabolizers seem to be prone to CNI nephrotoxicity and suffer more likely from BKV infection[70].

SUMMARY

Although TAC is a cornerstone in the immunosuppressive regimen after solid organ transplantation, nephrotoxic site effects and a narrow therapeutic window may limit its application. Elimination, dose reduction, or replacement of TAC is often foiled by increased rates of BPAR[14,38], occurrence of adverse events[8] or considerable rise in the costs caused by replacing immunosuppressive drugs like belatacept[71]. Due to the fact, that CNI nephrotoxicity can also occur in regimens with low TAC target levels[26], a tool to identify patients at risk for developing an inferior kidney function is desirable.

We were able to demonstrate a strong association between a low TAC C/D ratio (< 1.05 ng/mL*1/mg) and

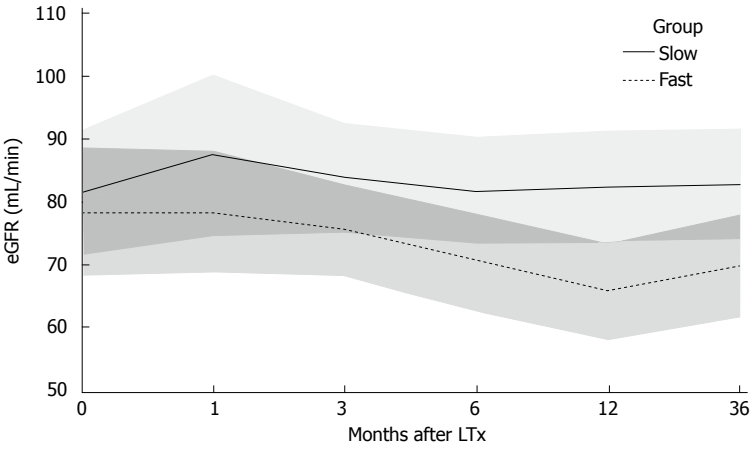

Figure 2 Estimated renal function measured by estimated glomerular filtration rate (Cockcroft-Gault eGFR, mL/min) after liver transplantation. There was no noticeable difference between fast and slow tacrolimus metabolizers at liver transplantation at 1 mo or 3 mo after liver transplantation. After 6, 12, and 36 mo, slow tacrolimus metabolizers had a significantly better renal function than fast metabolizers. Mean estimates and corresponding 95% confidence intervals from the multivariable linear mixed model are plotted; overlapping areas are shown in dark grey[59]. eGFR: Estimated glomerular filtration rate.

Table 1 Medication doses and blood trough concentrations

	Fast metabolizers (n = 97)	Interm metabolizers (n = 78)	Slow metabolizers (n = 73)	P value
Tacrolimus mean trough level (ng/mL)	8.2 ± 1.6	9.2 ± 1.8	9.5 ± 1.8	< 0.001[a]
After 1 mo	9.4 ± 3.2	10.5 ± 2.7	11.0 ± 3.2	0.002[a]
After 3 mo	7.8 ± 2.1	9.1 ± 2.9	9.5 ± 2.8	< 0.001[a]
After 6 mo	7.2 ± 2.3	7.8 ± 2.4	8.0 ± 2.8	0.079[a]
Tacrolimus mean daily dose (mg)	11 (6-27)	8 (4-14)	6 (2-12)	< 0.001[b]
After 1 mo	14 (6-40)	10 (4-22)	8 (2-20)	< 0.001[b]
After 3 mo	10 (4-23)	7 (4-13)	4 (2-12)	< 0.001[b]
After 6 mo	9 (3-21)	5 (2-10)	3 (2-8)	< 0.001[b]
Prednisolon mean daily dose (mg)	15 (4-37)	14 (5-70)	13 (0-40)	0.06[b]
After 1 mo	20 (15-90)	20 (15-70)	20 (0-50)	0.155[b]
After 3 mo	14 (3-30)	13 (5-30)	13 (0-30)	0.496[b]
After 6 mo	10 (5-30)	9 (5-20)	8 (0-20)	0.114[b]

Tacrolimus (TAC) trough levels and doses and prednisolone doses after renal transplantation. Fast metabolizers revealed noticeable lower TAC trough levels but higher TAC doses compared to intermediate and slow metabolizers. Prednisolone doses did not differ noticeably between the groups. [a]*P*-value is from the one-way ANOVA; [b]*P*-value is from the Kruskal-Wallis test; interm., intermediate; modified according to Thölking *et al*[60].

reduced renal function after a follow-up of 24 and 36 mo after RTx and LTx, respectively[59,60]. Furthermore, a low C/D ratio (fast TAC metabolism) led to more indication biopsies, more CNI nephrotoxicity and more BKV infection after RTx[62].

In this context, *CYP3A* genotyping has improved our knowledge on TAC metabolism and might explain why patients present as slow or fast metabolizers but its predictive value in terms of TAC dose requirement or renal function is still unsatisfactory[58]. Currently, genetic testing does not deliver relevant data and counteracts our simplification strategy in the daily routine.

CONCLUSION

In conclusion, fast TAC metabolism is associated with a reduced renal function after RTx and LTx. Higher rates of CNI nephrotoxicity and BKV infections/BKVN are assumed to be at least partly responsible for these results. Calculation of the TAC C/D ratio is a simple clinical tool that may assist transplant clinicians in individualizing immunosuppressive regimens.

Controlled, prospective, multicenter trials are needed to confirm the predictive value of the TAC C/D ratio.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Risk factors and outcomes of delayed graft function in renal transplant recipients receiving a steroid sparing immunosuppression protocol

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Abstract

AIM

To analyse the risk factors and outcomes of delayed graft function (DGF) in patients receiving a steroid sparing protocol.

METHODS

Four hundred and twenty-seven recipients of deceased donor kidney transplants were studied of which 135 (31.6%) experienced DGF. All patients received monoclonal antibody induction with a tacrolimus based, steroid sparing immunosuppression protocol.

RESULTS

Five year patient survival was 87.2% and 94.9% in the DGF and primary graft function (PGF) group respectively, $P = 0.047$. Allograft survival was 77.9% and 90.2% in the DGF and PGF group respectively, $P < 0.001$. Overall rejection free survival was no different between the DGF and PGF groups with a 1 and 5 year rejection free survival in the DGF group of 77.7% and 67.8% compared with 81.3% and 75.3% in the PGF group, $P = 0.19$. Patients with DGF who received IL2 receptor antibody induction were at significantly higher risk of rejection in the early post-transplant period than the group with DGF who received alemtuzumab induction. On multivariate analysis, risk factors for DGF were male recipients, recipients of black ethnicity, circulatory death donation, preformed DSA, increasing cold ischaemic time, older donor age and dialysis vintage.

CONCLUSION
Alemtuzumab induction may be of benefit in preventing early rejection episodes associated with DGF. Prospective trials are required to determine optimal immunotherapy protocols for patients at high risk of DGF.

Key words: Allograft failure; Deceased donors; Delayed graft function; Cold ischaemic time; Rejection; Steroid sparing; Alemtuzumab

Core tip: Alemtuzumab induction may help mitigate the early rejection risk associated with delayed graft function following renal transplantation. This may help with the management of recipients of transplants at high risk of delayed graft function, as it may lessen the need for repeated histological sampling.

Willicombe M, Rizzello A, Goodall D, Papalois V, McLean AG, Taube D. Risk factors and outcomes of delayed graft function in renal transplant recipients receiving a steroid sparing immunosuppression protocol. *World J Transplant* 2017; 7(1): 34-42 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/34.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.34

INTRODUCTION

Delayed graft function (DGF) is associated with adverse allograft and patient outcomes[1-6]. The incidence of DGF has increased over the recent years in concordance with the expanding use of marginal donors[4,7]. Risk factors for DGF are well established and include both recipient and donor characteristics mediated through immunological and non-immunological mechanisms[1,4,6,8,9]. Strategies to reduce the incidence of DGF are imperative in order to improve transplant outcomes and minimise cost. Hypothermic machine perfusion has been shown to reduce the risk and severity of DGF but whether this will translate into beneficial long term outcomes is not known[10-15]. There are also numerous trials currently in progress which are focusing on immunomodulation of the transplant prior to engraftment in order to reduce the ischaemic reperfusion injury, which is thought to be the pathological mechanism behind DGF and its sequelae[16,17]. Such agents include complement (*e.g.*, Mirococept® and Eculizumab®) and chemokine (*e.g.*, Reparixin®) inhibitors[16,17].

Rejection has been shown to be associated with DGF with a reported incidence as high as 40%-50%[2-4,6,18,19]. Therefore, the type of immunosuppression protocol used may impact on the natural corollary of DGF and there is evidence to show that DGF outcomes may be improved with the use of lymphocyte depleting antibody induction[4,19-23]. Neither ATG nor alemtuzumab have been shown to reduce the risk of DGF, however it has been demonstrated that their use is associated with a decrease in the incidence of rejection episodes[4,19-24]. Conversely, even in the absence of DGF steroid avoidance protocols have been shown to be associated with a higher number of rejection episodes despite good medium term allograft survival[25-29]. The additional risk posed by using steroid sparing regimens to the incidence of rejection and outcomes in DGF has not been formally trialled.

The aim of this study is to describe the risk factors and outcomes of DGF in a large cohort of ethnically diverse, deceased donor recipients treated with monoclonal antibody induction and a steroid sparing immunosuppression protocol.

MATERIALS AND METHODS

Patients

We retrospectively analysed 427 patients who received a deceased donor transplant at Imperial College Kidney and Transplant centre between 2005 and 2012. We excluded all patients who had lost their graft within 24 h due to technical reasons, recipients of living donor kidneys and simultaneous kidney-pancreas grafts. We included both deceased donor following circulatory death (DCD) and deceased donor following brain death (DBD) donors. All patients were CDC (T and B cell) and T cell flow cytometry cross match negative at the time of transplantation; patients with preformed donor specific antibodies detected by luminex methods only were included. Patient demographics are shown in Table 1.

All patients received monoclonal antibody induction with either anti-CD52 antibody [alemtuzumab (Mabcampath, Genzyme, United Kingdom)] or an anti-CD25 antibody [daclizumab (Zenpax®, Roche Inc, NJ) or basiliximab (Simulect®, Novartis Pharma Corp, NJ)]. All patients receive alemtuzumab induction unless they have a relative contraindication, which includes a past history of malignancy, hepatitis or previous significant immunosuppressive burden, when they receive an anti-CD25 antibody. Historically, patients enrolled into a clinical trial may also have received an anti-CD25 antibody at induction[29]. Maintenance immunosuppression included a steroid sparing, tacrolimus based regimen of tacrolimus monotherapy in the alemtuzumab induced patients and tacrolimus with the addition of mycophenolate mofetil in the anti-CD25 induced patients. All patients received a steroid sparing protocol of 500 mg methylprednisolone at the time of transplantation followed by one week of oral corticosteroids, which consists of 3 d of 30 mg prednisolone twice a day followed by 4 d of 30 mg once daily. Rejection episodes were diagnosed by biopsy and classified using the Banff 07 Classification of Renal Allograft Pathology[30]. Donor specific antibodies were detected using LABScreen® single antigen beads.

DGF was defined as the need for dialysis in the first week post-transplant.

Statistical analysis

All analyses were performed using Medcalc version 10.4.3. Comparisons of means and frequencies of normally

Table 1 Patient demographics

Factor		DGF *n* = 135 (%)	PGF *n* = 292 (%)	*P* value
Recipient age	Years (mean)	51.43 ± 12.19	47.45 ± 13.93	0.0046
Donor age	Years (mean)	51.56 ± 13.05	47.00 ± 15.99	0.0041
Recipient gender	Male	105 (77.8)	178 (61.0)	0.0009
	Female	30 (22.2)	114 (39.0)	
Donor gender	Male	69 (51.1)	123 (42.1)	0.12
	Female	66 (48.9)	167 (57.2)	
Ethnicity	Black	35 (25.9)	41 (14.0)	0.004
	Non-black	100 (74.1)	251 (86.0)	
Time on RRT	Years (mean)	6.37 ± 5.44	5.00 ± 5.07	0.012
Regrafts	1st	114 (84.4)	261 (89.4)	0.2
	> 2nd	21 (15.6)	31 (10.6)	
Donation type	DCD	45 (33.3)	32 (11.0)	< 0.00001
	DBD	90 (66.6)	259 (88.7)	
CIT	Hours (mean)	24.70 ± 7.82	21.29 ± 7.58	0.000023
HLA mismatch	Mean	3.47 ± 1.30	3.19 ± 1.58	0.079
Preformed DSA	DSA+	17 (12.6)	18 (6.2)	0.039
	DSA-	118 (87.4)	274 (93.8)	
Induction	Alemtuzumab	113 (83.7)	292 (84.9)	0.86
	IL2RA	22 (16.3)	44 (15.1)	
Recipient Diabetes	Yes	35 (25.9)	46 (15.8)	0.02
	No	100 (74.1)	246 (84.2)	

CIT: Cold ischaemic time; DGF: Delayed graft function; PGF: Primary graft function; DBD: Brain death; DCD: Circulatory death.

distributed variables were calculated using *t*-tests and χ^2/Fisher's exact tests. Kaplan-Meier survival analysis was used to calculate time of event from index biopsy and statistical significance was determined by log rank testing. Cox proportional regression plots were used for multivariable analyses, variables with a significance level of $P < 0.1$ on univariant analysis were included in the multivariable analysis using a stepwise method selection. A *P* value of < 0.05 was deemed statistically significant.

RESULTS

The 135/427 (31.6%) of recipients of a deceased donor renal allograft experienced DGF. Patient and allograft outcomes were compared between the DGF and PGF (primary graft function) group, with a mean follow up was 42.62 ± 19.96 mo.

Patient survival

Patient survival was negatively impacted by the development of DGF post-transplant. Overall patient survival at 1, 3 and 5 years post-transplant was 96.3%, 87.2% and 82.5% in the DGF group and 97.9%, 95.0% and 94.2% in the PGF group, $P < 0.01$ as shown in Figure 1A. Censoring at the time of allograft failure, 1, 3 and 5 year patient survival was 98.4%, 90.2% and 87.2% in the DGF group and 97.9%, 95.7% and 94.9%, $P = 0.047$ in the PFG as shown in Figure 1B. The causes of death in the 11/135 (8.1%) DGF patients who died with a functioning graft were cardiovascular 4/11 (36.4%), sepsis 4/11 (36.4%), malignancy 1/11 (9.1%), autoimmune disease 1/11 (9.1%) and unknown 1/11 (9.1%).

Allograft outcomes

Allograft survival was also inferior in the DGF group. Censored allograft survival in the DGF group was 90.3%, 84.7% and 77.9% at 1, 3 and 5 years compared with 99.0%, 95.5% and 90.2% in the PGF group, $P < 0.001$ as shown in Figure 2. The causes of allograft failure in the 23/135 (17.0%) of patients with DGF were late technical losses in 4/23 (17.4%) (2 renal vein thrombosis, 2 ureteric complications), rejection in 6/23 (26.1%), BK nephropathy in 1/23 (4.3%), progressive scarring in 6/23 (26.1%) and multifactorial aetiologies in 6/23 (26.1%).

The development of DGF but not donor type impacted on allograft survival. Overall allograft survival in recipients of DBD and DCD kidneys with PGF was 90.3% and 90.7% respectively, which was significantly higher than recipients of DBD and DCD kidneys with DGF, which was 75.3% and 65.8% respectively, $P = 0.0016$ as shown in Figure 3. Comparing outcome by donor type, there was no difference in survival between DBD and DCD kidneys with PGF, $P = 0.84$ or with independently, DBD and DCD kidneys with DGF, $P = 0.73$.

Patients with preformed DSA were at increased risk of rejection when compared with patients with no DSA, with a one year rejection free survival of 58.9% and 82.1% in the DSA+ and DSA-groups respectively, $P < 0.001$. Preformed DSA were also more frequent in the DGF group, with 17/135 (12.59%) and 18/292 (6.16%) patients having preformed DSA in the DGF and PGF groups respectively, $P = 0.03$. Censoring for DSA positive patients, the overall rejection free survival was no different between the DGF and PGF groups. The 1, 3 and 5 year rejection free survival in the DGF group

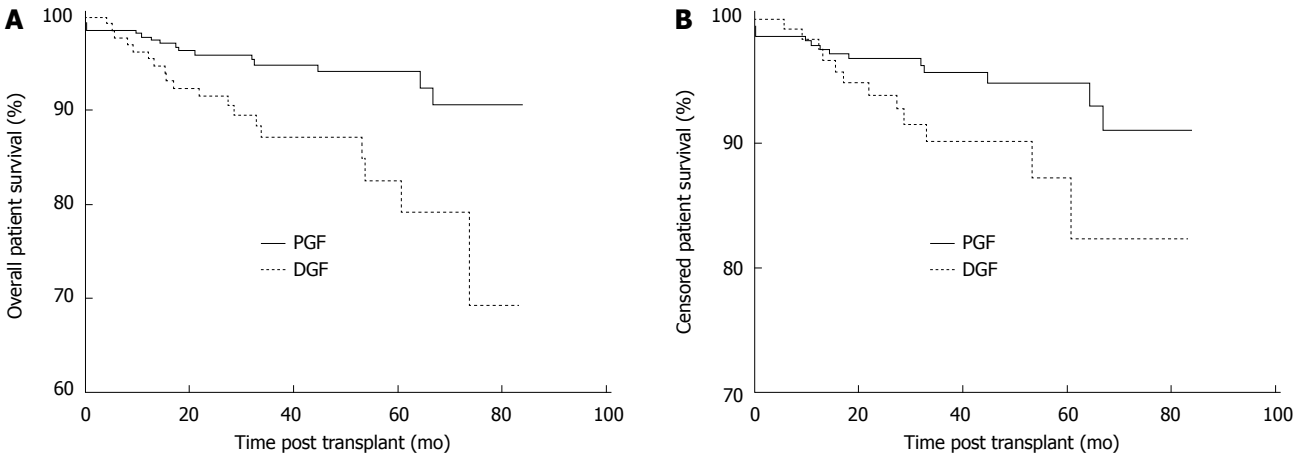

Figure 1 Patient survival in patients with delayed graft function. The 1, 3 and 5 year patient survival post-transplant: A: Overall patient survival: 96.3%, 87.2% and 82.5% in the DGF group and 97.9%, 95.0% and 94.2% in the PGF group, $P < 0.01$; B: Patient survival censored at the time of allograft failure: 98.4%, 90.2% and 87.2% in the DGF group and 97.9%, 95.7% and 94.9%, $P = 0.047$. DGF: Delayed graft function; PGF: Primary graft function.

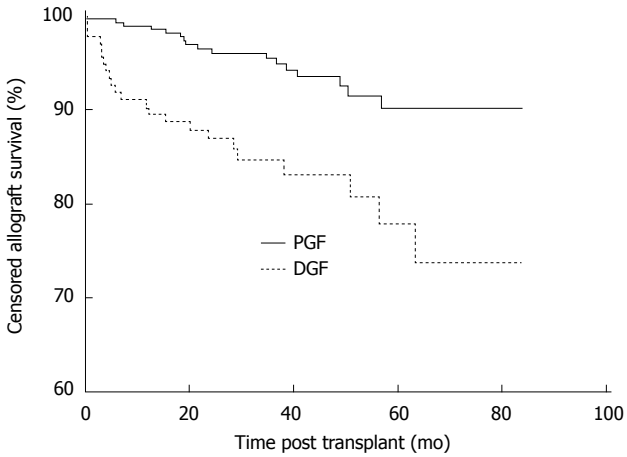

Figure 2 Censored allograft survival. Censored allograft survival in the DGF group was 90.3%, 84.7% and 77.9% at 1, 3 and 5 years compared with 99.0%, 95.5% and 90.2% in the PGF group, $P < 0.001$. DGF: Delayed graft function; PGF: Primary graft function.

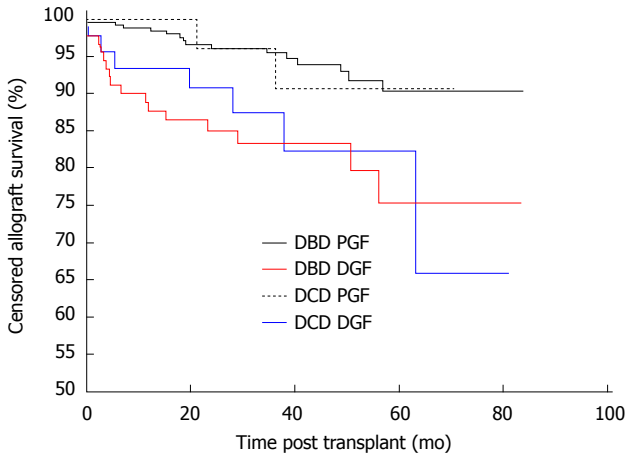

Figure 3 Allograft survival by donor type and delayed graft function. Allograft survival in the DBD and DCD donors with PGF was significantly higher than the recipients of DBD and DCD kidneys with DGF, with an allograft survival of 90.3%, 90.7%, 75.3% and 65.8% respectively, $P = 0.0016$. DGF: Delayed graft function; PGF: Primary graft function; DBD: Brain death; DCD: Circulatory death.

was 77.7%, 72.2% and 67.8% compared with 81.3%, 77.7% and 75.3% in the PGF group, $P = 0.19$. However, comparing early rejection episodes by induction agent used and the occurrence of DGF, patients receiving an IL2RA who had DGF (IL2-DGF) were at significantly higher risk of rejection than the alemtuzumab-DGF (C-DGF) group in the first 3 mo post-transplant as shown in Figure 4A. The 3 mo rejection free survival was 93.0%, 92.9%, 92.5% and 77.8% in the C-PGF, C-DGF, IL2RA-PGF and IL2RA-DGF groups respectively, $P = 0.03$. However, this effect was not maintained and the overall rejection free survival was no different, with a rejection free survival of 76.4%, 71.5%, 76.5% and 70.7% in the C-PGF, C-DGF, IL2RA-PGF and IL2RA-DGF groups respectively, $P = 0.75$ as shown in Figure 4B. Induction agent had no subsequent impact on graft loss and patients with DGF had inferior allograft survival to those with PGF in the alemtuzumab and IL2RA groups, $P = 0.0014$. Allograft survival in the C-DGF group compared with the IL2-DGF group was 73.6% and 76.6%, $P = 0.78$ and 89.7% and 89.7% in the C-PGF and IL2-PGF groups respectively, $P = 0.58$.

De novo DSA free survival was lower in the DGF group in the first month only, with a DSA free survival of 89.8% and 95.3% in the DGF and PGF groups respectively, $P = 0.04$. At 3, 12, 36 and 60 mo the DSA free survival was 88.1%, 83.0%, 77.3% and 77.3% in the DGF group and 92.3%, 86.8%, 81.6% and 78.5% in the PGF group, $P = 0.16$, 0.29, 0.26 and 0.38 respectively.

Allograft function of patients who remained dialysis independent was inferior in the DGF groups in the short to medium term as shown in Figure 5. Mean serum creatinine was 203.4 ± 120.0, 172.3 ± 86.6, 161.9 ± 74.9, 167.2 ± 86.1 and 149.6 ± 59.4 μmol/L at 1, 6, 12, 36, 60 mo post-transplant in the DGF group compared with 132.4 ± 48.6, 133.8 ± 56.7, 127.8 ± 43.6, 138.4 ± 47.7 and 143.0 ± 65.2 μmol/L in the PGF group; giving a P value of < 0.01 at 1 to 12 mo, a P value of 0.015 at 36

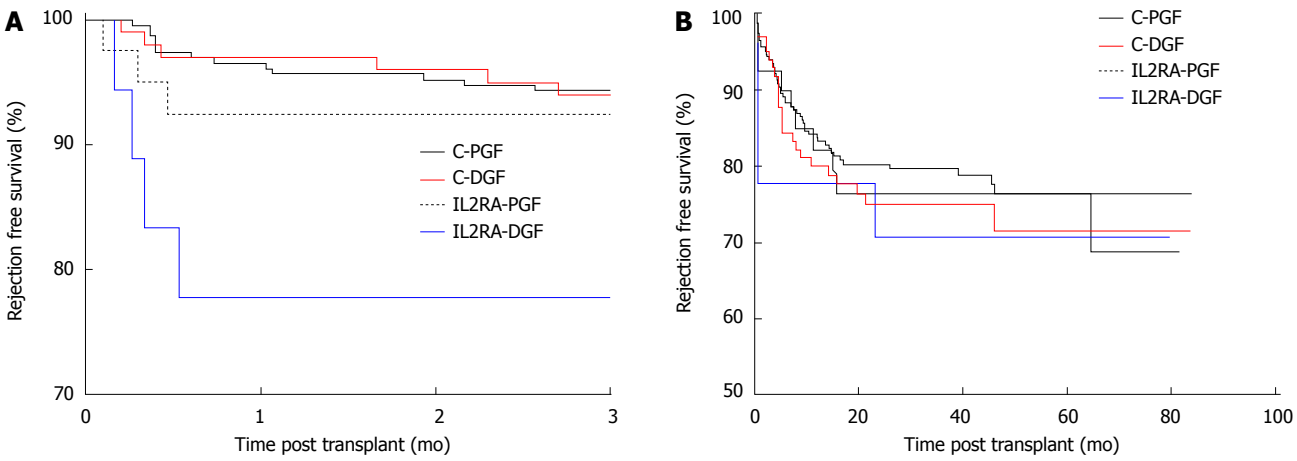

Figure 4 Three month and overall rejection free survival by induction agent and delayed graft function. Rejection free survival, censored for DSA+ in patients with alemtuzumab induction and PGF (C-PGF), alemtuzumab induction and DGF (C-DGF), IL2RA induction and PGF (IL2RA-PGF) and IL2RA induction and DGF (IL2RA-DGF) at A: 3 mo: 93.0%, 92.9%, 92.5% and 77.8% respectively, P = 0.03 and B: 5 year: 76.4%, 71.5%, 76.5% and 70.7%, P = 0.75. DGF: Delayed graft function; PGF: Primary graft function.

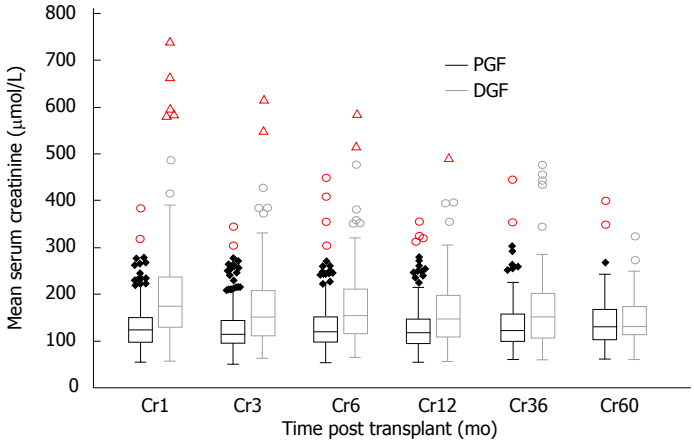

Figure 5 Allograft function according to delayed graft function or primary graft function. Mean serum creatinine was 203.4 ± 120.0, 172.3 ± 86.6, 161.9 ± 74.9, 167.2 ± 86.1 and 149.6 ± 59.4 μmol/L at 1, 6, 12, 36, 60 mo post-transplant in the DGF group compared with 132.4 ± 48.6, 133.8 ± 56.7, 127.8 ± 43.6, 138.4 ± 47.7 and 143.0 ± 65.2 4 μmol/L in the PGF group; giving a P value of < 0.01 at 1 to 12 mo, a P value of 0.015 at 36 mo and 0.70 at 60 mo. DGF: Delayed graft function; PGF: Primary graft function.

mo and 0.70 at 60 mo.

new onset diabetes after transplant (NODAT) free survival at 1, 3 and 5 years in the DGF group was 91.0%, 87.7% and 80.3% which was no different from the PGF group, which had a 1, 3 and 5 year NODAT free survival of 92.6%, 87.0% and 82.4%, P = 0.88.

Risk factors associated with the development of DGF

The baseline demographics for the patients who developed DGF are shown in Table 2. On univariate analysis we found that both older donor and recipient age was associated with risk of DGF. The mean age of the recipient in the DGF and PGF groups was 51.4 ± 12.2 and 47.5 ± 13.9 respectively, P < 0.01; whilst the mean donor age was 51.6 ± 13.1 and 47.0 ± 16 respectively, P < 0.01. Male recipients were at higher risk of DGF, with 105/135 (77.8%) of the DGF group being male compared with 178/292 (61.0%) of the PGF group, P < 0.001. Donor gender did not influence DGF. Black recipients were more likely to experience DGF when compared with recipients of other ethnicities, with 35/135 (25.9%) of the DGF and 41/292 (14.0%) of the PGF group being of Black ethnic origin P = 0.004. Patients with DGF had spent longer on dialysis therapy pre-transplantation, with a mean dialysis vintage of 6.37 ± 5.44 and 5.00 ± 5.07 years in the DGF compared with PGF groups, P = 0.012. Recipients with DGF were more likely to be diabetic, with 35/135 (25.9%) of patients with DGF having diabetes compared with 46/292 (15.8% of the PGF group, P = 0.02). There were a significantly higher proportion of DCD donors in the DGF group, with 45/135 (33.3%) of the DGF patients receiving a DCD graft compared with 32/292 (11.0%) of the PGF group, P < 0.001. There was also a significant difference in the mean cold ischaemic time (CIT) between the groups, with a CIT of 24.70 ± 7.82 and 21.29 ± 7.58 h in the DGF and PGF groups respectively, P < 0.001.

Statistically significant variables by univariate analysis were placed into a multivariable model. These included donor and recipient age, recipient being of male gender and black ethnicity, diabetic recipients, the presence of preformed DSA, DCD donors, CIT and dialysis vintage. Independent categorical risk factors for DGF were found to be black ethnicity [OR = 2.27 (1.3-4.0), P = 0.005], receiving a DCD graft [OR = 4.1 (2.3-7.2), P < 0.001], the presence of preformed DSA [OR = 2.36 (1.1-5.2), P

Table 2 Independent risk factors for delayed graft function

Variable	OR	95%CI	*P* value
Black Ethnicity	2.27	1.28-4.00	0.0047
Female gender	0.43	0.25-0.73	0.0017
DCD donor	4.09	2.33-7.20	< 0.0001
Preformed DSA	2.36	1.07-5.18	0.0326
CIT	1.05	1.02-1.08	0.0009
Donor age	1.02	1.01-1.04	0.0049
Time on dialysis	1.07	1.02-1.11	0.0023

CIT: Cold ischaemic time; DCD: Circulatory death.

= 0.03, with female gender being protective [OR = 0.43 (0.25-0.7), *P* = 0.002]. Continuous variables associated with DGF were CIT [HR = 1.05 (1.0-1.1) *P* < 0.001], with a CIT of > 20 h being most predictive of DGF; donor age [OR = 1.02 (1.01-1.04), *P* = 0.005] with a donor age of > 36 years being most predictive and time on dialysis [OR = 1.07 (1.02-1.11), *P* = 0.002], with risk increasing after 3.1 years. Recipient age and diabetes were not retained in the model.

DISCUSSION

In this descriptive study of the outcomes of DGF in a large series of ethnically diverse, deceased donor recipients receiving a steroid sparing immunosuppression protocol, we found that DGF is associated with inferior allograft and patient survival. This is in accordance with published DGF studies incorporating the use of corticosteroids[1-6]. Rejection was not increased in patients who experienced DGF compared with the PGF group, however we found that the rejection patterns differed depending upon the type of induction antibody used. Patients receiving IL2RA induction who had DGF were more likely to have rejection in the first 3 mo compared with those patients who received alemtuzumab induction. Risk factors associated with the development of DGF in our cohort were consistent with other studies and included donor age, recipients of a DCD organ, CIT, recipient gender and ethnicity, length of time on dialysis and the presence of preformed DSA[8,9]. This highlights CIT as a modifiable risk factor for DGF and efforts to reduce CIT are crucial in order to prevent DGF.

According to registry data, the incidence of DGF has increased over the past 2 decades, with an incidence of 21.3% reported in the United States in 2011[7]. Single centre series, depending on their patient population have reported an incidence of up to 45%[1]. The incidence of 27.4% we found in our deceased donor recipients despite steroid sparing is within this reported range. Inferior allograft outcomes are widely reported following DGF with increased risk of graft failure, rejection and poor function[1,2,4-6]. Less studies have analysed patient survival following DGF. Although there are individual series in which patient survival has been shown to be reduced, a meta-analysis did not demonstrate a significant association between DGF and death[1-3,23]. However, Narayanan *et al*[3] found that DGF following live donation was associated with death with a functioning graft.

To date no immunosuppression protocol has been shown to influence the development of DGF. However, it is recognised that immunosuppression and more precisely, the type of induction agent used can impact on the subsequent outcomes of DGF[4,20-23]. It has been shown that DGF is associated with increased risk of rejection[1,4,6,31]. However, this risk may be dependent upon the immunosuppression protocol as several studies have shown that the use of lymphocyte depleting induction agents, either ATG or alemtuzumab may reduce the risk of rejection in patients with DGF[4,20-23]. The effectiveness of ATG in preventing rejection in DGF may be dose dependent, which has not been reported post alemtuzumab[4,21,23,32]. Regarding further comparisons between ATG and alemtuzumab, in a prospective RCT in which the effectiveness of alemtuzumab *vs* ATG induction was examined in high risk patients with early steroid withdrawal, the incidence of early biopsy proven acute rejection (BPAR) was less in the patients who received alemtuzumab[26]. Despite, the overall incidence of DGF in that particular study being low due to the exclusion of marginal donors, the results might favour alemtuzumab over ATG to prevent early DGF associated rejection[26]. Several other studies have shown that alemtuzumab may mitigate the rejection risk of DGF[20,33,34]. Knechtle *et al*[20] in a retrospective study comparing alemtuzumab, thymoglobulin and anti-CD25 antibody induction, showed that alemtuzumab reduced the incidence of rejection in patients with DGF and improved allograft survival however the patients in this study were receiving maintenance corticosteroids. Tyson *et al*[31] in a RCT comparing ATG and alemtuzumab induction, had a similar proportion of marginal donors and DGF between the two arms and showed the incidence of BPAR to be less in the alemtuzumab arm. It should be noted that although alemtuzumab is associated with reduced early BPAR, alemtuzumab has been shown to be associated with a higher incidence of late BPAR resulting in equivocal rejection rates between ATG and alemtuzumab overall[26,35]. However, the use of alemtuzumab may be useful in the management of patients at high risk of DGF given the low early rejection risk, which may reduce the need for frequent biopsies.

Steroid sparing protocols have been shown to be associated with an increased rejection rate, although there is no adverse impact on allograft survival[25]. Conversely, corticosteroids use post-transplant is associated with NODAT, hypertension, hypercholesterolaemia and patient death secondary to cardiovascular and infectious complications[25-29]. The patient demographic at risk of DGF, which include older males and ethnic minorities are already more likely to have many of these complications and therefore steroids might confound the problem. Diabetes is a relatively new risk factor to be reportedly associated with DGF and peri-operative hyperglycaemia has been shown to exacerbate the ischaemic reperfusion

injury in both animal and human models[36,37]. Steroid avoidance or early withdrawal might therefore help with diabetic control in the crucial recovery period.

Irish *et al*[8,9] formulated a predictive model of DGF by performing a multivariable logistic regression analysis of 24337 deceased donor transplant recipients in the United States. Given the relationship between DGF and allograft loss, their model predicts not only patients with increased risk of DGF but also those at risk of subsequent graft failure[8,9]. They found that the most significant risk factors for DGF to be CIT, donor creatinine, recipient body mass index, donor age and recipients of DCD organs[8,9]. They did not address the risk of low level preformed DSA, however they did find that the contribution to the overall risk according to the level of peak panel reactive antibodies (%) and previous transplantation diminished between two consecutive eras of immunosuppression[8,9]. Minimising CIT is an important variable in the lowering risk of DGF and improving outcomes and we accept that our mean CIT is higher than the average reported[38,39]. One study indicated a CIT of > 18 h was strongly associated with DGF and allograft failure[39]. Although cold storage slows the ischaemic damage, even in hypothermic conditions prolonged ischaemic times result in a more severe ischaemic reperfusion injury[17,40]. The superiority of hypothermic machine perfusion over static cold storage in preventing DGF is still an area of controversy and the long term benefit is not known[10-13]. The mechanisms through which machine perfusion is thought to minimise ischaemic injury include maintaining the patency of the vascular bed, providing nutrients and low level oxygen along with the ability to remove metabolic toxins[41]. In practice, machine perfusion is not universally available, therefore the most important modifiable factor in reducing DGF remains minimising CIT[38,40,42].

In conclusion, DGF is associated with inferior allograft and patient outcomes in patients receiving monoclonal antibody induction and a steroid sparing protocol. There is an increased risk of early rejection in patients with DGF receiving IL2RA compared with alemtuzumab induction, which implies that type of immunosuppression is important in the management of patients at risk of DGF. With an increase in the use of marginal donors, prospective studies into optimal immunotherapy protocols for these high risk patients are needed. Donor and recipient characteristics also contribute to the risk of DGF and CIT remains an important modifiable risk factor.

COMMENTS

Background

Delayed graft function (DGF) post renal transplantation is associated with adverse patient and allograft outcomes. The incidence of DGF has been increasing with the use of extended criterion donors, and strategies to reduce DGF are required in order to improve outcomes. Risk factors for DGF are well established and include both recipient and donor characteristics mediated through immunological and non-immunological mechanisms.

Research frontiers

Significant research has been carried out to establish methods of optimising extended criterion allografts pre-implantation in order to provide the best outcomes. Most of these methods either involve hypothermic machine perfusion or immunomodulation of the transplant prior to engraftment in order to reduce ischaemic reperfusion injury. This study highlights the importance of immunosuppression post-transplant as a means to reduce any further injury to these allografts secondary to alloimmune responses.

Innovations and breakthroughs

In this study the authors directly compare the outcomes of DGF in patients receiving a steroid sparing immunosuppressive protocol but who either receive an IL2 receptor antibody or alemtuzumab induction.

Applications

This study shows how the type of induction immunosuppression may help in managing patients at high risk of DGF. By reducing the risk of early rejection in these patients, it may help with long term outcomes by preventing a secondary injury due to alloimune responses. If risk of rejection is low, it may also reduce the need for frequent histological examination.

Terminology

Delayed graft function can be described in many ways, but the authors use one of the most common definitions, which is the need for dialysis in the first 7 d post renal transplant.

Peer-review

The paper is very exciting and instructive article.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Effectiveness and versatility of biological prosthesis in transplanted patients

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Abstract

AIM

To emphasize the effectiveness and versatility of prosthesis, and good tolerance by patients with incisional hernia (IH).

METHODS

From December 2001 to February 2016, 270 liver transplantations were performed at San Camillo Hospital. IH occurred in 78 patients (28.8%). IH usually appeared early within the first year post-orthotopic liver transplantation. In the first era, fascial defect was repaired by primary closure for defects smaller than 2.5 cm or with synthetic mesh for greater defects. Recently, we started using biological mesh (Permacol™, Covidien). We present a series of five transplanted patients submitted to surgery for abdominal wall defect correction repaired with biological mesh (Permacol™, Covidien).

RESULTS

In our cases, the use of biological prosthesis (Permacol™, Covidien) have proven to be effective and versatile in repairing hernia defects of different kinds; patients did not suffer infections of the prosthesis and no recurrence was observed. Furthermore, the prosthesis remains intact even in the years after surgery.

CONCLUSION

The cases that we presented show that the use of biological mesh (Permacol™, Covidien) in transplanted patients may be safe and effective, being careful in the management of perioperative immunosuppression and

renal and graft function, although the cost of the product itself has been the main limiting factor and there is need for prospective studies for further evaluations.

Key words: Incisional hernia; Liver transplantation; Heart transplantation; Biological mesh; Surgery; Morbidity; Risk factors; Immunosuppression; Infection; Recurrence

Core tip: Incisional hernia (IH) following abdominal organ transplantation have a high rate, and even more in immunosuppressed patients. Several factors have been described to be associated with IH in transplant patients. Herein, we present our preliminary experience with porcine dermal collagen mesh.

Vennarecci G, Mascianà G, De Werra E, Sandri GBL, Ferraro D, Burocchi M, Tortorelli G, Guglielmo N, Ettorre GM. Effectiveness and versatility of biological prosthesis in transplanted patients. *World J Transplant* 2017; 7(1): 43-48 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/43.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.43

INTRODUCTION

Incisional hernia (IH) following abdominal organ transplantation have a high rate. Every year thousands of transplant procedures are performed worldwide. Equally, the number of IH in this population is growing every year. This post-operative complication rate is estimated for kidney transplant, liver transplant and pancreas transplant as ranging from 1.6% to 18%[1,2], from 1.7% to 32.4%[3,4] and 13% to 34.8%[5,6] respectively.

Different causes have been proposed to increase IH risk. Among them are: Pre-transplant malnutrition, presence of abundant ascites for liver candidates, type of incision and type of wall closure, co-morbidities such as diabetes and obesity, multiple surgeries, and male sex. Compromised wound healing process is major in patients with an immunosuppressive regimen; nonetheless, this therapy increases the infections rate. The European Hernia Society recommend to use a porcine dermal collagen (PDC) mesh in these cases. In spite of this, no proven benefit *vs* synthetic mesh (SM) has been described.

Recent studies have shown that biological prostheses have a greater ability to integrate into tissues, resist bacterial colonization, reduce cytotoxic or allergic reactions, and provide similar functional results, compared with SM[7,8]. This article shows the experience of our surgical division in the use of PDC mesh (Permacol™, Covidien) in transplanted patients, emphasizing their effectiveness and versatility, and good tolerance by the patients.

MATERIALS AND METHODS

From December 2001 to February 2016, 270 liver transplantations were performed at San Camillo Hospital. The transplant procedures were performed with the piggy-back technique without venous-venous bypass. Surgical access was obtained by a bilateral subcostal laparotomy with a cranial midline extension or a J-shaped (Makuuchi) laparotomy. Closure of the abdomen was performed with a slowly absorbable two-layer running sling suture. All patients received a triple immunosuppressive therapy with steroid, tacrolimus and mycophenolate. Everolimus has been used since 2010 in patients with renal dysfunction and/or associated hepatocellular carcinoma (HCC). IH occurred in 78 patients (28.8%). IH usually appeared early within the first year post-orthotopic liver transplantation (OLT). The elective surgical repair of the abdominal defect was delayed until the patient recovered good general condition. On average, repair was performed at a median of 29 mo (range: 22-45 mo) after OLT. IH was diagnosed by physical examination. In the first era, the fascial defect was repaired by primary closure for defects smaller than 2.5 cm or with SM for greater defects. Whenever possible, the sublay technique with implantation of the mesh between the closed posterior fascia and the muscle in the majority of patients was used. Otherwise, a dual-mesh prosthesis was implanted intraperitoneally. Recently, we started using PDC mesh (Permacol™, Covidien). The patient's management included everolimus withdrawal before surgery, early nasogastric tube removal to facilitate oral feeding, administration of immunosuppressive therapy, peri-operative antibiotic administration, monitoring "graft function", monitoring patient for local or chest infections, and *e.v.* fluid administration to avoid dehydration and renal dysfunction. In our practice, we applied a third-generation cephalosporin until the tube-drain removal.

Herein, we present a case series of OLT patients submitted to surgery for abdominal wall defect correction repaired with PDC mesh (Permacol™, Covidien), including: 1 case of subcostal/epigastric IH; 1 case of paraombelical IH; 1 case of reconstruction of the diaphragm in a patient with HCC recurrence infiltrating the diaphragm; 1 case of large-for-size liver graft mismatch; and 1 case of epigastric IH in a heart transplant (HT) patient (Table 1).

RESULTS

A 52-year-old male was admitted to the hospital with a giant IH in the epigastrium region 4 years after OLT. A PDC (10 cm × 15 cm) mesh (Permacol™, Covidien) was positioned without tension to the edges of the fascia defect, and fixed with 2-0 interrupted polypropylene sutures. We used a Jackson-Pratt drain (Cardinal Health™) above the mesh construct. The skin was closed with interrupted sutures. Prophylactic antibiotics were given until post-operative d (POD) 5. The patient continued immunosuppressive therapy without any changes. The drain was removed

Table 1 Patient characteristics

Case No.	Age/sex	Type of transplant	Immunosuppressive therapy	Hernia size, cm	Time from transplantation to repair	Recurrence	Follow-up duration
1	52/male	Liver	Tacrolimus + Everolimus	10 × 8	8 mo	None	2 yr
2	58/male	Heart	Steroids + Tacrolimus	10 × 10	5 yr	None	3 yr
3	55/male	Liver	Steroids + Tacrolimus + Everolimus	8 × 8	6 mo	None	5 yr
4	58/female	Liver	Steroids + Tacrolimus + Everolimus	20 × 15	3 d	None	3 mo
5	70/male	Liver	Tacrolimus	6 × 7	4 yr	None	6 mo

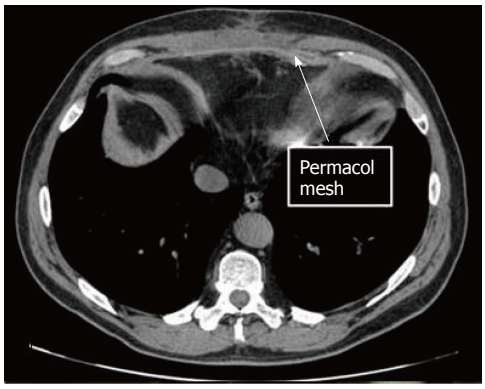

Figure 1 Computed tomography scan at 6 mo after abdominal wall repair. Arrow: Biological prosthesis.

and the patient was discharged on POD 5 without complications. No hernia recurrence was observed at 2-year follow-up after surgery.

A 58-year-old male was admitted with a subxiphoid-epigastric IH 5 years after a HT. The surgical access was a sternotomy with a subxiphoid extension. The abdominal IH occurred within 1 year from HT. The patient was on an immunosuppressive regimen with steroids, once-daily tacrolimus and everolimus. Everolimus was stopped 2 mo before surgery. Physical examination showed that the defect was about 20 cm in diameter. The operative procedure started with incision xypho-supraumbilical. The hernia sac was prepared and isolated by adhesions with cutaneous scar to the back-end of the rectus abdominis without opening the sac. The dissection was continued with the preparation of the rear end of the rectum to the lateral margin; the fascia was sutured on midline obtaining the reduction of the hernia sac in subfascial position. Permacol™ mesh (molded with diameter 15 cm × 13 cm) was implanted using the sublay technique and sutured with 0 interrupted polypropylene sutures. We placed 1 drain in the subfascial over the prosthesis and then sutured the front fascia of the rectus abdominis. Everolimus was restarted 2 wk after surgery. The drain was removed and the patient was discharged on POD 5 without complications. No hernia recurrence was observed at 3-year follow-up after surgery (Figure 1).

A 55-year-old male received a liver transplant 6 years earlier for autoimmune-related liver cirrhosis. At the time of the transplant procedure, the patient's giant umbilical hernia (10 cm × 8 cm) was not repaired. The hernia sac was opened carefully, and no adhesions were found. The PDC mesh (Permacol™, Covidien) was fixed with not-absorbable sutures at the muscle-aponeurotic plane, bridging the defect without primary fascial apposition. A drain was placed in the subcutaneous plain. The subcutaneous tissue and skin were closed with interrupted sutures. Antibiotics were given until POD 6. The patient continued immunosuppressive therapy without any changes, including steroids at 7.5 mg daily. The drain was removed and the patient was discharged on POD 6 without complications. At 5 years after the surgery no hernia recurrence was observed.

A 58-year-old female received a liver transplant in November 2015 for a primary biliary cirrhosis. The surgical access was a bilateral subcostal laparotomy with a cranial midline extension. Due to large-for-size liver graft mismatch, with a graft-to-recipient-weight-ratio of 3.3%, and presence of bowel edema, abdominal wall closure was not possible at the end of procedure. In order to prevent the onset of a compartment syndrome, a temporary wound closure with Bogota Bag was performed. After 3 d, a PDC mesh (Permacol™, Covidien) was molded (28 cm × 18 cm) and sutured at the muscle-aponeurotic plane with 0 interrupted polypropylene sutures (Figure 2A). We placed 1 drain in the subcutaneous plain and the skin was closed with continuous sutures above the mesh (Figure 2B). Post-operative course was characterized by respiratory distress (classified as Dindo-Clavien Grade Ⅱ) resolved at POD 3. The patient was discharged on POD 5 and followed as out-patient. Three mo after the liver transplant, a CT scan showed the complete integrity of the biological prosthesis, and the patient had an excellent functional result (Figure 2C) and a normally perfusioned graft.

Four years after OLT for HCC, a 70-year-old male was admitted to the hospital with a recurrence of HCC infiltrating the peritoneum pericardium and diaphragm. Abdominal exploration showed a neoplasm of left lobe liver graft with infiltration of the diaphragm which extended to the pleura and pericardium. The operative procedure included a left lobectomy of the graft with resection of the diaphragm "*en bloc*" with the adjacent portion of right pleura and pericardium. The resection created a wide pleura-pericardial wall defect (Figure 3A). The wall defect was sheltered by apposition of a PDC mesh (Permacol™, Covidien) sutured to the diaphragm with 2-0 continuous polypropylene sutures. At the end of procedure, the subcostal wall defect was repaired by apposition of the same prosthesis used before. Everolimus therapy was discontinued 7 d before

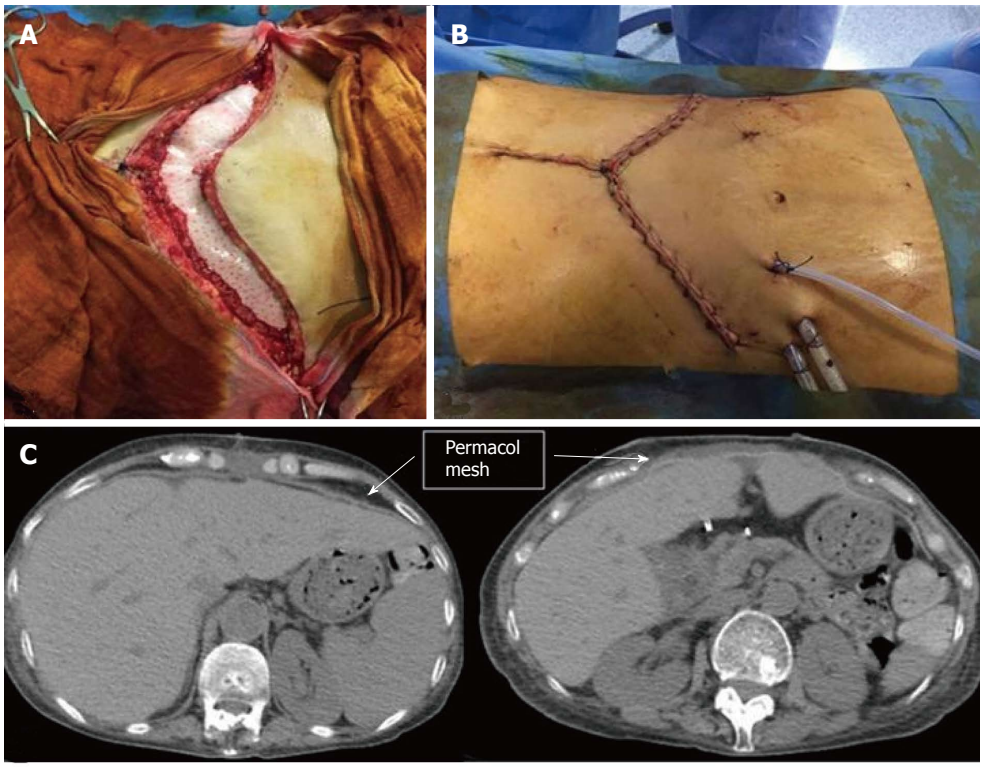

Figure 2 In order to prevent the onset of compartment syndrome, a temporary wound closure with Bogota Bag was performed. A: Implantation of Permacol™ mesh; B: Skin closure after Permacol™ mesh implantation; C: Computed tomography scan at 3 mo after abdominal wall repair (arrow: Biological prosthesis).

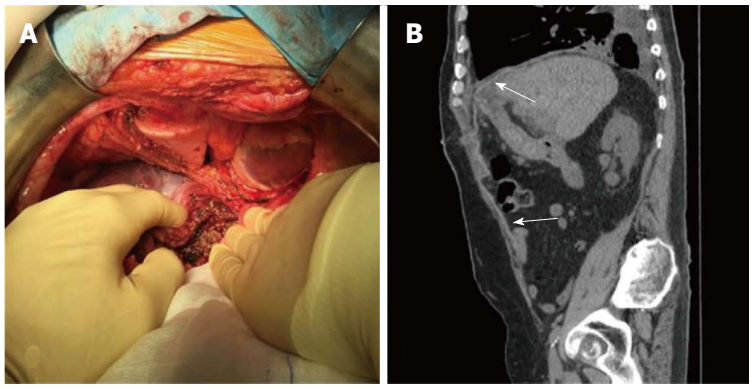

Figure 3 The abdominal exploration showed a neoplasm of left lobe liver graft with infiltration of the diaphragm which extended to the pleura and pericardium. A: Left liver lobectomy of the graft with resection of the diaphragm "*en bloc*" with adjacent portion of right pleura and pericardium; B: Computed tomography scan at 6 mo after abdominal wall repair (arrow: Biological prosthesis).

IH repair until POD 7. A mild pleural effusion (Figure 3B) was observed as post-operative complication.

DISCUSSION

The rate of IH after OLT is estimated to range from 1.7% to 32.4%[9,10]. In OLT patients several risk factors have been defined, including male sex, elevated body mass index, wound infection, hematoma, ascites, repeat interventions, immunosuppressive drugs, low platelets count, abdominal wall closure technique, diabetes mellitus and smoking history[11,12]. Different techniques are available to repair the IH, including open techniques with primary fascia closure and open or laparoscopic repair with synthetic or biological mesh[13]. Although permanent mesh prostheses are considered the best treatment for minimizing IH recurrence, they have been associated with a high risk of complications due to their non-absorbable characteristics, such as erosion into the abdominal viscera, protrusion, extrusion, adhesion, infection and bowel fistulae, that can lead to more complex and costly surgery[14].

Biological mesh was introduced as an alternative to SM in the 1990s[15]. The bioprosthetic materials are taken from several different species (bovine, porcine and equine) and from different organs (pericardium, skin and bowel submucosal)[14]. Biological mesh prostheses allow neo-vascularization and regeneration due to infiltration of native fibroblasts and they are incorporated into the surrounding tissue. During incorporation, they generate active neofascia to withstand the mechanical forces of the abdominal wall[16]. Recent studies have shown that biological prosthesis have a greater ability to integrate into tissues being colonized by host cells and blood vessels, resist bacterial colonization minimizing the risk of infection, reduce cytotoxic or allergic reactions, and provide similar functional results, compared with synthetic prosthesis. Porcine dermis is the closest to human dermis and it is not cytotoxic, hemolytic, pyrogenic or allergenic, and it does not elicit a foreign

body response[17]. It is soft and flexible, and it has bilateral smooth surfaces with high tensile strength[17]. It is sold in sheets, allowing it to be cut to shape, and provides the largest grafts available (maximum size, 28 cm × 40 cm)[16,17]. In animal studies, a porcine dermal collagen implant produced a substantially weaker inflammatory response and less extensive, less dense adhesions[17,18].

To date, no prospective studies have been performed for which surgical technique in abdominal closure in IH is best, neither in indications about use of PDC mesh (Permacol™, Covidien). Some retrospective studies have shown that the use of a biological prosthesis may improve clinical outcome[19]. Schaffellner *et al*[20] reported an experience of 3 cases of ventral IH after OLT, and they did not observe wound healing disorders or signs of post-operative infections.

Our experience is limited to the use of PDC mesh (Permacol™, Covidien) in patients who underwent liver transplant and HT. In our series, biological mesh has been also used to bridge fascial defects, defined as placement of the PDC between edges of the rectus sheath where primary closure was not feasible; although, the data reported in the literature are not in favor of the use of biological prostheses in bridge repairing[21,22]. Of the 2 cases examined, the first (case 5) had a follow-up that was too short to consider a recurrence of IH, and the other (case 2) showed a good outcome, with no hernia recurrence at 3-year follow-up after surgery.

A grading system to stratify patients according to their risk factors for adverse surgical site occurrences has been proposed by the Ventral Hernia Working Group (VHWG)[23]. In this grading system, the immunosuppressed transplanted patients are classified as grade 2, which suggests that a PDC mesh may improve the outcome[23].

An Italian study described the biological meshes as useful and found a lower rate of infection and recurrence in transplanted patients[24]. Nonetheless, the use of banked fascia lata allografts seemed to provide a biocompatible, safe and effective alternative to other biological meshes[15].

Biological prosthesis is related with decreased number of infections, recurrence and mesh removal, compared to SM. The cases that we have presented show that the use of PDC mesh (Permacol™, Covidien) in transplanted patients may be safe and effective, being careful of the management of perioperative immunosuppression and renal and graft function; although, the cost of the product itself has been the main limiting factor and there is a need for randomized controlled trials for further evaluations. Our experience with PDC has been successful for several reasons. The prostheses have proven to be effective and versatile in repairing hernia defects of different kinds; moreover, in our series, patients did not suffer infections of the prosthesis and no recurrence was observed, even in cases in which they were used to bridge fascial defects. Furthermore, the prosthesis has remained intact even in the years after surgery.

COMMENTS

Background

Incisional hernia (IH) is a common complication after organ transplantation. Considering the immunosuppressed status, transplanted patients may have an increased risk of post-operative morbidity.

Research frontiers

In this study, the use of biological mesh (Permacol™, Covidien) in transplanted patients, emphasizes its effectiveness and versatility, and good tolerance by the immunosuppressed patients.

Innovations and breakthroughs

To date, no prospective studies have been performed for surgical technique in abdominal closure in IH, neither regarding indications about use of porcine dermal collagen mesh.

Applications

IH following abdominal organ transplantation has a high rate and is related to the immunosuppressive status of the patient. Each year, thousands of new transplantations are performed and in the same way the number of IH has increased in these patients.

Terminology

A porcine dermal collagen mesh prosthesis has a greater ability to integrate into tissues, resist bacterial colonization, reduce cytotoxic or allergic reactions, and provide similar functional results.

Peer-review

It is a well-written paper.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Cardiovascular disease: Risk factors and applicability of a risk model in a Greek cohort of renal transplant recipients

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Author contributions: Anastasopoulos NA performed the study, collected data, wrote the paper; Dounousi E designed the study, analyzed data, wrote the paper; Papachristou E contributed important reagents; Pappas C contributed important reagents; Leontaridou E and Savvidaki E collected data; Goumenos D contributed important reagents; Mitsis M designed the study, contributed important reagents.

Institutional review board statement: The study was reviewed and approved by the Institutional Scientific Council and the Review Board of the University General Hospital of Ioannina, 6th District Health (Peloponnese, Ionian Islands, Epirus and Western Greece), Greece. All patients provided written inform consent.

Informed consent statement: All participants were informed of the study and its anonymity and provided written informed consent prior to study enrolment.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at evangeldou@gmail.com. Patients' consent on sharing data was not obtained but the presented data are anonymized and risk of identification is low.

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Abstract

AIM

To investigate the incidence and the determinants of cardiovascular morbidity in Greek renal transplant recipients (RTRs) expressed as major advance cardiac event (MACE) rate.

METHODS

Two hundred and forty-two adult patients with a functioning graft for at least three months and available

data that were followed up on the August 31, 2015 at two transplant centers of Western Greece were included in this study. Baseline recipients' data elements included demographics, clinical characteristics, history of comorbid conditions and laboratory parameters. Follow-up data regarding MACE occurrence were collected retrospectively from the patients' records and MACE risk score was calculated for each patient.

RESULTS

The mean age was 53 years (63.6% males) and 47 patients (19.4%) had a pre-existing cardiovascular disease (CVD) before transplantation. The mean estimated glomerular filtration rate was 52 ± 17 mL/min per 1.73 m^2. During follow-up 36 patients (14.9%) suffered a MACE with a median time to MACE 5 years (interquartile range: 2.2-10 years). Recipients with a MACE compared to recipients without a MACE had a significantly higher mean age (59 years *vs* 52 years, $P < 0.001$) and a higher prevalence of pre-existing CVD (44.4% *vs* 15%, $P < 0.001$). The 7-year predicted mean risk for MACE was 14.6% ± 12.5% overall. In RTRs who experienced a MACE, the predicted risk was 22.3% ± 17.1% and was significantly higher than in RTRs without an event 13.3% ± 11.1% ($P = 0.003$). The discrimination ability of the model in the Greek database of RTRs was good with an area under the receiver operating characteristics curve of 0.68 (95%CI: 0.58-0.78).

CONCLUSION

In this Greek cohort of RTRs, MACE occurred in 14.9% of the patients, pre-existing CVD was the main risk factor, while MACE risk model was proved a dependable utility in predicting CVD post RT.

Key words: Cardiovascular disease; Major advance cardiac event; Risk factors; Risk model; Kidney; Transplantation

Core tip: Cardiovascular disease being the leading cause of death with a functioning graft following renal transplantation. The aim of this study was to investigate the incidence and the determinants of cardiovascular morbidity in prevalent Greek renal transplant recipients (RTRs) expressed as major adverse cardiac event (MACE) rate. Additionally, we examined the applicability of a recently developed risk prediction model in our population. According to our results older age of recipient and pre-existing cardiovascular disease were the main risk factors for MACE. The applied risk model can be used for risk stratification in this database of RTRs.

Anastasopoulos NA, Dounousi E, Papachristou E, Pappas C, Leontaridou E, Savvidaki E, Goumenos D, Mitsis M. Cardiovascular disease: Risk factors and applicability of a risk model in a Greek cohort of renal transplant recipients. *World J Transplant* 2017; 7(1): 49-56 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/49.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.49

INTRODUCTION

Renal transplantation is the treatment of choice for patients with end stage renal disease (ESRD), as it enhances survival and quality of life and is also cost-effective. Nevertheless, cardiovascular disease (CVD) is the leading cause of death with functioning graft in renal transplant recipients (RTRs)[1,2]. Cardiovascular mortality rates in RTRs are significant lower than in an age stratified dialysis population but remain at least twice as high as in an age-stratified sample of the general population[3-5]. Although, successful renal transplantation results in the removal of the hemodynamic and uremic abnormalities associated with dialysis along with the improvement of cardiovascular indices such as left ventricular hypertrophy[6,7], by the time of renal transplantation, the majority of patients already have a heavy burden of atherosclerosis[8].

Knowledge of responsible cardiovascular risk factors has improved in RTRs but precise risk calculation and realistic prediction of a subsequent cardiovascular fatal or non-fatal event still remains a challenge among transplant physicians. In this direction, risk prediction models for cardiovascular events, based on traditional cardiovascular risk factors, have been validated and applied in the general population but their validity remains controversial in RTRs. Accordingly, the Framingham risk score which is a simple and easily accessible tool for the prediction of the risk of a coronary event within the following 10 years has been shown to underestimate cardiovascular risk in RTRs[9]. Given this gap in prediction, transplant-related risk factors have been investigated in large multicenter databases of RTRs, showing that cardiovascular comorbid conditions and risk factors linked to graft function explain much of the variation in coronary heart disease after kidney transplantation[10].

More recently, Soveri *et al*[11] developed and internally validated major adverse cardiac event (MACE) and mortality risk calculators for prevalent RTRs by using Assessment of Lescol in Renal Transplantation (ALERT) data from the extension trial. The same group of investigators subsequently externally validated the risk equation in an international transplant database using RTRs from the patient outcomes in renal transplantation (PORT) cohort and successfully applied the risk estimator in the Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT) and BENEFIT-EXT ended criteria donors trial (BENEFIT-EXT)[12].

In our study, we sought to investigate the incidence and the determinants of cardiovascular morbidity in Greek RTRs expressed as MACE rate. Additionally, we examined the applicability of a validated risk prediction model for MACE in our population.

MATERIALS AND METHODS

Patient characteristics

The full database consisted of 293 RTRs. Adult patients with a functioning graft for at least three months and available data that were followed up on the August 31, 2015 at the two transplant centers of the 6th District Health (Renal Transplant Units of the University Hospital of Patras and University General Hospital of Ioannina), were included in this study. The final analysis included 242 RTRs as for the rest of the patients detailed data regarding coronary heart events and potential CVD risk factors were insufficient.

Recipients' data elements included demographics, clinical characteristics, time on dialysis prior to transplant, history of comorbid conditions such as diabetes [including new onset diabetes after transplantation (NODAT)], hypertension, cardiac ischemic heart disease [myocardial infarction (MI) based on electrocardiography or troponin rise, coronary angioplasty or artery bypass grafting], congestive heart failure, cerebrovascular accident, transient ischemic attack and peripheral artery disease, pre- and post-transplant smoking status and immunosuppression therapy. Laboratory parameters included renal function markers [serum creatinine, 24 h urine protein content (UPR, mg/24 h)], glucose, hemoglobin, lipid profile [total cholesterol (TChol) and low density lipoprotein-(LDL)], C-reactive protein (CRP) and mineral bone disease markers [calcium, phosphate, parathyroid hormone (PTH)]. Estimated glomerular filtration rate (eGFR) was calculated using the four variable modification of diet in renal disease study equation (MDRD)[13]. Clinical characteristics, laboratory parameters, cardiovascular disease and immunosuppressive medications recorded closest to 3 mo post-transplant were used in the analysis. All data were collected retrospectively and were obtained from the patients' medical files.

MACE definition and risk calculation

Major adverse cardiac event was strictly defined as one or more of nonfatal MI and/or invasive coronary artery revascularization (angioplasty or coronary artery bypass grafting), that occurred 3 mo post-transplant in a RTR with a functioning allograft on the cross-sectional database review as of August 31, 2015. Follow-up data regarding MACE occurrence were collected retrospectively from the patients' records. Time to event was defined as time from transplant to the earliest date of MACE.

For prediction of a subsequent MACE, the MACE risk calculator, recently described by Soveri *et al*[11], was applied in the study. It is a seven variable calculator using age, previous cardiac event, history of diabetes mellitus (DM) including NODAT, pre- and post-transplantation smoking habits, number of renal grafts received, serum creatinine and LDL levels to predict 7-year risk of MACE. The area under the receiver operator curve (ROC) in the original study was 0.738[11]. The MACE risk was calculated for all 242 participants (http://www.medsci.uu.se/forskning/Inflammation_och_autoimmunitet/Njurmedicin/Projekt/ risk-calculator/).

This study was approved by the Institutional Scientific Committee and the Review Board of the University General Hospital of Ioannina, 6th District Health (Peloponnese, Ionian Islands, Epirus and Western Greece), Greece.

Statistical analysis

Data are expressed as mean and standard deviation (for normally distributed data), median and interquartile range (IQR) (for not-normally distributed data), or as percentage frequency (for binary variables). Differences in baseline characteristics of RTRs without (group A) and with MACE (group B) were compared by using the Mann Whitney *U* test for continuous variables and the chi-square test for categorical variables.

Univariate and multivariate Cox proportional hazards models were used to assess effects of potential risk factors on the primary outcome, first MACE. Tested covariates in the univariate analysis included, age, sex, pre- and post-transplant smoking status, hypertension, systolic blood pressure (BP), DM, pre-existing CVD, total time on dialysis and transplantation, number of grafts, serum creatinine, UPR, TChol, LDL, PTH, CRP and calculated MACE risk. Risk factors with a P value $\leqslant$ 0.1 in the univariate analysis were included in the multivariate model. In the Cox analysis data were expressed as hazard ratio (b), 95%CI and P value.

The validation for discrimination was performed externally using the Greek cohort of RTRs. The discriminatory power of MACE risk model for identifying patients with from those without the primary outcome was assessed by calculating the area under the ROC curve (c-statistics). A value of AUC of 50% is considered as the threshold of prognostic usefulness.

All the statistical analyses were performed by using a standard statistical package (IBM SPSS Statistics for Windows, version 22.0).

RESULTS

Characteristics of RTRs

Demographics, clinical characteristics and laboratory parameters of the 242 RTRs overall and classified in the two groups are shown in Table 1. In the whole group, the mean age was 53 years and 63.6% were males. The vast majority of RTRs were hypertensive patients (87.6%), 29.4% of them were diabetics (including NODAT) and 47 patients (19.4%) had a positive history of CVD before transplantation. The percentage of active smokers in the whole cohort was almost halved after transplantation (previous smokers 35.1% *vs* current smokers 17.8%, $P < 0.001$). The mean time on dialysis before transplantation was 4.8 ± 3.9 years. Most of the patients received one renal graft (90%), while 23 patients received two grafts and one patient three grafts. The mean eGFR of the functioning graft was 52 ± 17 mL/min per 1.73 m^2 and the median UPR level was 309 mg/24 h (IQR, 167-600 mg/24 h). Immunosuppression regimen was effectively recorded in 209 patients (Table

Table 1 Demographics, clinical characteristics and laboratory parameters in all renal transplant recipients and among the two groups

	Total	Group A	Group B	*P*
No. of patients (*n*, %)	242	206 (85.1)	36 (15)	
Age (yr)	53 ± 12	52 ± 12	59 ± 10	< 0.001
Male sex (*n*, %)	154 (63.6)	126 (61.2)	28 (77.8)	0.056
Previous smoker (*n*, %)	85 (35.1)	69 (33.5)	16 (44.4)	0.2
Current smoker (*n*, %)	43 (17.8)	37 (17.5)	7 (19.4)	0.77
Hypertension (*n*, %)	212 (87.6)	178 (86.4)	34 (94.4)	0.56
Systolic BP (mmHg)	140 ± 18	141 ± 18	137 ± 19	0.25
Diabetes mellitus (*n*, %)	71 (29.3)	57 (27.7)	14 (38.8)	0.17
Previous CVD (*n*, %)	47 (19.4)	31 (15)	16 (44.4)	< 0.001
Time on dialysis (yr)	4.8 ± 3.9	4.7 ± 3.6	5.6 ± 3.8	0.16
Received allografts > 1 (*n*, %)	24 (9.9)	22 (10.7)	2 (5.6)	0.6
Time since transplant (mo)	9.8 ± 5.3	9.7 ± 5.3	10.5 ± 5.2	0.43
Creatinine (mg/dL)	1.45 ± 0.6	1.45 ± 0.57	1.44 ± 0.45	0.95
eGFR-MDRD (mL/min per 1.73 m^2)	51.9 ± 17.2	51.9 ± 17.3	52.1 ± 17.2	0.97
Urine protein (mg/24 h)	309 (167-600)	325 (166-604)	290 (189-374)	0.76
Total cholesterol (mg/dL)	209 ± 33	212 ± 34	194 ± 25	0.08
LDL (mg/dL)	107 ± 35	107 ± 37	103 ± 27	0.56
Haemoglobin (g/dL)	13.1 ± 1.7	13.1 ± 1.7	13.3 ± 1.7	0.61
Calcium (mg/dL)	9.56 ± 0.62	9.6 ± 0.7	9.5 ± 0.4	0.88
Phosphate (mg/dL)	3.06 ± 0.95	3.1 ± 0.9	2.7 ± 1.3	0.08
PTH (pg/mL)	118 ± 89	117 ± 88	127 ± 96	0.55
Glucose (mg/dL)	99 ± 27	98 ± 24	102 ± 39	0.44
CRP (mg/L)	0.8 (0.3-3)	0.8 (0.3-2.6)	0.8 (0.3-3)	0.78

Data are expressed as mean value and standard deviation, median value and interquartile range or absolute frequency and percentage as appropriate. Group A: Without MACE; Group B: With MACE. MACE: Major advance cardiac event; RTRs: Renal transplant recipients; BP: Blood pressure; eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease; LDL: Low density lipoprotein; PTH: Parathyroid hormone.

2). In total, out of the 209 RTRs, 196 (93.8%) received a three-drug regimen (steroids + Calcineurin inhibitor or Everolimus + Mycophenolate mofetil), while 13 received a two-drug regimen.

Of the 242 RTRs, with a mean time since transplantation 9.8 ± 5.3 years, 36 patients (14.9%) suffered a MACE with median time to MACE being 5 years. Recipients who sustained a MACE (group B) compared to recipients with no MACE (group A) post transplantation had a significantly higher mean age (59 years *vs* 52 years, $P < 0.001$), had a higher prevalence of CVD before transplantation (44.4% *vs* 15%, $P < 0.001$) and, with a marginal significance, were more likely to be men (77.8% *vs* 61.2%, $P = 0.056$) (Table 1). Patients among the two groups did not differ significantly as for the other clinical characteristics including smoking, hypertension, diabetes, time on dialysis, number of renal grafts, time with functioning graft, renal function markers and assessed laboratory parameters as well as immunosuppression, antihypertensive and hypolipidemic drugs (Tables 1 and 2).

MACE risk factors and calculator validation

The 242 RTRs included in the study had a mean follow-up of 9.8 years, and 69% of the patients had at least 7 years of follow-up with a functioning graft. Thirty six patients (14.9%) experienced a MACE (1.52 events/100 patient-years) before graft loss with a median time to event 5 years (IQR 2.2-10 years). The 7-year predicted mean risk for MACE by using the 7-variable calculator was 14.6% ± 12.5% in the whole cohort of 242 RTRs. In RTRs who experienced a MACE the predicted risk was 22.3% ± 17.1% and was significantly higher than in RTRs without a subsequent event 13.3% ± 11.1% ($P = 0.003$) (Figure 1).

Table 3 provides the results of the univariate and multivariate analysis with MACE as the dependent variable of interest. In the univariate Cox regression analysis we found that the calculated MACE risk (HR = 1.04, 95%CI: 1.02-1.06) was associated with a higher risk of a subsequent event. When the risk factors of the model and other factors were tested separately, older age (HR = 1.05, 95%CI: 1.02-1.10), male sex (HR = 0.45, 95%CI: 0.20-0.99) and pre-existing CVD (HR = 3.63, 95%CI: 1.88-7.01) were associated with an increased risk of MACE. In the multivariate model, pre-existing CVD was the main independent predictor for the occurrence of MACE (HR = 2.86, 95%CI: 1.45-5.62), while older age (HR = 1.05, 95%CI: 1.01-1.08) was associated with an increased risk of MACE as well.

The discrimination ability of the model in the Greek cohort of RTRs was good with an area under the ROC curve of 0.68 (95%CI: 0.58-0.78) (Figure 2).

DISCUSSION

The incidence of MACE before graft loss in our clinical database of RTRs was 14.9% with a median time to event 5 years. Recipients who suffered a MACE were older and had higher prevalence of pre-existing CVD. The first attempt to apply an externally validated risk MACE model in a Greek cohort of RTRs showed that the model can be used for risk stratification in this

Table 2 Immunosuppression and cardiovascular disease therapy in all renal transplant recipients and differences between the two groups

	Total RTRs	Group A	Group B	*P*
Steroids	199 (95.2)	167 (95)	32 (97)	0.61
Mycophenolate mofetil	207 (99)	175 (99.4)	32 (97)	0.18
Tacrolimus	56 (26.8)	49 (27.8)	7 (21.2)	0.43
Cyclosporine	146 (69.9)	122 (69.3)	24 (72.7)	0.69
Everolimus	6 (2.9)	4 (2.3)	2 (6.1)	0.23
CCB	134 (55.4)	116 (56.3)	18 (50)	0.65
Beta-adrenergic blockers	151 (62.4)	128 (62.1)	23 (63.9)	0.86
ARBs/ACEi	131 (54.1)	117 (56.7)	14 (38.9)	0.35
Diuretics	56 (23.1)	46 (21.8)	10 (27.8)	0.58
Other antihypertensive drugs	53 (21.9)	48 (23.3)	5 (13.9)	0.46
Hypolipidemic drugs	154 (63.6)	134 (65)	20 (55.6)	0.49

Immunosuppression therapy was recorded for 209 RTRs. Cardiovascular disease therapy was recorded in all 242 RTRs. Data are expressed as absolute frequency and percentage. Hypolypidemic drugs included statins, fibrates, ezetimibe or combinations of the aforementioned. Group A: With MACE; Group B: Without MACE. MACE: Major advance cardiac event; CCB: Calcium channel blockers; ARBs: Angiotensin receptor blockers; ACEi: Angiotensin converting enzyme inhibitors; RTRs: Renal transplant recipients.

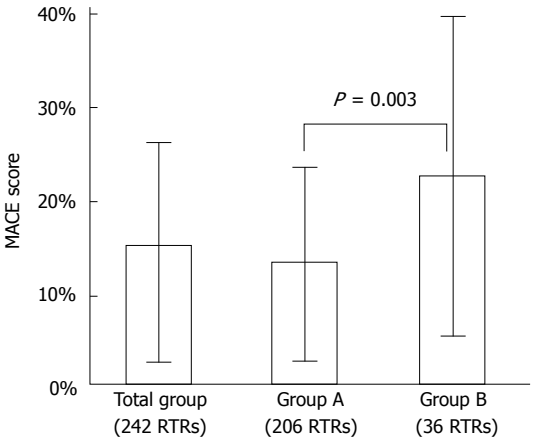

Figure 1 Calculated major advance cardiac event risk score in the 242 renal transplant recipients and in the two groups. MACE score for all the RTRs, group A, defined as RTRs without MACE and group B, defined as RTRs with MACE, is respectively 14.6% ± 12.5%, 13.3% ± 11.1% and 22.3% ± 17.1%. MACE: Major advance cardiac event; RTRs: Renal transplant recipients.

population.

Disproportionate increased cardiovascular burden is true since the early stages of chronic kidney disease, further increases during dialysis and although renal transplantation removes hemodynamic and uremic abnormalities associated with dialysis, the vast majority of RTRs with a functioning graft die due to a MACE. In our study, RTRs with a functioning graft who suffered a MACE had higher prevalence of CVD before transplantation, with pre-existing CVD being the most significant risk factor for MACE in this cohort. As regards traditional cardiovascular risk factors such as smoking, hypertension, diabetes and lipid profile their prevalence did not significantly differ between the two groups in our database of RTRs and separately each one could not predict the occurrence of a MACE. Our findings are in accordance with the results of an early study by Kasiske *et al*[14] showing that the strongest risk factors were pre-existing coronary heart disease, cerebrovascular and peripheral vascular, which were associated with an increase of three to nine times in cardiovascular risk. In this study, there was not a relation between traditional risk factors (smoking, hypertension, or dyslipidemia) and CVD in 1000 RTRs. In the more recent PORT study, a large scale clinical database of 23575 RTRs, it was found that among the significant predicting factors for MACE were age, male sex and pre-existing CVD, whereas traditional modifiable cardiovascular risk factors were very poor predictors of cardiac events[10]. On the other hand, the investigators of the ALERT study used *post-hoc* analyses and identified the determinants of specific cardiovascular endpoints such as MI being associated with age, hyperlipidemia and diabetes[8].

Unconventional and transplant-related risk factors, including immunological and non-immunological ones further increase the risk of CVD after transplantation[10,15]. In particular, the large multicentre PORT study found that a number of transplant-specific variables, such as delayed graft function, acute rejection and eGFR could predict cardiac events[10]. However, interventional studies which tried to normalize unconventional modifiable risk factors, such as haemoglobin and homocysteine, failed to reduce occurrence of CVD in RTRs[16,17]. Moreover, immunosuppressive drugs prescribed to RTRs, mainly corticosteroids and calcineurin inhibitors (cyclosporine, tacrolimus), which possess diabetogenic and atherogenic side effects exacerbate established cardiovascular risk factors such as dyslipidemia, hypertension, and diabetes[18].

Given the fact that traditional, non-traditional and transplant-related risk factors separately only partly can explain the increased burden of CVD and that the interplay between all these factors seems to be the core of the increased cardiovascular risk in RTRs many groups of investigators have tried to apply established risk models or to create new risk calculators in order to accurate predict a subsequent cardiovascular event in this population. In particular, the use of the Framingham risk score in RTRs underestimates cardiovascular risk,

Table 3 Univariate and multivariate analysis of risk factors for major advance cardiac event in renal transplant recipients

Variables (units of increase)	Univariate		Multivariate	
	b (95%CI)	*P*	b (95%CI)	*P*
MACE risk (1%)	1.04 (1.02-1.06)	< 0.001		
Age (1 yr)	1.05 (1.02-1.10)	0.001	1.05 (1.01-1.08)	0.005
Sex (male reference)	0.45 (0.20-0.99)	0.05	0.58 (0.28-1.37)	0.18
Previous smoker	1.51 (0.73-2.92)	0.21		
Current smoker	1.0 (0.44-2.29)	0.99		
Systolic BP (1 mmHg)	1.01 (0.99-1.02)	0.61		
DM	1.53 (0.78-2.98)	0.21		
Previous CVD	3.63 (1.88-7.01)	< 0.001	2.86 (1.45-5.62)	0.006
Number of grafts (first graft reference)	0.50 (0.12-2.02)	0.33		
Total time on dialysis and transplantation (1 yr)	0.99 (0.92-1.01)	0.33		
Creatinine (1 mg/dL)	0.90 (0.48-1.68)	0.74		
Urine protein (1 mg/24 h)	0.99 (0.99-1.00)	0.28		
Total cholesterol (1 mg/dL)	0.99 (0.99-1.00)	0.3		
LDL (1 mg/dL)	0.99 (0.98-1.01)	0.46		
Hemoglobin (1 g/dL)	1.14 (0.93-1.40)	0.21		
PTH (1 pg/mL)	1.00 (0.99-1.00)	0.25		
CRP (1 mg/L)	1.01 (0.92-1.09)	0.88		

MACE: Major advance cardiac event; BP: Blood pressure; DM: Diabetes mellitus; CVD: Cardiovascular disease; LDL: Low density lipoprotein; PTH: Parathyroid hormone; CRP: C-reactive protein.

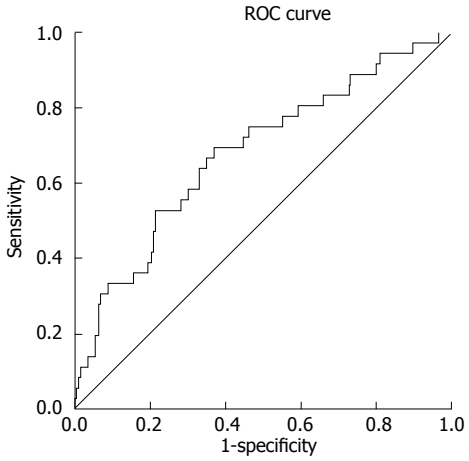

Figure 2 Discrimination. Receiver operating characteristics for major adverse cardiac event in the cohort of RTRs. Area under the curve is 0.68 (95%CI: 0.58-0.78). RTRs: Renal transplant recipients; ROC: Receiver operator curve.

although the addition of renal function in the Framingham equation was shown to improve the prediction of MACE[9,19]. More recently, Soveri *et al*[11] used data from the ALERT trial[8], a large scale multicenter trial and constructed a seven year, seven variable MACE risk equation with an area under the ROC curve of 0.738[11]. Subsequently they externally validated the 7-year risk calculator for discrimination and calibration in the PORT study database, which was an observational study[10]. Although the calculator was derived from the ALERT trial, a transplant population with moderate CVD risk, it was validated in the high risk RTRs of the PORT study and found suitable for this population with an area under the ROC curve of 0.740[12].

In this study we applied the MACE risk calculator in our cohort of RTRs from two transplant centers in Western Greece. According to the results the predicted risk was significantly higher in RTRs who experienced a MACE than in RTRs without a subsequent event and the calculator by preserving the discrimination ability is suitable for risk stratification in our population. The incidence of MACE in our database was 14.9%, while the incidence of MACE in ALERT trial was 11.8%. It should be noted that there were important differences in the composition of populations among the two studies as ALERT trial included moderate CVD risk RTRs from North Europe and Canada.

Nevertheless, our study has potential limitations which should be taken into consideration. First of all, this is a retrospective study conducted in a small sample population. Additionally, we did not report on data about graft survival and patients' cardiovascular and total mortality as we included only RTRs with a functioning kidney graft at the time of the cross-sectional database review. Finally, we did not assess the possible effect of transplant-related risk factors, such as delayed graft function, acute rejection, on the occurrence of MACE.

In conclusion, pre-existing CVD was found to be the most important risk factor of a subsequent MACE, which necessitates holistic approach prevention strategies of CVD starting early in the course of chronic kidney disease. In our study, a validated MACE risk calculator was successfully tested in a Greek cohort of RTRs and was found to be suitable for the prediction of MACE in this patient group. Considering the fact that RTRs are a heterogenous population as well as the identification of new emerging transplant related risk factors, patient approach should always be individualized. Nevertheless, the application of cardiovascular risk prediction equations potentiates increased level of alertness among caregivers as well as improved interventional strategies in high risk

patients.

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COMMENTS

Background

Kidney transplantation offers a significant improvement in all the cardiovascular parameters of end stage renal disease (ESRD) patients, reduces mortality risk and boosts quality of life.

Research frontiers

To determine the risk factors for cardiovascular disease after kidney transplantation and validate a major advance cardiac event (MACE) risk model to a Greek renal transplant recipients (RTRs) cohort.

Innovations and breakthroughs

In this study, the authors found that older age, pre-existing cardiovascular disease (CVD) and MACE risk score, were significant predictors of post-transplant cardiovascular risk. So long as, there are modifiable components to the risk factors/scores, it is the belief that prevention of CVD early in chronic kidney disease along with control of these factors in ESRD patients and RTRs, could possible reduced cardiovascular burden to some degree.

Applications

The externally validated equation can be used in any appropriate RTR population to calculate MACE risk.

Terminology

MACE was defined as one or more of nonfatal myocardial infarction and/or invasive coronary artery revascularization (angioplasty or coronary artery bypass grafting).

Peer-review

It is a well-written study about the event of cardiovascular disease after renal transplantation.

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ORIGINAL ARTICLE

Retrospective Study

Dengue in renal transplant recipients: Clinical course and impact on renal function

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Institutional review board statement: The study was reviewed and approved by Hospital Universitário Walter Cantídio/Universidade Federal do Ceará.

Informed consent statement: As the data collected was anonymous and retrospective, the Institutional Board waived it from this research.

Conflict-of-interest statement: The authors report no any conflicts of interest. This study is the result of our daily work in the HUWC renal transplant ward and received no funding whatsoever.

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Abstract

AIM

To present clinical characteristics from renal transplant recipients with dengue fever and its impact on graft function.

METHODS

We retrospectively evaluated 11 renal transplant recipients

(RTR) with dengue infection confirmed by laboratory test, between January 2007 and July 2012, transplanted in the Renal Transplant Center of Walter Cantídio University Hospital from Federal University of Ceará.

RESULTS

Positive dengue serology (IgM) was found in all patients. The mean time between transplant and dengue infection was 43 mo. Fever was presented in all patients. Nine patients presented with classical dengue and two (18%) with dengue hemorrhagic fever. All cases had satisfactory evolution with complete recovery of the symptoms. The time for symptom resolution varied from 2 to 20 d, with an average of 9 d. An increase of creatinine after the infection was observed in three (27.2%) patients with no clinically impact on the kidney graft function.

CONCLUSION

RTR with dengue infection seems to have a clinical presentation and evolution similar to those seen in the general population, with no long-term damage to patient and to the graft.

Key words: Kidney; Renal; Transplant; Dengue; Clinical; Brazil

Core tip: Dengue is a viral arthropod-borne disease transmitted by mosquitoes of the genus Aedes, mainly Aedes aegypti. The kidney is the most transplanted solid organ in the world with approximately 79000 transplants performed annually. Data are lacking on the clinical presentation of dengue in renal transplant recipients. We retrospectively evaluated 11 renal transplant recipients with dengue infection confirmed by laboratory test, between January 2007 to July 2012, transplanted in the Renal Transplant Center of a tertiary hospital in northeast Brazil.

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INTRODUCTION

Dengue is an arthropod-borne disease caused by a *Flaviviridae* virus transmitted by mosquitoes of the genus *Aedes*, mainly *Aedes aegypti*. Most of dengue cases are asymptomatic, which explains the high number of under diagnosed cases[1-3]. Ceará is a hyperendemic state, in 2015; there were 55400 confirmed dengue cases and 72 deaths in Ceará State[4]. In the last years, organ transplant programs have been expanding in Brazil, with increase of specialized centers and number of organ donations. In 2015, 5556 kidney transplants were conducted in the country, of which 264 were in Ceará[5].

Kidney transplant patients who travel to or live in endemic areas are under higher risk of acquiring the disease. However, few dengue cases are reported in this population. Dengue viral infection in the immunosuppressed population may be more severe as compared with immunocompetent hosts, with reports of fatal cases in our environment[6]. Conversely, severe dengue infection, witch is hypothesized to be the result of the immune-mediated mechanisms, may not occur in transplant recipients who have a muted immune response. Only a few case series of dengue in renal transplant recipients have been reported, with most describing a mild disease[7-10].

The aim of our study was to determine the clinical presentation of dengue in kidney transplant patients and the impact of this disease in patients and allograft outcomes.

MATERIALS AND METHODS

We retrospectively evaluated dengue in renal transplant patients in the Renal Transplant Center of Walter Cantídio University Hospital (HUWC) from Federal University of Ceará, in Northeast of Brazil. The ethics committee of the institution approved the study. They were diagnosed in the period from January 2007 to July 2012. The inclusion criteria were all kidney transplant patients who had dengue confirmed by laboratory test attended in our center with clinical suspicion. Laboratory diagnosis of dengue was made by IgM enzyme-linked immunosorbent assay (ELISA) using commercially available kits or by polymerase chain reaction (PCR). The HUWC Renal Transplant Center works since 1977; it has performed 1255 transplants, with a mean of 100 transplants per year in the last 5 years, and 95% of the donators are deceased.

Patients were classified according to the World Health Organization (WHO) classification from 1997[11], which was then adopted by the Brazilian Ministry of Health[12]. Since 2014, Brazil started adopting the WHO 2009 new classification for dengue[13].

The classic dengue fever (DF) was characterized by a febrile condition that lasts 7 d, followed by at least two unspecific signs and symptoms (headache, malaise, retro-orbital pain, exanthema, myalgia and arthralgia). Dengue hemorrhagic fever (DHF) was characterized by increased vascular permeability leading to a bleeding diathesis or disseminated intravascular coagulation, with at last one of the following signs: Hemorrhagic manifestations, hemoconcentration due to capillary leak, hipoproteinemia, and pleural effusion or ascites. Dengue shock syndrome (DSS) was all severe cases that do not follow the WHO DHF criteria, and when the classical dengue classification is unsatisfactory, presence of one of the following findings characterizes the clinical condition:

Table 1 Characteristics of kidney transplant patients with dengue diagnosis in the period from January 2007 to July 2012 *n* (%)

Characteristics	*n* = 11
Age in years-mean (variation)	41.3 (19-61)
Female gender	7 (63.3)
Transplant time in years - mean (variation)	3.6 (1 mo-9 yr)
Deceased donor	9 (82.0)
Thymoglobulin induction	4 (36.6)
Immunosuppressive regimens	
PRED + TAC + MMF	4 (36.3)
PRED + CYA + AZA	2 (18.1)
PRED + TAC + MPS	2 (18.1)
TAC + MMF	1 (9.0)
PRED + AZA + SRL	1 (9.0)
CYA	1 (9.0)
Rejection before dengue	3 (27.2)
Clinical findings	
Fever	11 (100.0)
Myalgia	10 (91.0)
Headache	6 (54.5)
Abdominal pain	3 (27.2)
Bleedings	3 (27.2)
Nauseas and vomiting	2 (18.1)
Postural hypotension	2 (18.1)
Pleural effusions	2 (18.1)
Laboratory outcomes	
Thrombocytopenia	9 (81.8)
Severe Thrombocytopenia (< 50000/mm^3)	4 (36.6)
Leucopenia	4 (36.6)
Hemoconcentration	4 (36.6)
Transaminases increase (AST;ALT)	7 (63.6)
AST value, mean (variation) UI/L	130 (17-360)
ALT value, mean (variation) UI/L	100 (14-230)
Hospitalization	9 (81.8)
Hospitalization time in d, mean(variation)	14.2 (3-45)
Classification of dengue cases	
Classical dengue	9 (81.8)
DHF	2 (18.1)
Dengue with complication	0

PRED: Prednisone; TAC: Tacrolimus; MMF: Mycophenolate mofetil; CYA: Cyclosporine; AZA: Azathioprine; MPS: Mycophenolate sodium; SRL: Sirolimus; AST: Aminotransferase alanine; ALT: Aminotransferase aspartate; DHF: Dengue hemorrhagic fever.

several changes in the nervous system; cardiorespiratory dysfunction; liver failure; thrombocytopenia equal or lower than 20000/mm^3; digestive hemorrhage; pleural effusions; global leukocyte count equal or lower than 1000/m^3; suspicious dengue case evolving to death.

Software Excel 2010 was used for data tabulation and analysis. Clinical and laboratory data were obtained from the revision of patients' kidney post-transplant ambulatory follow-up forms and medical records.

RESULTS

Among the 416 medical records of the assessed patients, from January 2007 to July 2012, we found 27 cases with clinical suspicious dengue, with only 11 confirmed through laboratory exams. Among these 11 patients, seven (60%) were female with mean age of 41.3 years old (19 to 61 years old). All patients lived in an endemic area, in the city of Fortaleza, State of Ceará, Brazil.

All cases were confirmed through the ELISA test for IgM antibody detection. One patient also presented positive polymerase chain reaction (PCR). Two patients received the graft from living donors and other nine were from deceased donors. In three patients, there was graft rejection before dengue diagnosis. The mean time between kidney transplant and dengue infection was of 43 mo. The most used immunosuppressive regimen was the association of tacrolimus, prednisone, and mycophenolate mofetil (36.3%). The immunosuppressive drugs, especially mycophenolate mofetil, had its doses reduced and in some cases and temporarily suspended in severe leucopenia and thrombocytopenia.

The clinical and laboratory characteristics, as well as the patients' evolution, are summarized in Tables 1 to 3. All patients had fever varying from 37.8 ℃ to 40 ℃; headache and myalgia were also present in most cases. Among 11 patients from the study, 9 showed thrombocytopenia, which was seen right in the moment of patient's admission, with absolute mean value of 135390/mm^3. Only four patients (3, 9, 10 and 11) achieved levels lower than 50000/mm^3, one of whom (Patient 3) needed platelet transfusion due to level below 10000 and presence of active gastrointestinal bleeding. The lowest mean count of patients' platelets was of 90818/mm^3. Four patients (36.4%) presented hemoconcentration (hematocrit increase > 20%) throughout the infection. Only four subjects showed light leucopenia, with a mean of leukocytes of 5103/mm^3. The minimum level of leukocytes had an average of 3898/mm^3. One patient developed pancytopenia (Patient 9), with severe leukopenia (775 leukocytes) and sepsis secondary to urinary tract infection, and needed critical care support.

Seven patients had increased liver enzymes above three times the reference value of Alanine transaminase (ALT) and Aspartate transaminase (AST). The AST maximum value registered was 360 UI/L, with mean of 130 UI/L, and maximum ALT registered was 230 UI/L, with mean of 100 UI/L. Nine patients had classical dengue and two followed DHF criteria (Patients 7 and 9) through the old WHO classification. Using the most recent classification, we found 3 cases of dengue with warning signs (Patients 1, 3 and 6). Hemoconcentration, blood hypertension, persistent abdominal pain, and pleural effusion were seen in such patients. There were two cases with severe dengue (Patients 7 and 9) due to the presence of postural hypotension and shock.

All cases had satisfactory evolution with complete recovery of the symptoms. The time for symptom resolution varied from 2 to 20 d, with an average of 9 d. Only two patients needed hospitalization, with a mean of hospital stay of 9 d. Among the hospitalized patients, only one (patient 9) was admitted in intensive care unit due to urinary sepsis, not directly associated with dengue infection.

Table 2 Clinical and kidney graft evolution of 11 kidney transplant patients with dengue *n* (%)

Characteristics	*n* = 11
Resolution of symptoms	11 (100)
Death	0
Time for resolution of symptoms in d, mean (variation)	9 (2-20)
Creatinine before dengue, mean(variation)	1.35 mg/dL (0.8-2.2)
Increase of creatinine > 20% and < 50% of baseline	3 (27.2)
Increase of creatinine > 50% of baseline	3 (27.2)
Creatinine after dengue, mean (variation) mg/dL	1.1 (0.8-1.7)
Creatinine 1 mo after dengue, mean (variation) mg/dL	1.3 (0.8-1.8)

With regard to kidney function, the mean creatinine value of patients at admission time was 1.35 mg/dL (0.8 to 2.2 mg/dL). The mean creatinine value at infection time was of 2.5 mg/dL, and the maximum creatinine value presented was 10 mg/dL, which was seen in Patient 7, who developed acute kidney failure with the need of transitory dialytic support. After the infection, values varied from 0.85 to 1.75 mg/dL with an average value of 1.33 mg/dL. An increase of creatinine after the infectious condition was observed in three (27.2%) patients. Nevertheless, there was no clinically significant impact on the kidney graft function, which returned to the baseline creatinine in almost all patients after 1 mo of symptom resolution.

DISCUSSION

In the present study, we found 11 dengue cases in kidney transplant patients throughout almost 6 years, in a single center located at a hyperendemic area. Based on the high number of cases reported in our State in such period[4], we expected a higher number of cases in this specific population. However, it is very difficult to assess the real prevalence of the disease in these patients, since most of the cases present as flu-like syndrome with spontaneous resolution, with high sub-notification. The largest Brazilian casuistic of dengue in kidney transplant patients was reported by Azevedo *et al*[9] with 27 cases in 10 years achieved through inquiries sent to 182 renal transplant centers in the country. Comparing to our study, we can see a much more expressive casuistic comprised of 11 cases in only one center, with almost half of the evaluated period. The largest series of cases published until now was conducted by Nasim *et al*[8] with 102 cases diagnosed from January 2009 to December 2010, in a kidney transplant center in Karachi, Pakistan, which is a hyperendemic country for the disease. In 2015, Costa et al published a dengue series with 10 cases, this article was produced with data from a tertiary hospital in the same city from our own, not surprisingly, it showed similar results[10]. After literature review, we found several other series of cases, such as those from Singapore[14] (six cases) and India[7] (eight cases), among many others. Most of them described dengue as a benign disease in this population.

Dengue asymptomatic infection is commonly seen in Brazil. A serologic survey carried out in the city of Salvador (BA), Brazil, in 1998[15], showed a 69.7% seroprevalence in a sample with 1515 people.

When these data are extended for the city population, 560000 people could have been infected with the virus, which is different from the only 360 cases that were reported in the same period[15].

The mean time of dengue symptoms, especially thrombocytopenia, in our study was of 9 d, which is higher than the general population. This fact was also seen by Nasim *et al*[8] with mean thrombocytopenia duration of 11 d compared to 3.6 d in the general population. This longer evolution can be associated with use of immunosuppressive medications and slower viral clearance that is seen in immunocompromised patients. Another important fact of Nasim *et al*[8] study was the absence of fever in 20% of their patients. This was mainly seen in subjects using larger immunosuppressive doses, thus concealing a notable manifestation of the disease and making its diagnosis more difficult. This finding has not been seen in our area, in which 100% of our patients had fever.

In our study, thrombocytopenia was found in most of the cases, with only 33.6% in the severe scale. Most of our patients presented the classical form of the disease with only two (18%) evolving to DHF, without any deaths. Comparing with data from the general population in our state, we observed a 0.2% incidence of DHF in the year of 2013, which is much lower than that seen in our study. This can be justified by the small size of our analyzed population and by the non-inclusion of other 16 suspected cases without confirmation. Similarly, Azevedo *et al*[9] reported only 1 DHF case among the 27 dengue cases. However, in their sample, one patient died, corresponding to a 3.7% mortality, which is similar to ours. Nassim *et al*[8] also noticed an 11% incidence of DHF (12 cases among the 102 reported ones).

Several hypotheses attribute the severe forms of the disease to an immunopathological process mediated by T cells and interleukins[16].

The immunosuppressive drugs given to transplant patients may modify both cellular and humoral immune system, which possible explain a more benign clinical evolution of dengue seeing in this population[17].

Table 3 Characteristics of kidney transplant patients diagnosed with dengue, from January 2007 to July 2012

	Patient										
	1	2	3	4	5	6	7	8	9	10	11
Age	52	58	58	61	31	41	25	32	41	19	36
Gender	Female	Male	Male	Female	Female	Male	Male	Female	Female	Female	Female
Pre-Tx baseline diseases	CGN	CGN+HN	FSG	DN	M G	IN	FSG	SEL	HN	BWT	DN
Tx period until dengue	1 yr	10 mo	1 mo	4 mo and a half	3 yr	3 yr and 6 mo	7 yr	2 yr and a half	5 yr	7 yr and 8 mo	9 yr
Kind of donator	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased	Alive	Deceased	Alive	Deceased
Induction	Thymoglobulin + methyl-prednisolone	Basiliximab + methyl-prednisolone	Thymoglobulin + methyl-prednisolone	Thymoglobulin + methyl-prednisolone	Thymoglobulin + methyl-prednisolone	Basiliximab + methyl-prednisolone	Methyl-prednisolone	Basiliximab + methyl-prednisolone	Methyl-prednisolone	Methyl-prednisolone	Methyl-prednisolone
IMS drugs on use (during dengue period)	T + M + P	P + C + A	T + M + P	T + M	T + M + P	P + C + A	T + M + P	T + M + P	T + M + P	P + A + S	C
Dengue symptoms	Fever, myalgia, headache	Fever, myalgia	Fever, myalgia, abdominal pain, bleedings (enter-orrhagia)	Fever, myalgia, headache	Fever, headache, abdominal pain	Fever, myalgia	Fever, myalgia, headache, hypotension, postural hypotension	Fever, myalgia, headache, vomiting, abdominal pain	Fever, myalgia, hypotension, postural hypotension	Fever, myalgia, headache	Fever, myalgia, vomiting
Bleeding	No	No	Yes	No	No	No	Yes	No	Yes	No	No
Dengue diagnosis	IgM^+	IgM^+	IgM^+	IgM^+	IgM^+	IgM^+	IgM^+	IgM^+ and serum PCR	IgM^+	IgM^+	IgM^+
Hemoconcentration	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No
Pleural effusions	Yes	No	No	No	No	No	No	No	Yes	No	No
Hospitalization time	3 d	None	15 d	8 d	13 d	None	1 mo and a half	4 d	20 d (ICU)	10 d	10 d
Evolution	Symptom resolution in 3 d	Symptom resolution in 20 d	Symptom resolution in 15 d	Symptom resolution in 8 d	Symptom resolution in 5 d	Symptom resolution in 6 d	Symptom resolution in 16 d	Symptom resolution in 2 d	Symptom resolution in 5 d	Symptom resolution in 10 d	Symptom resolution in 8 d
Baseline creatinine before dengue	1	1.1	2	1.1	2.2	0.85	1.4	1.45	1.6	1.25	1
Maximum creatinine throughout dengue	2	1.2	2	1.1	2.2	1	10	1.8	3.3	2.1	1.4
Creatinine immediately after dengue	1	1.175	1.5	1.1	1.75	0.85	1.6	1.55	1.5	1.5	1.2
Creatinine 1 mo after dengue	0.9	1.2	1.7	0.8	1.7	0.8	1.8	1.5	1.8	1.6	1

Tx: Transplant; IMS: Immunosuppressive; PCR: Polymerase chain reaction; ICU: Intensive care unit; CGN: Chronic glomerulonephritis; HN: Hypertensive nephropathy; DN: Diabetic nephropathy; FSG: Focal segmental glomerulo-sclerosis; EL: Systemic eritematous Lupus; MG: Mesangiocapillary glomerulonephritis; IN: IgA nephropathy; BWT: Bilateral Wilms Tumor; T: Tacrolimus; M: Mycophenolate; P: Prednisone; C: Cyclosporine; A: Azathioprine; S: Sirolimus.

In agreement with other studies, even though a higher percentage of severe forms of the disease have been found, we observed in our cases that dengue tends to follow the usual course of the disease. Thus, we must pay attention to thrombocytopenia, even if no fever is seen in this group of patients, since it could be dengue virus infection with sub-clinical presentation.

In our study, we could not find any information about previous dengue infection in these subjects, neither through medical record nor laboratory exams, like the detection of IgG antibodies. It is also important to notice that in some patients who live in endemic areas, there is a persistence of IgM, which makes it even harder to diagnose acute infection[9].

Nasim *et al*[8] demonstrated that 25% of the severe cases seen were in primary infections, which can be associated with the immunosuppression given to these patients that predisposes more severe clinical conditions. Azevedo *et al*[9] also found a higher mortality (3.7%) than that of the general population, associated with clinical conditions of secondary bacteremia with sepsis.

Azevedo *et al*[9] also showed a transitory dysfunction of the kidney graft in the course of dengue. After using the level of serum creatinine as an assessment of the kidney function, we also found in our sample an increase of the mean value of creatinine level from 1.35 to 2.5 mg/dL in the infectious period. Although one of our patients reached creatinine levels of 10 mg/dL, with the need of dialytic support, the baseline creatinine levels were completely re-defined, thus no damage was seen in grafts at medium or long term in both studies. Recovery of all our patients was satisfactory with a mean value of 1.1 mg/dL in the post-infectious period. This standard behavior might not be due to the direct lesion of the virus in the kidney parenchyma, because there has not a study yet that proves this fact; however, this might happen due to factors associated with dehydration/hypovolemia caused by capillary leakage, vomiting, or bleedings[18].

Prasad *et al*[7] also pointed out the transitory dysfunction of the kidney graft with complete recovery after infection in kidney transplant patients that did not evolve to death. However, Nasim *et al*[8] found a 66.7% rate of kidney graft dysfunction, which was higher in patients who already had some degree of impairment. Both the percentage of increase in the serum creatinine level and the duration of return rate to baseline of kidney function were higher in subjects that developed the severe forms of dengue. In our study, we found the same behavior with regard to the temporary dysfunction of the kidney graft in the infectious period.

The present study had several limitations and potential bias. This was a retrospective series of cases with data collected through a review of medical records, without follow-up of the patients by the investigator. In addition, many patients with suspicion of the disease were not included in the study due to lack of laboratory confirmation with high rate of sub-diagnosis.

The renal transplant recipients with dengue infection have a clinical presentation and evolution similar to those seen in the general population. Due to the lack of serological surveys in this population and non-performance of routine serological screenings in asymptomatic patients, we do not know the real prevalence of the disease in these patients. Thus, assessing the impact on disease morbidity and mortality on these patients, based on our series of cases, was not possible.

Nonetheless, as seen here and in other studies, development of most of the cases seemed benign without evidence of higher mortality. Likewise, renal function is generally well preserved, with transitory graft dysfunction seen in most of the patients, without negative impact lifelong. It is very clear that dengue hypothesis should always be in the differential diagnosis of fever and thrombocytopenia or leucopenia in kidney transplant patients who lived or were from endemic areas.

Hence, new studies with better design and a larger amount of patients are needed to find the dengue impact on kidney transplant patients.

COMMENTS

Background

Dengue is an arthropod-borne disease caused by a *Flaviviridae* virus transmitted by mosquitoes of the genus *Aedes*, mainly *Aedes aegypti*. Most of dengue cases are asymptomatic. However the immunosuppressive drugs given to renal transplant patients may modify both cellular and humoral immune system, thus, modifying the disease characteristics and prognosis.

Research frontiers

Dengue fever in endemic in most tropical areas, the kidney is the most transplanted solid organ in the world. Data on renal transplant recipients with dengue fever is limited. This case series is important to update the clinical experience.

Innovations and breakthroughs

This is a well-documented case series of Brazilian renal transplant recipients with dengue fever and serves as an update of previous published cases.

Applications

This study concluded that renal transplant recipients with dengue infection have a clinical presentation and evolution similar to those seen in the general population and should be managed as regular patients.

Terminology

RTR: Renal transplant recipients; DF: Classic dengue fever; ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction; DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome.

Peer-review

A very informative case series of post kidney transplant recipients who developed dengue fever. Basically they were managed as regular patients and had similar outcomes.

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ORIGINAL ARTICLE

Retrospective Study

International kidney paired donation transplantations to increase kidney transplant of O group and highly sensitized patient: First report from India

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Abstract

AIM

To report the first international living related two way kidney paired donation (KPD) transplantation from India which occurred on 17th February 2015 after legal permission from authorization committee.

METHODS

Donor recipient pairs were from Portugal and India who were highly sensitized and ABO incompatible with their spouse respectively. The two donor recipient pairs had negative lymphocyte cross-matching, flow cross-match

and donor specific antibody in two way kidney exchange with the intended KPD donor. Local KPD options were fully explored for Indian patient prior to embarking on international KPD.

RESULTS

Both pairs underwent simultaneous uneventful kidney transplant surgeries and creatinine was 1 mg/dL on tacrolimus based immunosuppression at 11 mo follow up. The uniqueness of these transplantations was that they are first international KPD transplantations in our center.

CONCLUSION

International KPD will increases quality and quantity of living donor kidney transplantation. This could be an important step to solving the kidney shortage with additional benefit of reduced costs, improved quality and increased access for difficult to match incompatible pairs like O blood group patient with non-O donor and sensitized patient. To the best of our knowledge this is first international KPD transplantation from India.

Key words: Kidney paired donation; International kidney paired donation; Living donor kidney transplantation

Core tip: Kidney paired donation (KPD) has rapidly increased the access to living donor kidney transplantation (LDKT) in the last decade. The participation in the international kidney exchange registries will expand the donor pool for kidney transplantation. We report first Indian international living related KPD transplantation which occurred on 17th February 2015 after legal permission from authorization committee between a pair from Portugal and India who were highly sensitized and ABO incompatible with their spouse respectively. International KPD will increases quality and quantity of LDKT. This could be an important step to solving the kidney shortage with additional benefit of reduced costs, improved quality and increased access for difficult to match incompatible pairs like O blood group patient with non-O donor and sensitized patient.

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INTRODUCTION

There is growing incidence of chronic kidney disease in India and worldwide[1,2]. There is imbalance between organ supply and demand. Indian chronic kidney disease registry reported in 2010 that only 2% of end stage renal disease patients received kidney transplantation. The majority (61%) of patients did not afford renal replacement therapy[2]. There is lack of compliance to maintenance dialysis therapy (32% on hemodialysis and 5% on peritoneal dialysis) due to poverty and lack of uniform access to renal replacement therapy resulting in higher morbidity and mortality[1,2]. It is difficult to expand deceased donor kidney transplantation in India due to various problems including lack of awareness. The ABO compatible living donor kidney transplantation (LDKT) is the cost effective way for Indian end stage renal disease patients[3-5].

Kidney paired donation (KPD) has rapidly increased the access to LDKT in the last decade[3-11]. KPD avoids the cost and complications of desensitization therapies for ABO incompatible and human leukocyte antigen (HL A) incompatible LDKT with best long term outcome. Currently, national KPD program exist in many countries including South Korea, The Netherlands, United States, Canada, Australia, United Kingdom, and Spain[6,11]. Twenty percent increase in KPD transplants can be achieved with domino paired donation. ABO blood type O group patients and highly sensitized patients have less chance to get LDKT in kidney exchange program[11]. The large donor pool could increase transplant rate for such patients. The participation in the international kidney exchange registries will expand the donor pool for LDKT[12,13].

MATERIALS AND METHODS

We report international two way KPD transplantations which occurred on 17th February 2015 after legal permission from authorization committee between a donor recipient pair from Portugal and India who were highly sensitized and ABO incompatible with their spouse respectively. Authorization committee permission was obtained for this overseas donor from Government of Portugal, authorization committee of our hospital and the state authorization committee of Government of Gujarat, India. The lymphocyte cross-matching (LCM), T and B cell flow cytometry crossmatch (FCM) and donor-specific antibodies (DSA) titers were performed for immunolocial compatibility. Lymphocyte cross-matching > 20%, T cell and B cell FCM above 50 and 100 median channel shift (MCS) and donor-specific antibody > 1000 mean fluorescent intensity (MFI) were considered positive and contraindication for transplantation in our transplant center. The patient from Portugal had lymphocyte cross-matching of 90% positive, T and B cell FCM were 186, 231 MCS respectively with his wife as donor. The class 1 donor-specific antibody was 11600 MFI (Table 1).

Patient 1 and 2 were registered with our KPD registry due to sensitization and ABO incompatibility respectively. The manual allocation was performed by a Nephrologist under supervision of authorization committee to ensure proper allocation. Sensitized patients, O group patients

Table 1 Human leukocyte antigen data of patient and donor

	A		B		Bw		Cw		DR B1		DR B3, 4, 5		DQ B1	
Patient 1	1	24	15	37	4	6	6	8	10	12	52	-	5	7
Donor 2	1	11	40	-	6	-	15	-	8	11	52	-	7	4
Patient 2	2	33	15	51	6	-	1	12	4	8	53	-	7	8
Donor 1	1	68	15	55	6	-	7	0	7	14	52	53	2	6

Patient 1: Donor specific antibody in mean fluorescence intensity with donor 1, A68 = 9870; B55 = 7736; CW7 = 11600 and no donor specific antibody with donor 2; Patient 2: No donor specific antibody with donor 1 and 2.

with non-O donor, HLA match, dialysis time, donor age and waiting time were considered in this allocation. We demonstrated absence of DSA in the each recipient using data of blood groups, HLA antibody profile of recipients and HLA report of donor and recipient. All the three immunologic tests (LCM, FCM, and DSA) were negative and acceptable with intended KPD donor for both the recipients. Thus virtual cross-match approach has maximized the matching in sensitized patients in KPD program.

The donor-recipient pairs have negative LCM, FCM and DSA in two way kidney exchange with the intended KPD donors. There was no DSA even at low titer prior to transplant. Both the donors were of similar age group with similar creatinine, glomerular filtration rate and renal vessel anatomy (Table 2). Each pair underwent uniform pre-transplant evaluation of patient and donor by transplant team costing 1000 USD and $\leqslant$ 2 wk time. The total cost of kidney transplantation in our hospital is 5000 USD. Both the donors and patients underwent simultaneous donor nephrectomy and the transplantation surgery in our single center.

Immunosuppression

Induction immunosuppressive regimen included rabbit thymoglobulin (1.5 mg/kg single dose) and methyl prednisolone (500 mg/d × 3 d) and prednisolone, tacrolimus, and mycophenolate sodium (360 mg four times per day) were immunosuppressive agents in maintenance regimen. Tacrolimus trough level was 8-10 ng/mL during first 3 mo after transplantation and 4-8 ng/mL thereafter. Prednisolone dose was $\leqslant$ 20 mg/d during first 3 mo after transplantation and 5-10 mg/d thereafter. Patients were started on prophylaxis for pneumocystis jirovecii pneumonia (trimethoprim-sulfamethoxazole for 12 mo), fungal infections (fluconazole 100 mg/d for 3 mo) and cytomegalovirus infection (valganciclovir 450 mg/d for 3 mo).

RESULTS

Table 2 showed the demographics and outcome of two-way kidney exchange. Table 1 showed HLA data of patient and donor. Both pairs underwent uneventful kidney transplant surgeries and at 11 mo of follow up serum creatinine is 1 mg/dL on tacrolimus based immunosuppression. After transplantation monthly DSA for 3 mo and at 6, 9 mo were negative in sensitized patient.

DISCUSSION

The key feature of our case report is that this was the first international KPD transplantations in our center. The Portugese patient came to our transplant center for directed kidney transplantation with his wife as kidney donor. He came to our transplant unit with the information about our transplant center from the social media website and one of his friends was working in our hospital. On the initial pre-transplant evaluation, he was found to be sensitized with his wife as kidney donor. They were not registered in Portugese kidney sharing scheme. The mis-matched antigens against which sensitized Portugese recipient had DSA were avoided. The anti-A antibody titer in blood group O Indian recipient with husband as donor was 1:256. ABO incompatible kidney transplantation was not considered due to patients was having pulmonary tuberculosis, higher cost and risk of infections. The single center KPD program which is commonly practiced in India has inherent limitations to expand the donor pool. Each state, region and the entire country of India needs a more robust, organized kidney sharing scheme and efforts should be made to establish a national/regional pool of kidney sharing registry as is the case with the European, North American and other developed countries. There is no national KPD program in India. Local and regional kidney sharing options were fully explored for the Indian patient prior to embarking on international kidney sharing.

The ethical challenges

As per transplant human organ act 2014 (India), authorization committee of hospital or district or state can approve legal permission of KPD transplantation when the kidney donors are near relatives of the swap recipients. In our report both the donors are near relatives (spouses).

The authorization committee permission was obtained for an overseas donor from Government of Portugal, hospital and the state authorization committee of Government of Gujarat. All the steps were taken to ensure adherence to transplant human organ act and the Declaration of Istanbul principles with the exchange of equivalent kidneys in size, function, anatomy, immunology and donor age. This allowed exchange of equivalent kidney between donor-recipient pairs with positive cross-

Table 2 Demographics and outcome of two way kidney exchange

	Patient 1	Patient 2	Donor 1	Donor 2
Patient data				
Age (yr)	40	30		
Gender	Male	Female		
Original disease - ESRD	Hypertension	Hypertension		
ABO blood group	A	O		
Dialysis duration (mo)	12	12		
Weight (kg)	68	40		
Original donor relation	Wife	Husband		
Reason for Joining KPD	Sensitized	ABO incompatible		
Time from KPD registration to find KPD donor (wk)	2	36		
Time from KPD donor to transplant	4 wk	4 wk		
Desensitization	No	No		
State	Portugal	Rajasthan, India		
Donor data				
Age (yr)			36	33
Gender			Female	Male
Weight (kg)			60	60
ABO blood group			O	A
Glomerular filtration rate (right/left)			56/54	54/54
Creatinine (mg/ dL)			0.6	0.7
Renal vessel (right/left)			1 artery and vein on each side	1 artery and vein on each side
Laparoscopic donor nephrectomy			Left	Left
Surgical details and outcome				
Warm ischemia time (s)			150	117
Cold ischemia time (min)			60	90
Anastomosis time (min)			43	35
Intraoperative urine (mL)			1800	500
Kidney transplant date			17 Feb 2015	17 Feb 2015
Creatinine (mg/dL)			1	1
Follow- up (mo)			11	11

KPD: Kidney paired donation.

match barrier to transplantation in Portugese pair and ABO incompatibility barrier to transplantation in Indian pair. Thus both the pairs get the reciprocal sharing of benefit. The health and well-being of Portugese living donor and patient was monitored at regular interval for early diagnosis of any medical or surgical problems due to donation and transplantation. This was performed by sharing of medical reports performed at local laboratory by email communication and in person at regular interval. The administration of such a program should be ensured with support of all transplantations centers and transplant societies using computer software, uniform allocation algorithm, central and dedicated coordination and team work. All should act today with team work for better tomorrow. International kidney paired exchange is usually done in the context of reciprocal sharing agreements - which does not exist in this case. However this is one step close to start such program between 2 or more countries to pool their respective KPD cohorts.

There are encouraging reports of international KPD transplantation all over the world[6,8]. It will increase the LDKT opportunity for sensitized and O group patients by direct benefit of increase in donor pool and benefit from differences in heterogeneity of blood types in the population, antigens and antibodies profile. Garonzik-Wang *et al*[14] reported international kidney exchange between the United States and Canada in a 10-way domino chain transplantation which were performed between September 2009 and July 2010. KPD sharing between United States and Canada was logistically possible due to close geographic location, similar language and culture. Three international KPD transplantations between May 2013, and March 2014 were reported in Turkey where national KPD program increased LDKT by 5%[15]. The international organ exchange from deceased donors substantially contributed (7.2% of deceased donor transplantations) to the Swiss transplant activity during the period 2009-2013[16]. The cold ischemia time < 8 h does not significantly affect long term graft survival. Therefor transport of living donor kidney can be preferred over donor travel in multicenter simultaneous KPD program where cold ischemia time < 8 h[17,18]. Despite prolonged cold ischemia time for interstate exchanges, the Australian kidney exchange program preferred to transport kidney over the travel of living kidney donor[19].

Indian society of organ transplantation in collaboration with international mentorship should take the lead role in expansion of KPD as it will increase LDKT > 25%. There should be is a formal agreement between 2 or more countries to pool their respective KPD cohorts. Together transplant community can make a significant difference in the lives of kidney patients around the

globe. International KPD will be better that national exchange which will be better than regional exchanges or single center kidney exchanges to expand the donor pool. The large donor pool will increase the transplant rate in kidney exchange. It allows an optimized donor-recipient match, due to an expansion of the donor and recipient pool. It will further optimize potential of this modality to increase transplantation of O group patients and sensitized patients.

In international KPD, there are several potential sources of increasing the donor pool by assembling a database of incompatible pairs, including more two-way exchanges, longer domino chains instead of short chains (2-way or 3-way pairs), integrating list exchange and non-directed donors with exchange among incompatible patient-donor pairs and lastly in near future integrating compatible pairs. Living donor KPD transplant also reduces the waiting list in deceased donor kidney transplantation for those who have no living donor available.

Global kidney exchange

In 2010 Indian chronic kidney disease registry reported that 61% of stage 5 end stage renal disease population did not receive dialysis or kidney transplant mainly due to poverty and lack of access[2]. Poor compatible donor-recipient pairs (A blood group patient and O blood group donor) in developing world could not undergo kidney transplantation due to poverty and lack of health insurance care despite having healthy willing kidney donor. Many donor-recipient pairs in developed world (O blood group patient and A blood group donor) could not undergo kidney transplantation due to immunological barriers despite availability of health insurance care. These two pairs could exchange kidney with each other after legal permission in global kidney exchange to overcome financial and immunological barriers to transplantation. The cost of both kidney transplantations is paid by the health insurance payer of the developed country. Legal and logistical problems should be addressed for the implementation of global kidney exchange. This provides gift of life for the poor patients who would otherwise die due to lack of kidney transplant despite having kidney donor. The advantages of global kidney exchange are reduced costs, increased access to kidney transplantation and improved quality of match[20,21]. More studied are required to address willingness of patients, health care professionals to participate in global kidney exchange. To ensure success, an effort is required to standardize transplant principals, practice, policies and legislation among various countries.

International KPD will increase quality and quantity of LDKT. It would best balance the principles of utility and justice. Our study showed that international KPD could be an important step to solving the kidney shortage with additional benefit of reduced costs, improved quality and increased access for difficult to match donor recipient pair like O blood group patient with non-O donor and sensitized patient. To the best of our knowledge this is first international KPD transplantation from India.

COMMENTS

Background

Kidney paired donation (KPD) has rapidly increased the access to living donor kidney transplantation (LDKT) in the last decade. KPD avoids the cost and complications of desensitization therapies for ABO incompatible and human leukocyte antigen incompatible LDKT with best long term outcome.

Research frontiers

The participation in the international kidney exchange registries will expand the donor pool for kidney transplantation.

Innovations and breakthroughs

Here the authors reported first international 2-way KPD transplantations from India.

Applications

International KPD will increase quality and quantity of LDKT. It would best balance the principles of utility and justice. The study showed that international KPD could be an important step to solving the kidney shortage with additional benefit of reduced costs, improved quality and increased access for difficult to match donor recipient pair like O blood group patient with non-O donor and sensitized patient. To ensure success, an effort is required to standardize transplant principals, practice, policies and legislation among various countries.

Terminology

LDKT: Living donor kidney transplantation; KPD: Kidney paired donation; DDKT: Deceased donor kidney transplantation; DSA: Donor specific antibody.

Peer-review

An important positive step in attempting to increase the number of acceptable kidney donor-recipient pairs using two collaborating countries. What might be added to the brief text is some assessment of the time and expense of conducting the pretransplant typing and evaluations required to select willing donor-recipient pairs.

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SYSTEMATIC REVIEWS

Lobar lung transplantation from deceased donors: A systematic review

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Abstract

AIM

To systematically review reports on deceased-donor-lobar lung transplantation (ddLLTx) and uniformly describe size

matching using the donor-to-recipient predicted-total lung-capacity (pTLC) ratio.

METHODS

We set out to systematically review reports on ddLLTx and uniformly describe size matching using the donor-to-recipient pTLC ratio and to summarize reported one-year survival data of ddLLTx and conventional-LTx. We searched in PubMed, CINAHL *via* EBSCO, Cochrane Database of Systematic Reviews *via* Wiley (CDSR), Database of Abstracts of Reviews of Effects *via* Wiley (DARE), Cochrane Central Register of Controlled Trials *via* Wiley (CENTRAL), Scopus (which includes EMBASE abstracts), and Web of Science for original reports on ddLLTx.

RESULTS

Nine observational cohort studies reporting on 301 ddLLTx met our inclusion criteria for systematic review of size matching, and eight for describing one-year-survival. The ddLLTx-group was often characterized by high acuity; however there was heterogeneity in transplant indications and pre-operative characteristics between studies. Data to calculate the pTLC ratio was available for 242 ddLLTx (80%). The mean pTLCratio before lobar resection was 1.25 ± 0.3 and the transplanted pTLCratio after lobar resection was 0.76 ± 0.2. One-year survival in the ddLLTx-group ranged from 50%-100%, compared to 72%-88% in the conventional-LTx group. In the largest study ddLLTx (n = 138) was associated with a lower one-year-survival compared to conventional-LTx (n = 539) (65.1% *vs* 84.1%, P < 0.001).

CONCLUSION

Further investigations of optimal donor-to-recipient size matching parameters for ddLLTx could improve outcomes of this important surgical option.

Key words: Lobar lung transplantation from deceased donors; Cadaveric lobar lung transplantation; Lung size matching; Primary graft dysfunction; Survival

Core tip: Deceased-donor-lobar lung transplantation (ddLLTx) is an important and so far underutilized surgical option for lung transplant candidates with small chest cavities. It is only performed at a few specialized centers and frequently performed in high urgency cases. Outcome is acuity-driven and is expected to improve as more elective cases are done. The size matching decision for ddLLTx is complex and based on varying parameters. Systematically using the predicted Total Lung Capacity ratio as the size matching tool could help to identify sizing thresholds to maximize the risk/benefit balance for ddLLTx.

Eberlein M, Reed RM, Chahla M, Bolukbas S, Blevins A, Van Raemdonck D, Stanzi A, Inci I, Marasco S, Shigemura N, Aigner C, Deuse T. Lobar lung transplantation from deceased donors: A systematic review. *World J Transplant* 2017; 7(1): 70-80 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/70.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.70

INTRODUCTION

Lung transplantation (LTx) is an established therapy for appropriately selected patients suffering from end-stage lung disease. 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 restrictive lung diseases (LAS diagnoses group D)[1,2]. As a consequence, wait-list mortality rates are again rising despite higher wait-list transplant rates compared to the pre-LAS era[3]. 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[3,4]. This often affects patients with cystic fibrosis and pulmonary fibrosis[4]. 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[5].

Three operative solutions exist to increase the utilization of available deceased donors for patients with small chest cavities[6-8]. These include: (1) deceased lobar lung transplant (ddLLTx)[6,8]; (2) split lung transplant (a form of ddLLTx, where the left lung allograft is divided and then each resulting lobe is implanted into the two hemithoraces)[9]; and (3) peripheral atypical resection. ddLLTx was first described by Bisson *et al*[8] in 1994. Subsequently, several single center reports on ddLLTx have been published[6,7,9-16].

The best size-matching parameter remains debatable. Chest X-ray parameters, calculation of the ratio between donor and recipient heights, calculation of the ratio of predicted total lung capacity (pTLC) between donor and recipient (pTLCratio) and estimation based on visual inspection in the operating room are commonly used strategies[17]. Amongst these the pTLCratio has the largest evidence base to support its use[17-30].

Therefore, we set out to systematically review reports on ddLLTx with the aim to describe the size matching between donor and recipient uniformly using the pTL-Cratio[31-33]. Specifically we intended to compare the pTLCratio that would have occurred using the entire donor lungs ($pTLCratio_{Full}$) to the pTLCratio that was transplanted *via* the lobar transplantation ($pTLCratio_{Lobar}$). The second objective was to perform a systematic review and meta-analysis of one-year survival after ddLLTx.

MATERIALS AND METHODS

Data sources

A health sciences librarian ran extensive literature searches in PubMed, CINAHL *via* Ebsco, Cochrane Database of

Systematic Reviews *via* Wiley (CDSR), Database of Abstracts of Reviews of Effects *via* Wiley (DARE), Cochrane Central Register of Controlled Trials *via* Wiley (CENTRAL), Scopus (which includes EMBASE abstracts), and Web of Science. No filters for date, language, or any other parameter were used. The PubMed strategy described below was modified as needed for use in other electronic databases. Full search strategies are available upon request.

The search strategy was for PubMed: (((((“Lung Transplantation”[Mesh] OR lung transplant*[Text Word] OR lung graft*[text word])) OR ((“Tissue and Organ Procurement”[Mesh] OR “Tissue Donors”[Mesh] OR “Organ Transplantation”[Mesh] OR organ procurement*[text word] OR tissue procurement*[text word] OR tissue donor*[text word] OR organ donor*[text word] OR organ transplant*[text word]) AND (Lung[Mesh] OR Lung[text word] OR Lungs[text word]))))) AND ((lobar[text word] OR lobe*[text word]))) AND ((“Cadaver”[Mesh] OR Cadaver*[text word] OR Dead[text word] OR Nonliving[text word] OR Non-living[text word])).

Study selection criteria

For an identified study to be included in the systematic review it had to: (1) involve human participants; (2) have full text available in English; and (3) report on recipients of ddLLTx. For an identified study to be included in the meta-analysis it had to meet the following additional criteria: one year survival data is available for: (1) a conventional lung transplant cohort (either in same study or from a contemporary publication from the same center); and (2) a ddLLTx cohort. When overlapping data, *i.e.,* several publications from same center, study selection favored most recent data. The corresponding authors of the studies selected for inclusion in the systematic analysis were contacted to seek unpublished updated center data.

Study quality assessment

The methodological quality of the selected studies was evaluated using criteria from the United States Preventative Services Task Force.

Data extraction

Data extracted included author name, year of publication, location of center, number of patients in ddLLTx cohort, number of patients in conventional-LTx cohort, study-years, indication for transplantation and acuity at time of transplant. Outcome data extracted included rate of primary graft dysfunction (PGD), ICU and hospital length of stay (LOS), FEV_1(%-predicted) at 6 mo and peak FEV_1, survival at 1 year and 5 years.

Assessment of donor to recipient size matching

The parameter(s) used for the size matching were extracted for each study. For all studies that did not report recipient pTLC (pTLCrecipient), full donor pTLC ($pTLC_{donor\,Full}$) and donor pTLC after lobar resection ($pTLC_{donor\,Lobar}$) the study authors were contacted and asked to provide: recipient age, height and sex (to calculate pTLCrecipient[18]); donor age, height and sex (to calculate $pTLC_{donor\,Full}$[18]) and information on donor lobes transplanted [to calculate $pTLC_{donor\,Lobar}$ = ($pTLC_{donor\,Full}$) × (number donor lung segments transplanted/19)] for each donor and recipient pair. From this the pTLCratio that would have occurred using the entire donor lungs was calculated as $pTLC_{ratio\,Full}$ = $pTLC_{donor\,Full}$/pTLCrecipient. The pTLC ratio that was actually transplanted *via* the lobar transplantation was calculated as $pTLC_{ratio\,Lobar}$ = $pTLC_{donor\,Lobar}$/pTLCrecipient, Figure 1.

Definitions of primary and secondary outcomes

The primary outcome of interest was one-year-survival. Secondary outcomes were occurrence of PGD, ICU and hospital LOS, FEV_1 (6 mo and peak) and 5-year survival.

Statistical analysis

We expressed $pTLC_{ratio\,Full}$ and $pTLC_{ratio\,Lobar}$ as means ± standard deviation for the entire cohort and stratified by transplant indication and transplant center. We assessed for differences in mean $pTLC_{ratio\,Full}$ and $pTLC_{ratio\,Lobar}$ between transplant indications and centers by one-way ANOVA analysis of variance, with bonferroni adjustment for multiple comparisons. We extracted dichotomous data for one-year-survival form all studies reporting number of patients with events and total participants. We performed a meta-analysis and pooled the one-year-mortality data to calculate relative risks (risk ratios, RRs) with 95% confidence interval (CI). We used the statistic of I^2 to test for the heterogeneity, with I^2 < 25%, 25%-75% and > 75% to represent low, moderate and high degree of inconsistency, respectively. In analyses, if the heterogeneity was low then we used a fixed-effect model, or else applied the random-effect model. We performed a sensitivity analysis, in which a study was removed at a time while the rest was analyzed, to evaluate whether the results could have markedly been affected by that single study. We used Egger’s linear regression test to find a potential publication bias. All analyses were performed with Stata (Version10.0, Stata Corporation, College Station, TX, United States). A 2-tailed *P* value of less than 0.05 was considered statistically significant.

RESULTS

Search results

Our search identified 155 unique citations. Of these, 32 abstracts and 18 full-text publications were assessed (Figure 2). Nine studies fulfilled our inclusion criteria for final review[6,7,10-16] (Table 1). Reviewer agreement on selection of abstracts was 100% (K = 1.0) and on inclusion of articles for the final review it was 100% (K = 1.0).

Study range and characteristics

All nine reports were single center retrospective cohort studies. Seven reports originated in Europe[6,7,10,12,14-16],

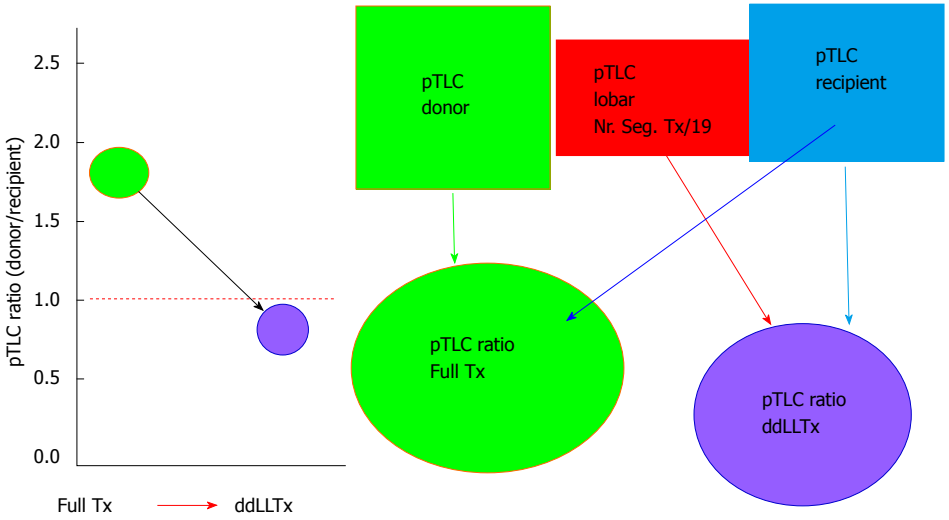

Figure 1 The parameter(s) used for the size matching were extracted for each study. For all studies that did not report recipient pTLC (pTLCrecipient), full donor pTLC (pTLCdonorFull) and donor pTLC after lobar resection (pTLCdonorLobar) the study authors were contacted and asked to provide: Recipient age, height and sex (to calculate pTLCrecipient); donor age, height and sex (to calculate pTLCdonorFull) and information on donor lobes transplanted [to calculate pTLCdonorLobar = (pTLCdonorFull) × (number donor lung segments transplanted/19)] for each donor and recipient pair. From this the pTLCratio that would have occurred using the entire donor lungs was calculated as pTLCratioFull = pTLCdonorFull/pTLCrecipient. The pTLC ratio that was actually transplanted *via* the lobar transplantation was calculated as pTLCratioLobar = pTLCdonorLobar/pTLCrecipient. ddLLTx: Deceased-donor-lobar lung transplantation.

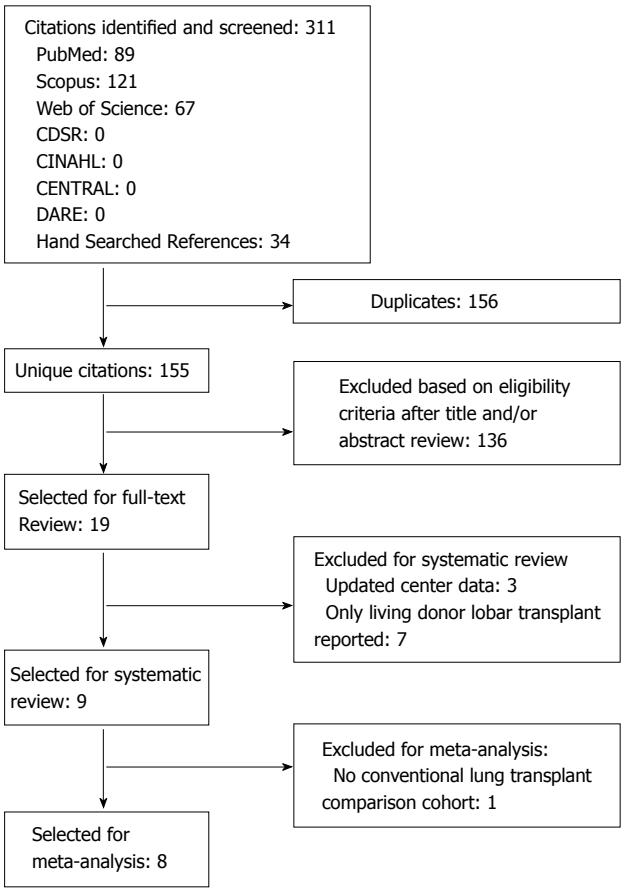

Figure 2 PRISMA diagram detailing study selection.

one in Australia[11], and one in North America[13]. The study period ranged from 1988-2012. Four centers reported on fewer than 10 recipients of ddLLTx, two had 20-35 ddLLTx recipients, and two reported 50 or more ddLLTx cases.

Indication for transplant and acuity

In the nine studies including 301 ddLLTx, the indications were available in eight studies (295 ddLLTx) and were predominantly cystic fibrosis (39%) and interstitial lung diseases (35%) (Figure 3). Six of the nine studies qualified the acuity of ddLLTx and these were often characterized by high acuity (Table 1).

Donor to recipient size matching

The size matching parameter used was the pTLCratio in five of nine studies, often in combination with visual inspection of fully inflated allograft and recipient chest cavity size in the operating room. Donor and recipient height and CXR characteristics were used in 2 studies (Table 2). Two studies reported $pTLCdonor_{Full}$, $pTLCdonor_{Lobar}$ and pLTCrecipient[6,11]. Data to calculate these parameters were provided for five additional studies[7,12,13,15,16] and $pTLCdonor_{Full}$, $pTLCdonor_{Lobar}$ and pLTCrecipient was then available for 242 of 301 donor-recipient pairs of ddLLTx (Figure 1). The mean $pTLCdonor_{Full}$ was 6.42 ± 1.0 L and after lobar resections was reduced to $pTLCdonor_{Lobar}$ 3.83 ± 0.8 L. The mean pLTCrecipient was 5.27 ± 1.0 L. The mean $pTLCratio_{Full}$ was 1.25 ± 0.3 and was reduced to a mean $pTLCratio_{Lobar}$ 0.76 ± 0.2. Stratified by transplant indication, the interstitial lung diseases group had the lowest mean $pTLCratio_{Full}$ (1.12 ± 0.03), which was significantly lower than COPD (1.37 ± 0.3) and CF (1.33 ± 0.3) (Figure 4). After lobar resections the transplanted mean $pTLCratio_{Lobar}$ was also the lowest in interstitial lung diseases group (0.70 ± 0.1) and significantly lower than COPD (0.87 ± 0.3) and CF (0.79 ± 0.2) (Figure 4). Stratified by transplant centers the $pTLCratio_{Full}$ ranged from 1.15 ± 0.4 to 1.68 ± 0.4 (Figure 5). The transplanted $pTLCratio_{Lobar}$ ranged between transplant centers from 0.69 ± 0.1 to 0.94 ± 0.3

Table 1 Study characteristics

Author	Year	Country	Center	Time	Nr	Indication/diagnosis					Acuity
						CF	IPF	IPAH	COPD	Other	
Couetil	1997	France	Paris	1993-1994	7	3	1	2	1	-	Not reported
Espinosa	2010	Spain	Reina Sofia	2003-2009	6	-	-	-	-	-	2 ICU, 2 Hosp, 2 Outpatient
Deuse	2011	Germany	Hamburg	2009-2012	7[1]	2	5	-	-	-	1 ECMO
Marasco	2012	Australia	Alfred	1990-2012	27[1]	6	5	-	4	12	Not reported
Inci	2012	Swiss	Zurich	2000-2012	23	10	8	-	3	2	3 ECMO, 1 MV,
Shigemura	2013	United States	UPMC	2010-2012	35[1]	4	17	-	-	14	7 ECMO, 9 MV, LAS 72-94
Mitilian	2013	France	Foch	1988-2012	50	35	7	-	3	5	2 ECMO
Aigner	2014	Austria	Vienna	2001-2012	138[1]	48	46	8	16	20	27 MV, 18 ECMO
Stanzi	2014	Belgium	Leuven	2005-2012	8	8	-	-	-	-	All outpatients

[1]Updated data provided. Nr: Number; CF: Cystic fibrosis; IPF: Idiopathic pulmonary fibrosis; IPAH: Idiopathic pulmonary arterial hypertension; OB: Obliterative bronchiolitis; COPD: Chronic obstructive pulmonary disease; ECMO: Extracorporeal membrane oxygenation; ICU: Intensive care unit; Hosp: Hospitalized; MV: Mechanical ventilation; LAS: Lung allocation score.

Table 2 Size matching parameters and characteristics

Center	Size matching parameter	pTLC donor (full)	pTLC donor (lobar)	pTLC recipient	pTLCratio (full)	pTLCratio (lobar)
Paris	pTLCratio	6.91 ± 0.7	3.11 ± 0.3	4.28 ± 1.1	1.69 ± 0.4	0.76 ± 0.5
Reina Sofia	Not reported	Not provided	Not provided	Not provided	Not provided	Not provided
Hamburg[1]	pTLCratio	6.96 ± 1.2	3.64 ± 0.7	5.27 ± 1.0	1.35 ± 0.3	0.69 ± 0.1
Alfred[1]	pTLCratio, CXR	6.82 ± 1.2	4.81 ± 1.1	5.12 ± 1.4	1.44 ± 0.5	0.94 ± 0.3
Zurich[1]	Visual inspection, height	7.21 ± 0.8	4.45 ± 0.7	5.04 ± 0.9	1.48 ± 0.4	0.90 ± 0.2
UPMC[1]	Height, CXR, visual inspection	6.28 ± 0.7	3.76 ± 0.7	5.22 ± 0.8	1.22 ± 0.9	0.73 ± 0.5
Foch	pTLCratio, visual inspection	Not provided	Not provided	Not provided	1.65	Not provided
Vienna[1]	pTLCratio, visual inspection	6.19 ± 1.1	3.80 ± 0.9	5.45 ± 1.0	1.15 ± 0.2	0.70 ± 0.1
Leuven[1]	Visual inspection, height	6.70 ± 1.2	4.11 ± 0.3	4.42 ± 0.4	1.52 ± 0.4	0.93 ± 0.3

[1]Centers provided additional size matching data for this systematic review. pTLC: Predicted total lung capacity; CXR: Chest X-ray.

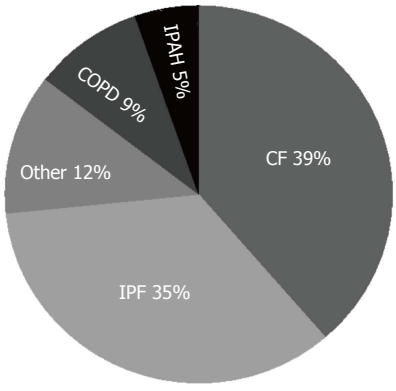

Figure 3 Pie chart of transplant indications. IPAH: Idiopathic pulmonary arterial hypertension; COPD: Chronic obstructive pulmonary disease;IPF: Idiopathic pulmonary fibrosis; CF: Cystic fibrosis.

(Figure 5).

Primary outcome: One year survival

Nine studies (301 patients) provided data on one-year survival after ddLLTx (Table 3). One-year survival in the ddLLTx groups ranged from 50%-100%. We identified survival information for a conventional-LTx comparison group within the same institution for eight studies. One-year survival was 72%-88% in the conventional-LTx groups, which was not statistically different within each individual study, with the exception of the largest study, where ddLLTx was associated with a higher risk of mortality (65.1% *vs* 84.1% one-year survival, $P < 0.001$)[15].

In pooled analysis of unadjusted data from eight studies, ddLLTx-recipients ($n = 284$) had a relative risk of one-year mortality of 1.85 (95%CI: 1.52-2.25, $P < 0.001$) compared with conventional-LTx-recipients ($n = 2777$) (Figure 6). There was low heterogeneity as indicated by an I^2 of 0% ($P = 0.47$). In an analysis for possible publication bias by performing a linear regression of the standard normal deviate against precision (Egger test) showed that the intercepts did significantly deviate from zero ($P = 0.007$, for one-year-survival), indicating the presence of publication bias. Visual inspection of the funnel plot showed asymmetry (Figure 7). This also indicated the presence of publication bias, limiting the interpretation of the meta-analysis.

Secondary outcomes

Five studies described the occurrence of primary graft dysfunction (PGD) and described rates ranging between 13%-56% in ddLLTx (Table 3). One study reported ddLLTx

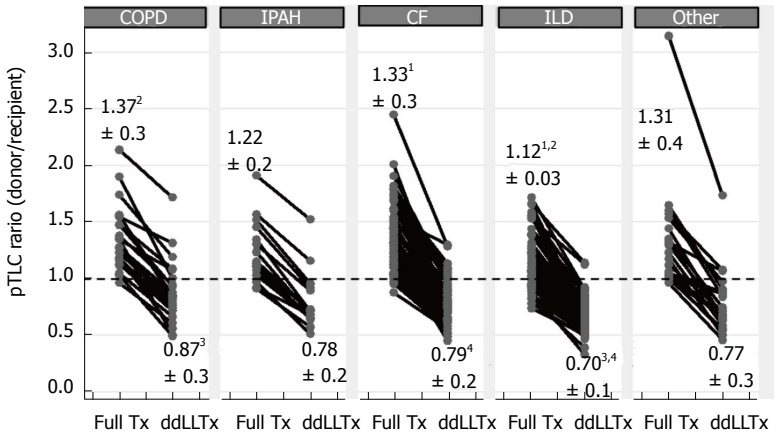

Figure 4 Donor to recipient size matching based on the donor to recipient predicted total lung capacity ratio, stratified by transplant indication. The predicted total lung capacity (pTLC) ratio that would have occurred using the entire donor lungs was calculated as $pTLCratio_{Full}$ = $pTLCdonor_{Full}$/pTLCrecipient. The pTLC ratio that was actually transplanted *via* the lobar transplantation was calculated as pTLCratioLobar = $pTLCdonor_{Lobar}$/pTLCrecipient, where $pTLCdonor_{Lobar}$ = [$pTLCdonor_{Full}$] × [number donor lung segments transplanted/19]. Each grey circle pair connected with black line represents one donor/recipient pair. The numbers represent the mean pTLCratio ± standard deviation. CF: Cystic fibrosis; IPF: Idiopathic pulmonary fibrosis; IPAH: Idiopathic pulmonary arterial hypertension; OB: Obliterative bronchiolitis; COPD: Chronic obstructive pulmonary disease; Tx: Lungtransplant; ddLLTx: Deceased donor lobar lung transplant. [1,2]Indicate a significant difference in pTLCratioFull (one-way-anova P-value < 0.05) of pairwise comparisons between transplant indications, after Bonferroni adjustment for multiple comparisons; [3,4]Indicate a significant difference in $pTLCratio_{Lobar}$ (one-way-anova *P*-value < 0.05) of pairwise comparisons between transplant indications, after Bonferroni adjustment for multiple comparisons.

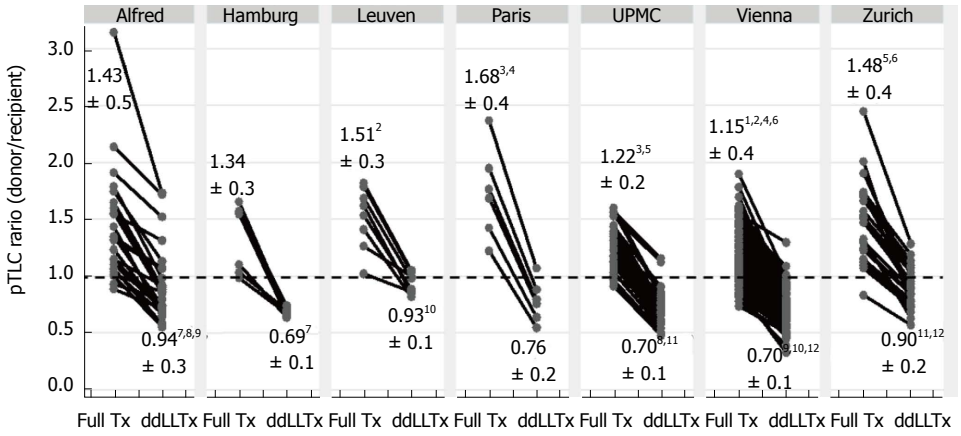

Figure 5 Donor to recipient size matching based on the donor to recipient predicted total lung capacity ratio, stratified by transplant center. See figure legend 3 for further details. [1,2,3,4,5,6]Indicate a significant difference in pTLCratioFull (one-way-anova *P*-value < 0.05) of pairwise comparisons between transplant centers, after Bonferroni adjustment for multiple comparisons; [7,8,9,10,11,12]Indicate a significant difference in pTLCratioLobar (one-way-anova *P*-value < 0.05) of pairwise comparisons between transplant centers, after Bonferroni adjustment for multiple comparisons. pTLC: Predicted total lung capacity.

PGD rates compared to conventional-LTx. At 48 h, PGD grade 3 rates were 25% in ddLLTx (n = 8), compared to 9% in the conventional-LTx (n = 66) group[16]; this difference, however, was not statistically significant in that study. Three studies reported on postoperative ECMO needs, which ranged from 20%-36% in the ddLLTx groups[13-15]. Four studies reported on ICU LOS. This ranged from 12 to 27 d in ddLLTx, compared to 4-6 d in conventional-LTx, Table 3. Five studies reported on FEV_1 in the post-ddLLTx period, Table 4. At 3-6 mo following ddLLTx FEV_1 (%-predicted) ranged from 52.6%-75.3%. Peak FEV_1 (%-predicted) following ddLLTX ranged from 67.3%-85.2%. Only one study compared FEV_1 (%-predicted) between ddLLTx (n = 8) and conventional-LTx (n = 66) cohorts[16]. In that study, at 3 mo ddLLTx FEV_1 (%-predicted) was 64.5%, compared to 76% (*P*-value non-significant) in conventional-LTx and peak FEV1 (%-predicted) was 80.5% and 99% (*P*-value non-significant) for the respective cohorts[16]. Two studies reported on the correlation between FEV_1(%-predicted) and the transplanted pTLCratio (= $pTLCratio_{Lobar}$) following ddLLTx and both studies found a significant correlation between the size of the transplanted lungs and FEV_1(%predicted), Table 4. Four studies reported on 5 year survival following ddLLTx and this ranged from 37.5%-54.9%, compared to 51%-69.9% in the conventional-LTx groups, Table 3[11,12,14,15]. Five-year-survival was not statistically different within each individual study, with the exception of the largest study, where ddLLTx was associated with a higher risk of mortality (54.9% *vs* 69.9% five-year survival, $P < 0.001$)[15].

DISCUSSION

The technique of deceased donor lobar lung trans-

Table 3 Outcomes of deceased donor lobar lung transplantation compared to conventional lung transplant within the same center

Center	Comparison Group with CLTx (number, diagnosis)	PGD (grade) PostOP-ECMO	ICU LOS (d)	Hospital LOS (d)	Survival 1 year	Survival 5 years
Paris	No	Not reported	Not reported	Not reported	ddLLTx: 86%	Not reported
Reina Sofia	Yes (149 - mixed)[1]	Not reported	Not reported	Not reported	ddLLTx: 50%, CLTx: 72%[1]	Not reported
Hamburg	Yes (28 - mixed)‡	Not reported	Not reported	Not reported	ddLLTx: 85%, CLTx: 72%[4]	Not reported
Alfred	Yes (329 - mixed)	ddLLTx: 56% ⩾ PGD (2)	LLT: 12; CLTx: 4	ddLLTx: 30 CLTx: 21	ddLLTx: 81%, CLTx: 84% (P = 0.115)	ddLLTx: 52%[5], CLTx: 37.5%[5] (P = 0.115)
Zurich	Yes (219 - mixed)	ddLLTx: 13% PGD (not spec.)	Not reported	Not reported	ddLLTx: 82%; CLTx: 88% (P = 0.56)	ddLLTx: 64%; CLTx: 69% (P = 0.56)
UPMC	Yes (691 - mixed)[2], Yes (65 - high LAS)[3]	ddLLTx: 36% ECMO	Not reported	Not reported	ddLLTx: 76%; CLTx: 83%[1]; (high LAS): 72%[2]	Not reported
Foch	Yes (445 - mixed)	ddLLTx: 54% ⩾ PGD (1) 20% ECMO	ddLLTx: 17	ddLLTx: 43	ddLLTx: 60%, CLTx: 78% (NS)	ddLLTx: 46%, CLTx: 51% (NS)
Vienna	Yes (778 - mixed)	ddLLTx: 44% ⩾ PGD1 32% ECMO	ddLLTx: 17; CLTx: 6	ddLLTx: 33.5 CLTx: 22	ddLLTx: 65.1; CLTx: 84.8% (P < 0.001)	ddLLTx: 54.9% CLTx: 69.9% (P < 0.001)
Leuven	Yes (66 - all CF)	ddLLTx: 25% PGD (3) at 48 h *vs* CLTx: 9%	ddLLTx: 12 CLTx: 5	ddLLTx: 37 CLTx: 24	ddLLTx: 100%; CLTx: 88.4% (NS)	Not reported

[1,2,3]From contemporary, but separate reports from same transplant center as the ddLLTx group; [4]Provided by center; [5]Estimated from Kaplan Meier survival curve. PGD: Primary graft dysfunction; ECMO: Extracorporeal membrane oxygenation; ICU: Intensive care unit; LOS: Length of stay; NS: Not statistically significantly different; ddLLTx: Donor lobar lung transplantation; CLTx: Compared to conventional lung transplant.

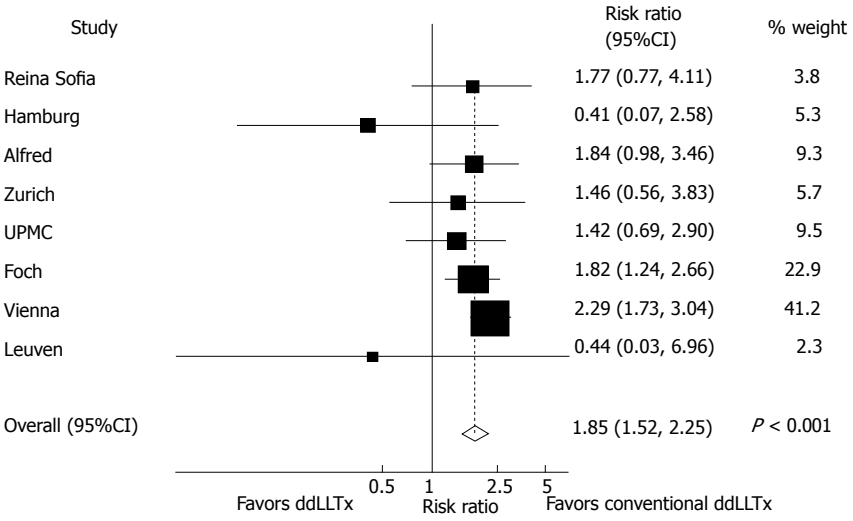

Figure 6 Forest plot for pooled analysis of 1 year survival comparing deceased donor lobar lung transplantation to conventional lung transplant. Vertical line is the "no difference" point in 1 year mortality between dLLTx and CLTx cohorts. Horizontal lines are 95%CI. ■ = Relative Risk (RR) and the size of each square denotes the proportion of information provided by each trial. ◊ = pooled RR for all studies combined. dLLTx: Donor lobar Lung transplantation; CLTx: Conventional lung transplant.

plantation (ddLLTx) is an important surgical option for LTx-candidates with small chest cavities and adds to our armamentarium of LTx techniques. The lung is a special organ that allows parenchyma resections to reduce its size without necessarily compromising the functionality of the remaining tissue. Amongst other solid organs, this remarkable feature is only shared by the liver, not by the heart or the kidneys and split liver transplants have already been established as a reliable tool to increase the donor pool for children[34]. After all, the anatomical organization of the graft and the number of individual lobes transplanted should be less of a concern than the total amount of lung parenchyma provided for the recipient.

Lobectomies are straightforward procedures, but are still rarely performed in the context of LTx. However lobectomies add to the surgical complexity of the LTx operation and may thus prolong the operative time. More importantly, when performed on the back-table, cooling may be impaired and the graft is exposed to warm ischemic time. These disadvantages need to be weighed against the advantages of significantly increasing the potential donor pool and reducing waiting times and waiting list mortality in LTx-candidates with small chest cavities[3]. Because prolonged waiting times often correlate with patient deconditioning, timely transplantation may also reduce the procedural risk for some patients. Differences in surgical strategies among centers include the preferred choice of lobes transplanted. Isolated lower and upper lobe transplants carry the fundamental advantage of not creating a bronchial stump as does bi-lobar transplantation of right

Table 4 Post-transplant FEV1 outcomes of deceased donor lobar Lung transplantation

Center (Nr of ddLLTx)	Comparison group (Nr)	FEV1 (%) 3-6 mo	Peak FEV1 (%)	Correlation to pTLCratio
Paris (7)	No	6 mo: 62%	81%	Not reported
Reina Sofia (6)	No	Not reported	Not reported	Not reported
Hamburg (3)	No	Not reported	Not reported	Not reported
Alfred (23)	No	6 mo: 52.6%	Not reported	Yes FEV1(%) at 3 mo correlates with $pTLCratio_{Lobar}$ (r = 0.549, P = 0.028)
Zurich (23)	No	6 mo: 75.3%	76.80%	Yes FEV1(%) at 3 mo correlates with $pTLCratio_{Lobar}$ (r = 0.485, P = 0.04)
UPMC (25)	No	Not reported	85.20%	Not reported
Foch (50)	No	6 mo: 61.1%	67.30%	
Vienna	No	Not reported	Not reported	Not reported
Leuven (6)	Yes	3 mo:	ddLLTx: 80.5	Not reported
	CLTx (66)	ddLLTx: 64.5% CLTx: 76%	CLTx: 99%	

ddLLTx: Donor lobar Lung transplantation; CLTx: Compared to conventional lung transplant.

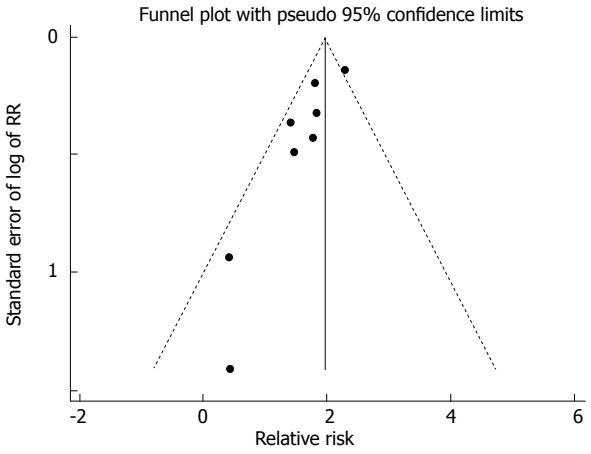

Figure 7 Funnel plot for assessment of publication bias in 1 year mortality results.

upper + middle or upper + lower lobes. Although there is a considerable size mismatch between the recipient main bronchus and a lobar graft bronchus, careful adjustment during surgery allows tension-free alignment in most of the cases. Airway complications have been described and in one study, anastomotic stenoses were reported to occur more frequently in ddLLTx than in full-size transplantation[7,10,11,14,16,35]. However, most airway complications were bronchial stenoses that were amenable for bronchoscopic treatment[14,35].

The size matching parameter utilized to make the decision to perform a ddLLTx varied between studies and some degree of surgeon-specific assessment based on visual inspection was repeatedly reported. However, among objective parameters, the pTLCratio was most frequently reported and offers the possibility to compare practices and results among centers. To our knowledge, this is the first study that uniformly analyzes size matching for ddLLTx based on the pTLCratio.

Although all 9 centers reporting ddLLTx for down-sizing have somewhat different patient populations and surgical philosophies, there were remarkable similarities. The mean recipient's pTLCs were mostly reported at around 5 L, only in two reports (Paris and Leuven) the mean recipient pTLCs were in the 4-4.5 L range, reflecting a higher proportion of pediatric recipients. Although the decision to perform a ddLLTx was based on different sizing considerations, the down-sizing performed as reflected by the $pTLCratio_{Lobar}$ was similar among centers and averaged at 0.76 ± 0.2. The general preference towards undersizing in the setting of fibrotic lung diseases[17,36] was also evident in this systematic review, where the interstitial lung diseases group had the lowest mean $pTLCratio_{Full}$ (1.12 ± 0.03) and after lobar resections the transplanted mean $pTLCratio_{Lobar}$ was also the lowest in interstitial lung diseases group (0.70 ± 0.1) (Figure 4).

In previous studies the pTLCratio was found to be an independent predictor of survival after LTx[21,22,25-28,37]. In an analysis of the SRTR database in the post-LAS era, the pTLCratio showed an independent and nonlinear association with one-year-survival after LTx, irrespective of LTx indication[27]. There was a declining risk of death with higher pTLCratio from 0.5 to about 1.3, where an inflection occurred with rising risk at pTLCratios > 1.3[27]. Furthermore, in an ancillary study to the Lung-Transplant-Outcomes-Group, oversized allografts were associated with a decreased risk of PGD grade 3 after bilateral-LTx[36]. This association was most apparent in recipients with risk factors for PGD[38]. There are concerns that in the intra-operative and early post-LTx period, hemodynamic compromise can occur in the setting of a profoundly oversized allograft secondary to a compartment-syndrome-like picture occurring after chest closure. Also, persistent atelectasis may hamper overall oxygenation and increase the risk for pulmonary infections. However in a single center study oversized allografts (mean pTLCratio 1.18 ± 0.14, range 1.01-1.63), when compared with undersized allografts (mean pTLCratio 0.89 ± 0.09, range 0.63-1.00), were not associated with an increase in post-LTx complications. On the contrary, oversized allografts were associated with a shorter hospital LOS after LTx

and lower resource utilization[20]. These previous data linking the pTLCratio to important post-LTx outcomes could suggest that for severely oversized $pTLCratio_{Full}$ (in excess of > 1.4) a ddLLTx could be an important surgical option however should be performed only in special circumstances in cases with lower $pTLCratio_{Full}$.

The principal finding was that the ddLLTx-group appeared to have a higher risk for one-year mortality than the conventional-LTx-group. In the meta-analysis the ddLLTx and conventional-LTx-groups were unmatched and the outcomes were unadjusted for confounders. Furthermore, the Egger test and visual inspection of the funnel plot for the 1 year survival meta-analysis indicated the presence of publication bias. In terms of publication bias, an underreporting of unsuccessful ddLLTx cases is or appears more likely than an underreporting of superior outcomes of ddLLTx compared to conventional LTx. Because of the above issues, the results of the meta-analysis need to be interpreted with caution. The majority of the included single center studies showed no statistically significant survival difference, although most studies suggested a trend towards higher one-year mortality in the ddLLTx-group. The largest single center study, however, showed a significantly higher risk for one-year mortality in the ddLLTx-group. Importantly, there are significant clinical differences between the ddLLTx and conventional-LTx-groups, which are not adjusted for in the pooled analysis. Because ddLLTx is more frequently used in very urgent cases to realize timely LTx, it is likely that the one-year-survival differences between ddLLTx and conventional-LTx groups are due to the high acuity of the ddLLTx-group. In the Vienna experience, for example, patients receiving ddLLTx were significantly more urgent and more frequently on mechanical ventilation or ECMO support pre-LTx[15]. The Pittsburgh experience also supports the notion of an acuity-driven mortality risk associated with ddLLTx. Only very urgent patients with LAS > 70 were considered as candidates for ddLLTx. This very high acuity ddLLTx group achieved a 76% one-year survival (n = 35)[13], which was similar to that of the high-LAS-cohort (LAS > 50) receiving full-sized lung transplants (72% one-year survival, n = 108)[39]. Resource utilization following ddLLTx seems to reflect the pre-transplant high acuity of the recipients. In three studies reporting on postoperative ECMO needs, this ranged from 20-36% in the ddLLTx groups[13-15]. Four studies reported on ICU LOS and this ranged from 12 to 27 d in ddLLTx, compared to 4-6 d in conventional-LTx (Table 3). It thus remains to be seen if elective ddLLTx in routine LTx-candidates achieves outcomes comparable to those of elective full-sized LTx. This is supported by the experience of the Leuven group, where a cohort of eight stable outpatient LTx-candidates with cystic fibrosis had a 100% one-year survival after ddLLTx[16]. Other centers also reported favorable results with ddLLTx in elective, non-urgent cases[40].

Our study has several limitations. All of the included reports were retrospective observational cohort studies. Although this study systematically analyzed size matching using the pTLCratio, data for its calculation was not available for all patients of the ddLLTx-cohort. Physiologically there a notable difference between a CF patient with short stature and a normal sized IPF patient with the exceptionally small chest cavity from the fibrotic lung disease. For this systematic review only aggregate data on outcomes was available and these two groups could not be analyzed separately. However the pTLC of the recipient would adequately reflect the "normal" chest cavity size of these two very different populations. Whereas using the actually measured total lung capacity or visual inspection of the chest cavities on imaging or in the operating room largely reflects the disease specific effects of the underlying lung diseases on the chest cavity size. However, such alterations in chest cavity size have been shown to be quickly reversible. Assessing chest cavity size *via* opto-electronic-plethysmography post-LTx demonstrated that, irrespective of LTx-indication, the chest volume and the response to exercise was not different from normal controls[41]. In this systematic review 2 studies reported on donor and recipient pTLC and both studies used regression equation based on sex and height to derive pTLC[6,11]. Whereas for the calculations of donor and recipient pTLC done as part of this systematic review from data provided by the authors of five of the included studies[7,12,13,15,16] were based on age, sex and height[18]. While the latter approach accounts for the main determinants of lung size, the race effect on lung size remains unaccounted for with both approaches. The best regression equation to calculate pTLC is not defined, but computed tomography (CT) and CT-volumetry is increasingly used to derive comprehensive and refined regression equations for pTLC[42]. There were wide variations in rates of PGD, likely in part due to variation in definitions, surveillance methods, and reporting. Despite between-institution variability, each individual institution reportedly treated ddLLTx and conventional-LTx cohorts similarly. The majority of the included reports originated in Europe[6,7,10,12,14-16] with only one originating from Australia[11] and one in North America[13]. The organ allocation mechanisms vary by region. Furthermore there were differences in the patient populations and surgical philosophies, which limit the interpretation of aggregate data. The optimal strategy for size matching decisions and thresholds to perform a ddLLTx, especially for recipient with restrictive lung disease, remains to be defined. Important open questions include: (1) Is there a threshold where the risk of implanting an oversized full allograft exceeds the risks of a ddLLTx and ddLLTx should be recommended? (2) When ddLLTx leads to a very undersized lobar allograft based on the $pTLCratio_{Lobar}$, is there a threshold where the risks of PGD and poor outcomes start to rise substantially? and (3) Would the risk of PGD and the overall outcome of reasonably matched ddLLTx compare to those of full-size allografts if performed routinely in elective cases?

In conclusion, ddLLTx is an important and so far underutilized surgical option for lung transplant candidates

with small pTLC. It is only performed at a few specialized centers and frequently performed in high urgency cases. Outcome is acuity-driven and is expected to improve as more elective cases are done. Systematically using the pTLCratio as the size matching tool could help to identify sizing thresholds to maximize the risk/benefit balance for ddLLTx.

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COMMENTS

Background

Lung transplantation (LTx) is an established therapy for appropriately selected patients suffering from end-stage lung disease. 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. Deceased-donor-lobar lung transplantation (ddLLTx) is an important and so far underutilized surgical option for lung transplant candidates with small chest cavities. The size matching decision for ddLLTx is complex and based on varying parameters.

Research frontiers

The best donor-to-recipient size-matching parameter in LTx remains controversial. Chest X-ray parameters, calculation of the ratio between donor and recipient heights, calculation of the ratio of predicted total lung capacity (pTLC) between donor and recipient (pTLCratio) and estimation based on visual inspection in the operating room are commonly used strategies. Amongst these the pTLCratio has the largest evidence base to support its use. Systematically using the pTLCratio as the size matching tool could help to identify sizing thresholds to maximize the risk/benefit balance for ddLLTx.

Innovations and breakthroughs

In this systematic review the authors' analyzed all reports on ddLLTx and uniformly described size matching using the donor-to-recipient predicted-total lung-capacity (pTLC) ratio and summarized reported one-year survival data of ddLLTx and conventional-LTx. Nine observational cohort studies reporting on 301 ddLLTx met the inclusion criteria for systematic review of size matching, and eight for describing one-year-survival. The ddLLTx-group was often characterized by high acuity; however there was heterogeneity in transplant indications and pre-operative characteristics between studies. Data to calculate the pTLCratio was available for 242 ddLLTx (80%). The mean pTLCratio before lobar resection was 1.25 ± 0.3 and the transplanted pTLCratio after lobar resection was 0.76 ± 0.2. One-year survival in the ddLLTx-group ranged from 50%-100%, compared to 72%-88% in the conventional-LTx group. In the largest study ddLLTx (n = 138) was associated with a lower one-year-survival compared to conventional-LTx (n = 539) (65.1% *vs* 84.1%, P < 0.001).

Applications

ddLLTx is an important and so far underutilized surgical option for lung transplant candidates with small pTLC. It is only performed at a few specialized centers and frequently performed in high urgency cases. Outcome is acuity-driven and is expected to improve as more elective cases are done. Systematically using the pTLCratio as the size matching tool could help to identify sizing thresholds to maximize the risk/benefit balance for ddLLTx.

Terminology

The technique of deceased donor lobar lung transplantation (ddLLTx) is an important surgical option for LTx-candidates with small chest cavities. The lung is a special organ that allows parenchyma resections to reduce its size without necessarily compromising the functionality of the remaining tissue. Amongst other solid organs, this remarkable feature is only shared by the liver, not by the heart or the kidneys and split liver transplants have already been established as a reliable tool to increase the donor pool for children.

Peer-review

The authors have prepared an excellent review of the literature concerning the lobar transplantation (LTx). That technique is one of the new possibility for improving the number of LTx and to save a larger number of patients in very poor respiratory condition. The work is absolutely important and deserves a priority publication.

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META-ANALYSIS

Contrast-induced acute kidney injury in kidney transplant recipients: A systematic review and meta-analysis

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Abstract

AIM

To evaluate the incidence of contrast-induced acute kidney injury (CIAKI) in kidney transplant recipients.

METHODS

A literature search was performed using MEDLINE, EMBASE, and the Cochrane Database of Systematic Reviews from the inception of the databases through July 2016. Studies assessing the incidence of CIAKI in kidney transplant recipients were included. We applied a random-effects model to estimate the incidence of CIAKI.

RESULTS

Six studies of 431 kidney transplant recipients were included in the analyses to assess the incidence of CIAKI in kidney transplant recipients. The estimated incidence of CIAKI and CIAKI-requiring dialysis were 9.6% (95%CI: 4.5%-16.3%) and 0.4% (95%CI: 0.0%-1.2%), respectively. A sensitivity analysis limited only to the studies that used low-osmolar or iso-osmolar contrast showed the estimated incidence of CIAKI was 8.0% (95%CI: 3.5%-14.2%). The estimated incidences of CIAKI in recipients who received contrast media with cardiac catheterization, other types of angiogram, and CT scan were 16.1% (95%CI: 6.6%-28.4%), 10.1% (95%CI: 4.2%-18.0%), and 6.1% (95%CI: 1.8%-12.4%), respectively. No graft losses were reported within 30 d post-contrast media administration. However, data on the effects of CIAKI on long-term graft function were limited.

CONCLUSION
The estimated incidence of CIAKI in kidney transplant recipients is 9.6%. The risk stratification should be considered based on allograft function, indication, and type of procedure.

Key words: Acute kidney injury; Kidney transplantation; Contrast-induced nephropathy; Contrast-induced acute kidney injury; Transplantation

Core tip: We conducted this meta-analysis to assess the incidence of contrast-induced acute kidney injury (CIAKI) in kidney transplant recipients. The estimated incidence of CIAKI is 9.6%. The estimated incidence of CIAKI in recipients who received contrast media is highest at 16% with cardiac catheterization, followed by 10% with other types of angiogram, and 6% with computed tomography scan. The findings from this study may impact the risk stratification for administration of contrast media and CIAKI prevention in kidney transplant recipients.

Cheungpasitporn W, Thongprayoon C, Mao MA, Mao SA, D'Costa MR, Kittanamongkolchai W, Kashani KB. Contrast-induced acute kidney injury in kidney transplant recipients: A systematic review and meta-analysis. *World J Transplant* 2017; 7(1): 81-87 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/81.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.81

INTRODUCTION

Contrast-induced acute kidney injury (CIAKI), or contrast-induced nephropathy (CIN), is associated with a significant increase in mortality and morbidity in patients with native kidneys[1-7]. The incidence of CIAKI has been reported from 2% in the general population without risk factors, to more than 20% in high-risk patients[1,8-12]. The overall frequency of CIAKI is approximately 150000 patients each year worldwide[13]. The number of diagnostic studies and procedures with iodinated contrast media including computed tomography (CT) imaging, coronary angiography, and other types of angiograms have increased for the past decade[14].

Renal transplant recipients are at an at increased risk for developing post-contrast AKI[15] since they have a lower average estimated glomerular filtration rate (GFR) and higher prevalence of diabetes and cardiovascular disease when compared to the general populations[16]. Furthermore, the majority of kidney transplant recipients are receiving calcineurin inhibitors, which are known to cause renal afferent vasoconstriction[17-20]. For these reasons, the American College of Radiology (ACR) Committee on Drugs and Contrast Media 2015 manual consider renal transplant recipients as a potentially higher risk population for CIAKI[21], and thus clinicians may be reluctant to administer iodinated contrast to renal transplant patients[22]. However, unlike the general population, the incidence and risk factors for CIAKI in kidney transplant recipients are not well studied.

The aim of this meta-analysis was to assess the incidence and risk factors of CIAKI in kidney transplant recipients.

MATERIALS AND METHODS

Cheungpasitporn W and Thongprayoon C individually examined published studies and conference abstracts indexed in MEDLINE, EMBASE, and the Cochrane Database from the inception of the databases through July 2016. The search strategy used is detailed in the supplementary material (Supplementary material 1). Further pertinent studies were retrieved by conducting a manual search using references from the articles that were identified 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[23] and previously published guidelines[24,25].

The inclusion criteria were as follows: (1) randomized controlled trials or observational studies; (2) patient population age $\geqslant$ 18 years old; and (3) and additional data on 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 above. Differing decisions were settled by joint agreement.

A standardized information collection form was utilized to derive the following data: The first author of each study, year of publication, study design, country where the study was conducted, number of kidney transplant recipients studied, definition of CIAKI, number of CIAKI patients, and age and gender of CIAKI patients.

Statistical analysis

MetaXL software (EpiGear International Pty Ltd)[26] was utilized for data analysis. The incidence rates (IRs) and 95%CIs of adverse effects were reported using a DerSimonian-Laird random-effects model[27]. 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[28]. Bias funnel plots to assess for publication were used[29].

RESULTS

Our search strategy yielded 1664 articles. Of these, 1495 articles were excluded following the review of title and abstract based on relevance and the eligibility criteria. The remaining 169 articles underwent full-

Table 1 Main characteristics of the studies included in this meta-analysis[19,30-36]

Characteristics	Light *et al*[30]	Peters *et al*[31]	Ahuja *et al*[32]	Agarwal *et al*[33]
Country	United States	United States	United States	United States
Year	1975	1983	2000	2009
Total number	34 (very early post-transplant (2-24 d)	93	33	57
Male sex	NR	NR	NR	74%
Mean age (yr)	NR	NR	42 ± 2.1	58.2 ± 10.1
Baseline creatinine (mg/dL)	NR	NR	2.3 ± 0.25	1.7 ± 0.8
Immunosuppression	Azathioprine, methylprednisolone with/without anti-thymocyte globulin	Azathioprine, prednisone with/without anti-thymocyte globulin	Cyclosporine (94%)	Mycophenolate (52.6%), tacrolimus (33.3%), azathioprine (26.3%), sirolimus (1.8%), cyclosporine (52.6%)
Procedure	Drip infusion urogram from 2-24 d post-transplantation	Intravenous pyelogram (87), allograft angiogram (6) during 2 mo post-transplantation	Coronary angiogram (6), CT scan (11), peripheral vascular angiogram (11), allograft angiogram with angioplasty (5), pulmonary angiogram (1), intravenous pyelogram (1)	Cardiac catheterization
Contrast used	30% meglumine diatrizoate	NR	High osmolar contrast (The volume of contrast used was not reported)	Low-osmolar contrast (36), iso-osmolar contrast (21)
Hydration	NR	NR	78.7% of patients received IV hydration	All patients received pre-procedural intravenous hydration with bicarbonate prophylaxis used in 14 patients
CIAKI definition	An increase of SCr > 0.4 mg/dL within 4 d after contrast	Oliguria or increase in creatinine within 12 d after contrast	An increase of SCr > 25% from baseline	An increase in SCr of ⩾ 25% or 0.5 mg/dL within 3 d post-catheterization
CIAKI (%)	11 (32.4%)	45 (48.4%)	7 (21.2%) Coronary angiogram 3/6 (50%)Angiogram 2/17 (11.8%) CT 1/11 (9.1%) IVP 1/1 (100%)	9 (15.8%) 13.2% in eGFR < 60% and 21.1% in eGFR > 60%
Dialysis (%)	2 (5.9%)	NR	0 (0%)	1 (1.8%) (temporary dialysis)
Risk factor for CIAKI	CIAKI was more common and more severe in those with impaired kidney function. Kidneys from older donors were at higher risk for CIAKI	CIAKI was common in the early post-transplant period, but no increased risk was found > 120 d post-transplant	IV hydration prior to contrast exposure was protective against CIAKI; 15% of patients who received IV hydration had CIAKI *vs* 49% in non-IV hydration group	Low osmolar contrast OR 7.75 (1.10-infinity) Use of NAC OR 0.29 (95%CI: 0.04-1.78)
Outcomes	NR	NR	NR	One patient received temporary dialysis

AKI: Acute kidney injury; CIAKI: Contrast-induced acute kidney injury; GFR: Glomerular filtration rate; NAC: N-acetylcysteine; NR: Not reported; SCr: Serum creatinine.

length review, and an additional 161 were excluded for failing to meet the eligibility criteria. Eight articles[19,30-36] that met all inclusion criteria were identified for our study of CIAKI in kidney transplant recipients (Table 1). Our search methodology and selection process were outlined in Figure 1.

CIAKI definition

All included studies[19,30-36] identified CIAKI occurrence by either change in serum creatinine (SCr), GFR, or the need for dialysis after administration of contrast media, as shown in Table 1. All included studies, except by Light *et al*[30] and Peters *et al*[31], defined CIAKI as an increase in SCr of > 25% from baseline and/or ⩾ 0.5 mg/dL after 48 to 72 h. This definition is also widely used for the diagnosis of CIAKI in general patient population[37].

incidence of CIAKI in kidney transplant recipients

The incidence of AKI and severe AKI requiring dialysis after contrast exposures in kidney transplant recipients within the eight individual studies ranged from 1.8% to 48.4% and 0% to 5.9%, respectively.

Two early studies by Light *et al*[30] and Peters *et al*[31] included patients who had contrast exposure in the early post-transplant period (within 1-2 mo) and reported incidences of CIAKI of 32.4% and 48.4%, respectively. Since AKI is common in the early post-transplant period, and it is difficult to differentiate CIAKI from other causes such as calcineurin inhibitor toxicity, dehydration, acute tubular necrosis, acute allograft rejection and surgical related etiologies[32], we omitted the aforementioned two studies and performed a meta-analysis of CIAKI incidence utilizing the remaining six

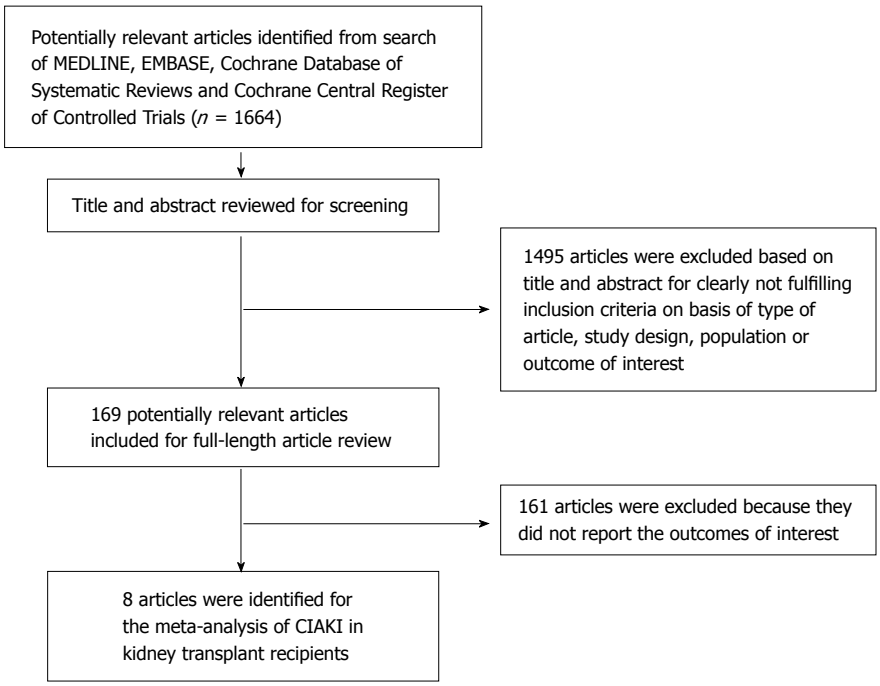

Figure 1 Search strategy. CIAKI: Contrast-induced acute kidney injury.

studies[19,32-36] with 431 kidney transplant recipients. These studies were conducted in the era of calcineurin inhibitor-based immunosuppression in kidney transplant patients with stable baseline serum creatinine before contrast administration. The estimated incidence of CIAKI was 9.6% (95%CI: 4.5%-16.3%) with evidence of a high level of heterogeneity (I^2 = 75%, P < 0.001; Figure 2). The estimated incidence of CIAKI requiring dialysis was 0.4% (95%CI: 0.0%-1.2%, I^2 = 0%). We performed a sensitivity analysis limited only to studies[19,33-36] that used low-osmolar or iso-osmolar contrast; this estimated incidence of CIAKI was 8.0% (95%CI: 3.5%-14.2%, I^2 = 72%).

Types of procedure or intervention with contrast media

The types of procedure or intervention with systemic contrast media administration in our meta-analysis of CIAKI incidence included CT scan (59.1%), coronary angiogram (23.1%), other types of angiogram (17.6%), and intravenous pyelogram (IVP) (0.2%).

Subgroup analyses by types of procedure were also performed. The estimated incidences of CIAKI in kidney transplant recipients who received contrast media with cardiac catheterization, other types of angiogram, and CT scan were 16.1% (95%CI: 6.6%-28.4%, I^2 = 40%), 10.1% (95%CI: 4.2%-18.0%, I^2 = 0%), and 6.1% (95%CI: 1.8%-12.4%, I^2 = 60%), respectively. Fananapazir *et al*[35] specifically studied the CIAKI in kidney transplant recipients who underwent allograft angiogram and reported the incidence of CIAKI of 8.1%. Data on the incidence of CIAKI in kidney transplant recipients, who underwent IVP, were limited as shown in Table 1. The incidence of CIAKI in patients who received IVP during early post-transplant period ranged from 32.4% to 100%[30-32].

Risk factors and prevention measures for CIAKI

Studies have identified early post-transplant period, older donor kidney, impaired baseline GFR, and lack of prophylactic volume hydration as potential important risk factors for CIAKI in kidney transplant recipients[30,31,38]. Ahuja *et al*[32] reported a CIAKI incidence of 15% in kidney transplant recipients with intravenous (IV) hydration before contrast exposure *vs* 49% in the non-IV hydration group. Despite limited data on the use of sodium bicarbonate and N-acetylcysteine (NAC), these studies did not find associated significant protective effects on the incidence of CIAKI[19,33,34,36].

Regarding the type of radiocontrast, high-osmolar contrast was associated with a higher incidence of CIAKI[32]. Compared to iso-osmolar contrast, Agarwal *et al*[33] found that low osmolar contrast was associated with increased CIAKI risk in kidney transplant recipients with an OR of 7.75 (1.10-infinity). In the setting of allograft angiogram, there was an increased incidence of CIAKI in recipients undergoing allograft angiogram alone (25%) compared to those who had allograft angiogram with stenting (0%).

Data on patients' comorbidities and the risk of CI AKI were limited. Abu Jawdeh *et al*[36] reported an association between low hemoglobin and increased risk of CIAKI[36]. Recently, Haider *et al*[34] found no significant effects of diabetes mellitus, age, race, gender, baseline SCr, ACE inhibitor, angiotensin receptor blocker, or diuretics use on the incidence of CIAKI. In addition, studies did not find a significant association between calcineurin inhibitor use and CIAKI[33,36].

Effects of CIAKI on renal allograft function and/or allograft failure

Although there were reported cases of severe CIAKI

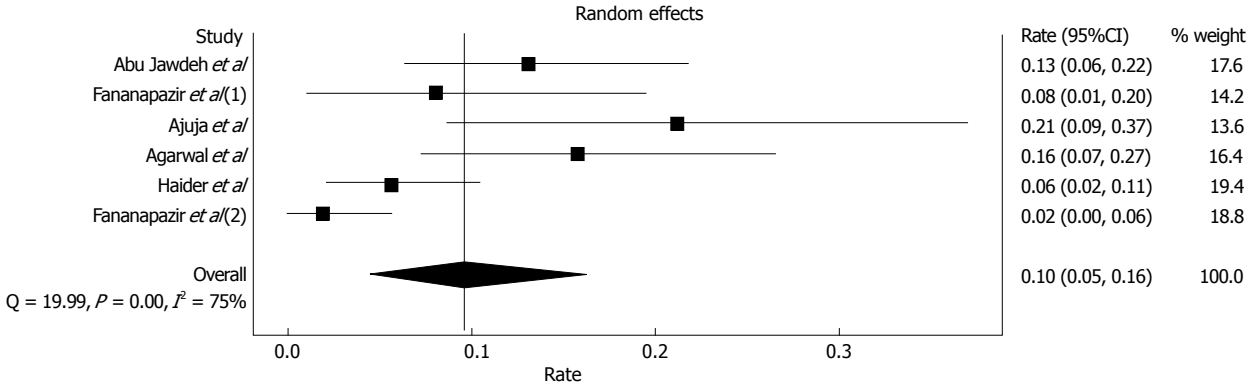

Figure 2 Forest plot of incidence of contrast-induced acute kidney injury in kidney transplant populations.

requiring dialysis[30,33], no studies reported persistent renal allograft failure requiring dialysis. Fananapazir *et al*[19,35] reported no graft loss at 30 d post contrast media administration with CT scan and renal allograft angiogram. Haider *et al*[34] reported that kidney allograft function returned to baseline in five of the seven patients who developed CIAKI within three weeks[34]. In two patients, SCr continued to be elevated due to recurrent AKI episodes from other causes. Data on the effects of CIAKI on long-term graft function or survival were limited.

Evaluation for publication bias

Funnel plots evaluating publication bias for the incidence of CIAKI in kidney transplant recipients demonstrated slight asymmetry of the graph and thus suggested the presence of publication bias for positive studies regarding the incidence of CIAKI.

DISCUSSION

In this meta-analysis, we demonstrated that overall incidence of CIAKI and CIAKI-requiring dialysis in kidney transplant recipients were 9.6% and 0.4%, respectively. The type of procedure with contrast media affected the CIAKI incidences, with estimated incidences undergoing cardiac catheterization, other types of angiogram, and CT scan of 16.1%, 10.1% and 6.1%, respectively. While no graft losses were reported within 30 d post-contrast media administration, data on the effects of CIAKI on long-term graft function were limited.

The incidence of CIAKI has been ranged from 1% in the general population without risk factors to 10%-20% among high-risk patients (especially those with diabetes and CKD)[1,2,8-12]. Not surprisingly, the incidence of CIAKI in kidney transplant recipients from our meta-analysis is relatively similar with those reported in the general adult high-risk populations since transplant recipients also have lower GFR and greater prevalence of diabetes and hypertension than the overall general population[17-20].

Our meta-analysis demonstrated higher rates of CIAKI in kidney transplant recipients who underwent cardiac catheterization and other angiograms than in those who had CT scans. These differences are likely due to intra-arterial contrast administration which may expose the kidney to higher contrast concentrations[39]. In addition, catheter manipulation may provoke atherosclerotic microemboli to the kidney[19]. Despite the higher rate of AKI and the requirement of temporary dialysis after cardiac catheterization[33], our study found no allograft failure noted at 30 d. After a CIAKI event, renal allograft function usually returns to baseline unless the patients develop recurrent AKI episodes from other causes[34]. Thus, our study supports findings from previous studies that coronary angiography is safe with respect to allograft function[40,41].

Renal allograft angiogram is performed for assessment and treatment of allograft renal artery stenosis, pseudoaneurysms, and arteriovenous fistulas[35]. Renal angiogram, which requires contrast media to be directly administered into the graft renal artery, correlates with a CIAKI risk of only 8.1% and is unassociated with any reported cases of dialysis or renal allograft failure[35]. Interestingly, allograft angiogram alone was associated with a higher incidence of CIAKI than allograft angiogram with stenting[35]. It is possible that improved renal allograft function from treating graft renal artery stenosis with stenting ameliorated the nephrotoxicity of iodinated contrast media[35].

Although renin-angiotensin-aldosterone system inhibitors/blockers and calcineurin inhibitors were studied as potential nephrotoxic medications that were commonly discontinued perioperatively, or before systemic contrast exposure due to concern for their afferent arteriolar vasoconstriction effect[42], the evidence from our study does not currently support withholding these medications prior to contrast studies. In addition, reduction of immunosuppression may put the recipients at risk of allograft rejection. Data on preventative measures for CIAKI in renal transplant recipients is limited. As in general patient populations, optimization of volume status with adequate hydration before contrast exposure may help prevent CIAKI. There was also no supported data on the use of sodium bicarbonate and NAC to prevent CIAKI in kidney

transplant recipients.

There are several limitations to our study. First, there were statistical heterogeneities in the analysis of the incidence of CIAKI. The potential sources of this heterogeneity included differences in baseline characteristics, types of procedure, and contrast media. Thus, we performed a sensitivity analysis of studies which only used low-osmolar or iso-osmolar contrast and a subgroup analysis of different procedure types, which yielded lower levels of heterogeneity. Second, selection bias may occur as contrast administration could have been avoided in patients with significantly reduced GFR. This effect may be due to the observation that most patients in the included studies had reasonable renal allograft function (eGFR > 30 mL/min per 1.73 m^2). In addition, most included studies assessed the incidence of CIAKI in a relatively low risk kidney transplant population. Although several studies have suggested safety of contrast administration in patients with significantly reduced GFR[35,43,44], more studies involving high risk patients are needed to make more definitive conclusions. Finally, data on the effect of CIAKI on long-term graft function and allograft survival are lacking. Further studies elucidating the impact of the incidence and severity of CIAKI on long-term allograft outcomes will influence clinical management.

In summary, our meta-analysis demonstrates that the estimated incidence of CIAKI in kidney transplant recipients is 9.6%. Risk stratification for the administration of contrast media in kidney transplant patients include GFR estimation or measurement, clinical indication, and type of procedure. Future studies are needed to further evaluate preventive strategies to reduce CIAKI and the effect of CIAKI on long-term graft function in kidney transplant recipients.

COMMENTS

Background

Renal transplant recipients have been considered at an increased risk for developing post-contrast acute kidney injury (AKI) because they have lower glomerular filtration rate (GFR), GFR and higher prevalence of diabetes and cardiovascular disease. In addition, the majority of kidney transplant recipients are currently on calcineurin inhibitors, which are known to cause renal afferent vasoconstriction. However, unlike the general population, the incidence and risk factors for contrast-induced acute kidney injury (CIAKI) in kidney transplant recipients are not well studied.

Research frontiers

It is necessary to assess the incidence of CIAKI and risk factors for CIAKI in kidney transplant recipients.

Innovations and breakthroughs

In this study, the authors demonstrated that an overall incidence of CIAKI and CIAKI-requiring dialysis in kidney transplant recipients was 9.6% and 0.4%, respectively. The estimated incidences of CIAKI in kidney transplant recipients undergoing cardiac catheterization, other types of angiogram, and computed tomography scan were 16.1%, 10.1% and 6.1%, respectively. No graft losses were reported within 30 d post contrast media administration.

Applications

The data in this study demonstrates an estimated incidence of CIAKI in kidney transplant recipients of 9.6%. Risk stratification for administration of contrast media in kidney transplant patients includes GFR, clinical indication, and type of procedure. While adequate hydration prior to contrast exposure may help to reduce CIAKI risk, there is currently no evidence for withholding renin-angiotensin system and calcineurin inhibitors prior to contrast studies. In addition, there is no supportive data on the use of sodium bicarbonate and N-acetylcysteine to prevent CIAKI in kidney transplant recipients.

Peer-review

Very well-written review article, the authors were investigating the incidence and risk factors for AKI in renal transplant recipients by reviewing what were published in this field.

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CASE REPORT

Allograft loss from acute Page kidney secondary to trauma after kidney transplantation

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Abstract

We report a rare case of allograft loss from acute Page kidney secondary to trauma that occurred 12 years after kidney transplantation. A 67-year-old Caucasian male with a past surgical history of kidney transplant presented to the emergency department at a local hospital with left lower abdominal tenderness. He recalled that his cat, which weighs 15 lbs, jumped on his abdomen 7 d prior. On physical examination, a small tender mass was noticed at the incisional site of the kidney transplant. He was producing a normal amount of urine without hematuria. His serum creatinine level was slightly elevated from his baseline. Computer tomography revealed a large subscapular hematoma around the transplant kidney. The patient was observed to have renal trauma grade Ⅱ at the hospital over a period of three days, and he was finally transferred to a transplant center after his urine output significantly decreased. Doppler ultrasound demonstrated an extensive peri-allograft hypoechoic area and abnormal waveforms with absent arterial diastolic flow and a patent renal vein. Despite surgical decompression, the allograft failed to respond appropriately due to the delay in surgical intervention. This is the third reported case of allograft loss from acute Page kidney following kidney transplantation. This case reinforces that kidney care differs if the kidney is solitary or a transplant. Early recognition and aggressive treatments are mandatory, especially in a case with Doppler signs that are suggestive of compression.

Key words: Page kidney; Kidney transplantation; Trauma; Subcapsular hematoma; Doppler ultrasound

Core tip: We experienced a rare case of allograft loss from acute Page kidney secondary to trauma that occurred 12 years after kidney transplantation. This case reinforces that care for a transplanted kidney differs from care of a native kidney. Early recognition and aggressive treatments are mandatory, especially when Doppler signs suggest there is compression of the transplanted kidney. To the best of our knowledge, our case is the third case of allograft loss from Page kidney following kidney transplantation.

Takahashi K, Prashar R, Putchakayala KG, Kane WJ, Denny JE, Kim DY, Malinzak LE. Allograft loss from acute Page kidney secondary to trauma after kidney transplantation. *World J Transplant* 2017; 7(1): 88-93 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/88.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.88

INTRODUCTION

The Page kidney (PK) phenomenon occurs with compression of the kidney by a hematoma or mass, leading to arterial hypertension[1]. More than 100 cases have been described in the literature[2-4]; however, no systematic review has focused on post-transplant PK. In this case, report, we describe a rare case of allograft loss from PK secondary to trauma that occurred 12 years after kidney transplantation. This is the third reported case of allograft loss from PK following kidney transplantation[5,6]. We describe this case alongside a review of the literature.

CASE REPORT

A 67-year-old Caucasian male presented to the emergency department at a local hospital for left lower abdominal tenderness. The patient had undergone a living unrelated kidney transplant into his left iliac fossa 12 years prior due to chronic glomerulonephritis. His stable immunosuppression regimen included tacrolimus (1 mg every 12 h), mycophenolate mofetil (500 mg every 12 h), and prednisone (5 mg daily). Except for one episode of acute cellar rejection a month after kidney transplantation, he had been doing well with a baseline serum creatinine level of 2.0 mg/dL. On arrival, his body temperature was 36.6 ℃, blood pressure was 163/54 mmHg, and pulse was 61 beats/min. He reported that he had been active until the day before without noticing any injuries, but he recalled his cat, weighing 15 lbs, jumped on his abdomen seven days prior. On physical examination, his abdomen was soft and flat without rebound or guarding, except for a small tender mass noticed at the incisional site of the kidney transplant. His hemoglobin was 7.1 g/dL. His serum creatinine level was elevated from his baseline to 2.5 mg/dL. He was producing a normal amount of urine without hematuria. Computed tomography (CT) without intravenous contrast revealed a 12 cm × 2.5 cm subcapsular hematoma around the transplanted kidney (Figure 1). Urology was consulted, and the decision was made to conservatively observe the patient, as he met criteria of a renal trauma grade Ⅱ according to the renal trauma grading system by the American Association for the Surgery of Trauma.

On admission, the patient received a red blood cell transfusion and was started on labetalol for hypertension. His systolic blood pressure was controlled within a range of 110-140. Within three days, his serum creatinine level increased to 5.4 mg/dL and his urine output decreased. His blood pressure was elevated up to 156/80 mmHg. The patient was transferred to a transplant center for further treatment.

At the transplant center, Doppler ultrasound (US) demonstrated an extensive peri-allograft hypoechoic area, abnormal arterial waveforms with absent diastolic flow in the arcuate arteries and a patent renal vein (Figure 2). He underwent emergent laparotomy for hematoma decompression. A substantial portion of the hematoma was evacuated by capsulotomy. Concurrent kidney biopsy showed no evidence of rejection. His postoperative course was uncomplicated and uneventful. The patient resumed tacrolimus, mycophenolate mofetil, and prednisone. However, his kidney function continued to deteriorate and he became dependent on hemodialysis. He is currently maintained with mycophenolate mofetil monotherapy and is awaiting a second kidney transplant.

DISCUSSION

PK was first described by Irvine Page in 1939, when he wrapped animal kidneys with cellophane and observed the development of acute hypertension[1]. The typical presentation of PK is distinguished by the presence of acute renal dysfunction in conjunction with hypertension. Trauma, spontaneous hemorrhage in patients with predisposing factors (anticoagulation), bleeding after interventions (surgery, biopsy, nephrostomy, and lithotripsy), tumors, renal cysts, urinoma, and lymphocele have been proposed as etiological factors[1-4]. Hypoperfusion and microvascular ischemia in the kidney are considered to stimulate the renin-angiotensin-aldosterone system and cause hypertension[1]. If the involved kidney is solitary, or if the contralateral organ is damaged, renal failure may ensue. There are a variety of treatment options, including conservative management as the hematoma is absorbed[7]; surgical decompression by capsulotomy as part of a laparoscopic intervention[8]; and, in extreme cases, nephrectomy[9,10]. Improvement of renal function after evacuation of the hematoma, in the absence of rejection or ureteral obstruction, confirms the diagnosis. In our case, CT demonstrated a large subcapsular hematoma compressing the parenchyma with a significant Doppler US finding of "absent arterial diastolic

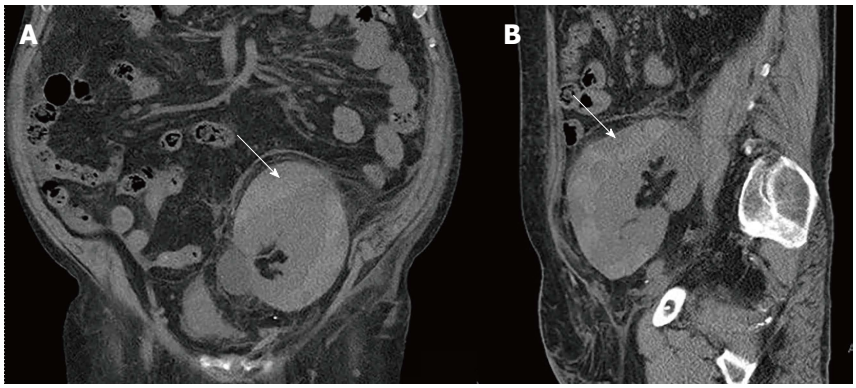

Figure 1 Computed tomography without intravenous contrast of the transplanted kidney. A: Coronal view; B: Sagittal view. A subscapular hematoma 12 cm × 2.5 cm in size was compressing the transplanted kidney (arrows).

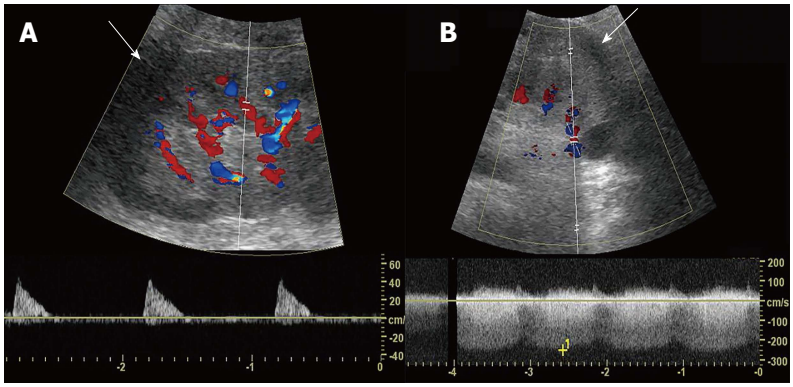

Figure 2 Presence of peri-allograft hematoma and Doppler ultrasound findings. A: Transplant arterial flow. Peri-allograft hypoechoic area (arrows) with absent diastolic flow in the arcuate arteries; B: Transplant venous flow. The transplant renal vein was patent.

flow with patent renal vein". Furthermore, a kidney biopsy failed to demonstrate evidence of rejection, which further supported the diagnosis of PK.

PK after kidney transplantation was first described in 1976 as "pseudorejection" by Cromie *et al*[11]. This was because PK causes acute deterioration of graft function, which resembles rejection. Since then, 30 cases of post-transplant PK have been reported in the literature (Table 1)[4-6,10-31]. The most common causes are iatrogenic (kidney biopsy in 18 cases, renal artery stenting in 1 case, ureteral stenting in 1 case, and nephrostomy in 1 case); trauma (3 cases)[5,6]; spontaneous (2 cases)[15,27]; and postoperative bleeding (2 cases)[11,12]. Surgical decompression with capsulotomy and evacuation of hematoma have been performed in most cases (25 cases), and interventional radiographic drainage was performed in 1 case[14], while 3 cases were conservatively observed with complete improvement of kidney function[19,26]. The diagnosis is most commonly made by Doppler US findings of "absent arterial diastolic flow, reversible arterial diastolic flow, or significant increase of arterial resistive index, with a large peri-allograft hypoechoic area," suggesting extrinsic compression of renal parenchyma and subsequent cortical ischemia. In most cases, these findings have prompted surgical or radiographic intervention. On the other hand, allograft losses have been reported in 2 cases[10,21]. One allograft was saved with a surgical intervention performed 2 d after the onset[11], while one allograft was lost despite immediate intervention[21]. In our case, the patient was observed at a local hospital and noted to have renal trauma grade Ⅱ; the patient did not undergo Doppler US evaluation for the first three days of hospitalization. He was finally transferred to a transplant center after his urine output significantly decreased. His graft loss may have been preventable if he had been evaluated with Doppler US upon presentation to the local hospital as well as if timely surgical intervention or transfer to a transplant center had been requested earlier. This case reinforces that care of the kidney differs if the kidney is solitary or a transplant. Early recognition of PK and aggressive treatments are mandatory, especially when Doppler findings suggest compression of a solitary or transplanted kidney.

We recommend the following care for acute PK. Patients without pre-existing kidney disease who have unilateral PK need to be admitted for monitoring of vitals, including blood pressure, heart rate, urine output, serum creatinine levels and hemoglobin levels. Abdominal/pelvic CT scan is preferable for accurate initial staging and diagnosis of the etiology of PK. Ongoing hemorrhage in a stable patient can be controlled by embolization with interventional radiology. After the initial diagnosis has been made, follow-up with US is appropriate. An initial attempt should be made to stabilize hypertension with antihypertensive medication. Conservative management and evaluation of the etiology are recommended as part of first-line treatment. Unstable patients might be more appropriate for surgery.

In the case of transplant patients, patients with a single kidney, and patients with bilateral PK, the patient should be transferred to a transplant center or a center capable of caring for the patient with acute PK and the underlying etiology. Vitals, including the blood pressure, heart rate and urine output, serum creatinine level and hemoglobin levels, should be carefully

Table 1 Acute Page kidney after kidney transplantation

Year	Ref.	Age/sex	Onset after transplant	Cause	Modality for diagnosis	Positive US sign[1]	Type of intervention	Intervention time after onset	Result
2016	Takahashi	67/M	12 yr	Trauma	US/CT	Yes	Surgical decompression	3 d	AL
2015	Sedigh *et al*[6]	67/M	12 yr	Trauma	US	Yes	Surgical decompression	12 h	CR
2015	Ay *et al*[12]	50/M	1 d	Postoperative bleeding	US	Yes	Surgical decompression	Immediately	CR
2014	Adjei-Gyamfi *et al*[13]	12/M	7 wk	Txp kidney biopsy	US/CT	No	Surgical decompression	Immediately	CR
2014	Adjei-Gyamfi *et al*[13]	18/F	1 yr	Txp kidney biopsy	US	No	Surgical decompression	Immediately	CR
2013	Hamidian Jahromi *et al*[14]	19/M	5 wk	Txp renal arterial stenting	US/Angio	Yes	IR drainage	6 h	CR
2012	Gandhi *et al*[15]	46/M	17 yr	Spontaneous	US	Yes	Surgical decompression	Immediately	CR
2011	Maurya *et al*[16]	30/M	7 d	Txp kidney biopsy	US/CT	Unknown	Surgical decompression	Immediately	CR
2011	Okecgukwu *et al*[17]	32/M	8 d	Txp ureter stenting	US	Unknown	Surgical decompression	Immediately	CR
2010	Butt *et al*[4]	61/F	24 d	Spontaneous	CT	-	Surgical decompression	Immediately	CR
2010	Posadas *et al*[18]	55/M	3 mo	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
2009	Kamar *et al*[19]	47/M	1 yr	Txp kidney biopsy	US	Yes	Observation	-	CR
2009	Kamar *et al*[19]	59/M	1 yr	Txp kidney biopsy	US	Yes	Observation	-	CR
2009	Caldés *et al*[20]	60/M	1 mo	Percutaneous nephrostomy	US	Yes	Surgical decompression	24 h	CR
2008	Chung *et al*[21]	27/F	11 d	Txp kidney biopsy	US/CT	Yes	Surgical decompression	Immediately	CR
2008	Chung *et al*[21]	39/F	Several days	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
2008	Chung *et al*[21]	35/M	4 d	Txp kidney biopsy	US/CT	Unknown	Surgical decompression	Immediately	AL
2008	Chung *et al*[21]	33/F	9 mo	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
2008	Heffernan *et al*[22]	64/M	4 mo	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
2007	Patel *et al*[23]	69/M	7 yr	Txp kidney biopsy	US/CT	Unknown	Surgical decompression	Immediately	CR
2005	Gibney *et al*[24]	32/M	1 yr	Txp kidney biopsy	US/Angio	Unknown	Surgical decompression	Immediately	CR
2000	Rea *et al*[25]	34/M	3 yr	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
1996	Machida *et al*[26]	32/M	4 mo	Txp kidney biopsy	CT/Scinti	-	Observation	-	PR
1996	Goyal *et al*[5]	41/M	12 yr	Trauma	CT/MRI/ Scinti	-	Unknown	Unknown	Unknown
1994	Nguyen *et al*[27]	26/M	12 h	Spontaneous	Scinti	-	Surgical decompression	Immediately	CR
1993	Dempsey *et al*[28]	19/F	2 yr	Txp kidney biopsy	US	Yes	Surgical decompression	Immediately	CR
1993	Ben Hamida *et al*[29]	32/M	7 mo	Heparin after renal vein thrombosis	US	Yes	Observation	-	CR
1991	Kliewer *et al*[10]	56/F	2 wk	Txp kidney biopsy	US	Yes	Nephrectomy	Unknown	AL
1988	Figueroa *et al*[30]	40/F	11 mo	Txp kidney biopsy	CT/Angio	-	Surgical decompression	30 h	CR
1988	Yussim *et al*[31]	40/F	5 mo	Postoperative lymphocele	US	Unknown	Surgical decompression	Unknown	CR
1976	Cromie *et al*[11]	35/M	10 d	Postoperative bleeding	US	Unknown	Surgical decompression	2 d	CR

[1]Absent diastolic flow, reversible flow, high resistive index at the transplant renal arteries, or increase in the RI from baseline by Doppler US. US: Ultrasound; CT: Computed tomography; IR: Interventional radiography; Txp: Transplant; AL: Allograft loss; CR: Complete resolution; Angio: Angiography; Scinti: Scintigraphy.

monitored. In addition to CT scanning for staging and diagnosis, Doppler US should be performed to evaluate parenchymal compression. Hypertension should be managed using antihypertensive medication and strict fluid balance. If the patient has an elevated serum creatinine level or a decrease in urine output as well as positive Doppler signs, prompt surgical intervention should be considered.

We experienced a rare case of allograft loss from acute PK secondary to trauma after kidney transplantation. The care of PK in a transplant kidney differs from PK in the native kidney. Early recognition and aggressive treatments are mandatory, especially in a case with positive Doppler signs.

COMMENTS

Case characteristics

A 67-year-old male with a past surgical history of kidney transplantation (12 years prior) presented to the emergency department for left lower abdominal tenderness after a cat jumped on his abdomen (seven days prior).

Clinical diagnosis

The abdomen was soft and flat without rebound or guarding, except for a small tender mass noted at the incision site of the kidney transplant.

Differential diagnosis

Lymphocele, urinoma, seroma, hematoma, renal cell cancer, renal cyst.

Laboratory diagnosis

On initial presentation, all labs were normal except for a hemoglobin of 7.1 g/dL and serum creatinine level of 2.5 mg/dL.

Imaging

Computed tomography without intravenous contrast revealed a 12 cm × 2.5 cm subcapsular hematoma around the transplanted kidney.

Pathological diagnosis

The transplant kidney biopsy showed no evidence of rejection.

Treatment

Emergent laparotomy for decompression of the hematoma.

Related reports

A renal trauma grade Ⅱ is usually observed according to the renal trauma grading system of the American Association for the Surgery of Trauma.

Term explanation

The Page kidney phenomenon occurs from kidney compression by a hematoma or a mass, leading to arterial hypertension. If the involved kidney is solitary, or the contralateral organ is damaged, renal failure may ensue.

Experiences and lessons

This case reinforces that kidney care differs if the kidney is solitary or transplanted. Early recognition and aggressive treatments are mandatory, especially in a case with Doppler signs suggestive of compression.

Peer-review

The topic is very interesting. The authors presented their experience with Page kidney phenomenon after kidney transplantation. It is relatively unfrequent complication but with possible serious complications on graft.

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CASE REPORT

Renoportal anastomosis in living donor liver transplantation with prior proximal splenorenal shunt

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Abstract

For transplant surgeons, end-stage liver disease with portal venous thrombosis and a previous splenorenal shunt (SRS) is a significant challenge during liver transplantation. Thrombosis of the portal vein can be corrected by surgical interventions, such as portal venous thrombectomy or surgical removal of the thrombosed portal vein. Even also placement of a graft between the mesenteric vein and the graft portal vein can be performed. If these maneuvers fail, a renoportal anastomosis (RPA) can be performed to achieve adequate graft inflow. A 51-year-old male patient who had a history of proximal SRS and splenectomy underwent living donor liver transplantation (LDLT) due to cryptogenic cirrhosis. LDLT was performed with RPA using a cadaveric iliac vein graft. The early postoperative course of the patient was completely uneventful and he was discharged 20 d after transplantation. To the best of our knowledge, this was the first patient to receive LDLT with RPA after surgical proximal SRS and splenectomy.

Key words: Liver transplantation; Portal vein thrombosis; Renoportal anastomosis; Proximal splenorenal shunt

Core tip: Renoportal anastomosis is such a feasible option during liver transplantation especially for patients having portal vein thrombosis. This case has a history of surgical proximal splenorenal shunting and splenectomy before liver transplantation which is a rare condition that makes surgery more complex and difficult. We reported how we

managed our patient.

Ozdemir F, Kutluturk K, Barut B, Abbasov P, Kutlu R, Kayaalp C, Yılmaz S. Renoportal anastomosis in living donor liver transplantation with prior proximal splenorenal shunt. *World J Transplant* 2017; 7(1): 94-97 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/94.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.94

INTRODUCTION

End-stage liver disease with portal venous thrombosis (PVT) and previous splenorenal shunt (SRS) presents significant challenges during liver transplantation[1]. The incidence of PVT was reported as 10% to 25% in patients with cirrhotic end-stage liver disease[2]. At different centers, the native PVT rate was between 2.1% and 26%[3]. PVT was as an absolute contraindication at the beginning of the liver transplantation era; nevertheless, adequate portal inflow during liver transplantation could be achieved by innovations in surgical techniques. Portal vein thrombosis can be corrected by surgical interventions, such as portal venous thrombectomy or surgical removal of the thrombosed portal vein. Even though bridging the mesenteric vein and the graft portal vein by placement of a vascular graft can be performed in order to maintain graft inflow[4]. In such cases, renoportal anastomosis (RPA) can also be performed in order to achieve adequate graft inflow. Sheil and colleagues were the first to describe this technique, and Kato *et al*[5] modified it for patients receiving orthotopic liver transplantation who had distal SRS[6]. We describe a case of successful living donor liver transplantation with RPA for a patient who had undergone proximal SRS and splenectomy 20 years ago.

CASE REPORT

A 51-year-old male with decompensated liver disease was admitted for liver transplantation. His viral hepatitis markers, including hepatitis B and C, were negative. He was also investigated for immune-mediated hepatic disorders; there was no positive test result and he was diagnosed as cryptogenic cirrhosis. He had a history of bleeding esophageal varices that were treated by endoscopic band ligation and also he had a history of proximal SRS and splenectomy from 20 years before. His Child-Pugh score was 11 (Grade C) and model for end-stage liver disease score was 33. Thrombosed portal vein was visualized on abdominal computed tomography and also active SRS draining from the splenic vein into the left renal vein was identified (Figure 1). The portal thrombus continued down to the mesenterico-splenic confluence. We planned to perform a right lobe living donor liver transplantation for him, and his 39-year-old male relative was prepared as a donor with the approval of the ethics committee. In the evaluation of the donor, the remnant liver volume was calculated as 34%. The graft weight was calculated as 580 g. The ratio of graft volume to recipient weight was 0.75.

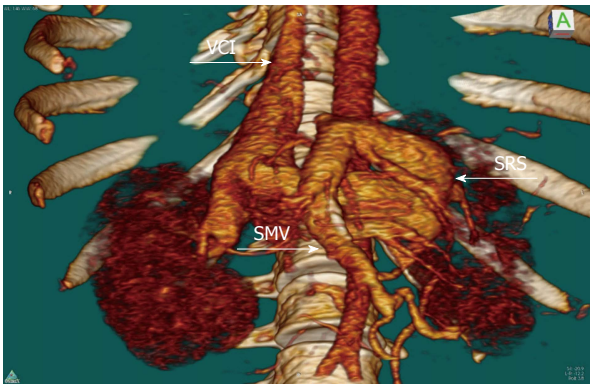

Figure 1 Active splenorenal shunt draining from the splenic vein into the left renal vein. VCI: Vena cava inferior; SRS: Splenorenal shunt; SMV: Superior mesenteric vein.

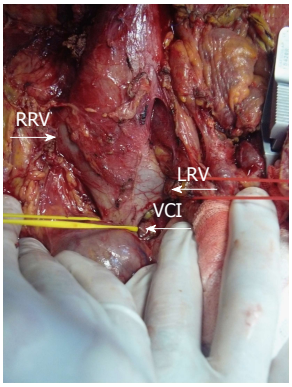

Figure 2 Anterior part of the infrahepatic vena cava was explored and dissected down to expose the bifurcation of the left renal vein. LRV: Left renal vein; VCI: Vena cava inferior; RRV: Right renal vein.

Recipient operation was started with a reverse L incision. There was no blood flow in the recipient's main portal vein during hilar dissection and we did not observe any bowel congestion. After total hepatectomy, the anterior part of the infrahepatic vena cava was explored and dissected to expose the bifurcation of the left renal vein (Figure 2). The duodenum was mobilized with a minimal Kocher maneuver to minimize bleeding from retroperitoneal collateral veins. We started the implantation of the liver graft with hepatic vein anastomosis, and then performed an end-to-end RPA between the left renal vein and the graft portal vein with 6-0 polypropylene-interrupted sutures using a cadaveric iliac vein as an interposition graft with sufficient forward flow (Figure 3). Finally, hepatic artery and biliary anastomosis were performed. Intraoperative Doppler ultrasound showed normal hepatic arterial, renoportal, and hepatic venous flow. The cold and warm ischemia times were 80 and 30 min. The total operation time and operative blood loss were 636 min and 2.4

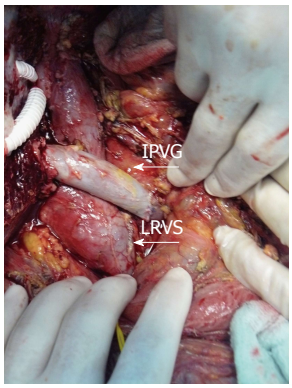

Figure 3 Right renal vein between left renal vein and graft portal vein with interposition vein graft. IPVG: Interposition vein graft; LRVS: Left renal vein stump.

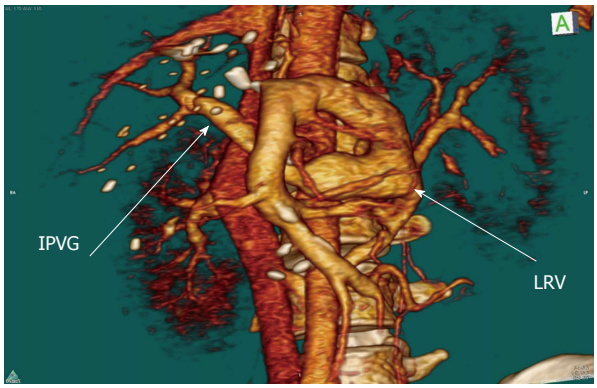

Figure 4 Computerized tomography scans visualize the patency of the right renal vein. IPVG: Interposition vein graft; LRV: Left renal vein.

L, respectively. The immediate postoperative course of the patient was uneventful. The amount of ascites drainage from abdominal drains decreased daily and we pulled out the drains ten days after liver transplantation. The INR, creatinine, and bilirubin levels of our patient reached normal ranges before they were discharged from the hospital. The computerized tomography scans confirmed the patency of the anastomosis at the 19th postoperative day (Figure 4). Unfortunately, we lost the patient due to biliary leakage and sepsis two months after transplantation.

DISCUSSION

It is critical to ensure adequate portal vein inflow for patients receiving liver transplantation with PVT. Possible surgical portal vein reconstruction strategies can be chosen according to Yerdel's classification, based on preoperative imaging data or intraoperative findings[7]. For partial (grade 1-2) PVT thrombectomy or thrombendvenectomy may be possible choices during LT[8,9]. On the other hand more complex surgical procedures such as using interposition grafts between the distal superior mesenteric vein and graft portal vein or portal vein arterialization can be performed for complete thrombosis of the portal vein (grade 3-4) in order to restore portal inflow[10-12]. However, patients with extensive PVT frequently have complex spontaneous porto-caval shunts[13]; the shunt vessels should be ligated to prevent this phenomenon. Unfortunately, ligation of these large, fragile shunt vessels is technically difficult and may cause significant bleeding. Two alternative surgical techniques can be used for patients with complete PVT: Cavoportal hemi transposition and RPA[14]. The graft's portal vein and inferior vena cava is anastomosed in an end-to-end, end-to-side, or side-to-end fashion in cavoportal hemi transposition. Nevertheless, lower limb edema and impaired renal functions due to obstruction of the vena cava are the risks of this surgical procedure.

RPA can be performed between the left renal vein and the graft's portal vein in an end-to-end or side-to-end fashion, with or without an interposition graft[15,16]. In RPA, adequate portal inflow without the steal phenomenon can be achieved easily in patients with major portosystemic shunts. There is no need for dissection or manipulation around large and fragile shunt vessels while performing RPA, so excessive bleeding can be avoided. We performed RPA in an end-to-end fashion with an interposition cadaveric iliac vein graft. Prosthetic grafts can also be used as interposition grafts, but using prosthetic grafts have some disadvantages because of their thickness and rigidity. Patients with prosthetic grafts must receive aspirin daily to prevent graft thrombosis. Moreover, they have the risk of graft infection due to immunosuppressive drugs.

Patients can develop small-for-size syndrome after RPA due to excessive portal inflow, which is characterized by the production of persistent ascites and prolonged hyperbilirubinemia[17]. Our patient's postoperative course was uneventful, and we did not observe excessive amount of ascites drainage; our patient's bilirubin level reached the normal range before they were discharged from the hospital. Congestion of the left kidney may be a problem because the manipulation of the left renal vein may affect the outflow of the left kidney. Lee *et al*[18] reported that temporary renal impairment can occur after the ligation of the proximal left renal vein in patients with large SRSs. We did not observe any renal impairment in our patient. To the best of our knowledge, our case is the first patient to receive LDLT with RPA after surgical proximal SRS.

PVT during liver transplantation is no longer a relative contraindication with today's surgical innovations. RPA is a feasible and efficient way to provide adequate inflow for the liver graft, even also in patients with portal vein thrombosis who underwent proximal SRS and splenectomy before.

COMMENTS

Case characteristics

A 51-year-old male who has the history of proximal splenorenal shunt (SRS) and splenectomy, had intractable ascites due to portal vein thrombosis and end

stage liver disease.

Clinical diagnosis
He had ascites and bleeding esophageal varices due to end stage liver disease.

Differential diagnosis
Upper GI tract endoscopy, imaging studies and biochemical laboratory analyzes were performed in order to make differential diagnosis.

Laboratory diagnosis
His Child-Pugh score was 11 (Grade C) and model for end-stage liver disease score was 33.

Imaging diagnosis
Thrombosed portal vein and also active SRS draining from the splenic vein into the left renal vein was visualized on abdominal computed tomography.

Treatment
The authors performed an end-to-end Renoportal anastomosis between the left renal vein and the graft portal vein with 6-0 polypropylene-interrupted sutures using a cadaveric iliac vein as an interposition graft with sufficient forward flow.

Related reports
Living-donor liver transplantation with renoportal anastomosis for the treatment of spontaneous splenorenal shunts in patients with end-stage liver disease is a life saving and a safe technique which was described before. The patient is the first case receiving living donor liver transplantation (LDLT) with renoportal anastomosis (RPA) after surgical proximal SRS and splenectomy.

Term explanation
RPA can be performed between the left renal vein and the graft's portal vein in an end-to-end or side-to-end fashion, with or without an interposition graft.

Experiences and lessons
RPA is a feasible and efficient way to provide adequate inflow for the liver graft, even also in patients with portal vein thrombosis who underwent proximal SRS and splenectomy before.

Peer-review
The case report is the first patient with end-stage liver disease to receive LDLT with RPA after surgical proximal SRS. The clinical experience is very important to treat the similar patients in the future.

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CASE REPORT

Mycophenolate mofetil toxicity mimicking acute cellular rejection in a small intestinal transplant

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Author contributions: Apostolov R, Asadi K, Lokan J and Testro A contributed to writing and revising the paper; all authors contributed to the acquisition and interpretation of data.

Institutional review board statement: This case report was exempt from ethics approval by our Institute's Ethics Committee.

Informed consent statement: The patient involved in this study gave his informed consent authorising use and disclosure of his anonymised health information and pathology slides.

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Abstract

Mycophenolate mofetil (MMF) is an important medication used for maintenance immunosuppression in solid organ transplants. A common gastrointestinal (GI) side effect of MMF is enterocolitis, which has been associated with multiple histological features. There is little data in the literature describing the histological effects of MMF in small intestinal transplant (SIT) recipients. We present a case of MMF toxicity in a SIT recipient, with histological changes in the donor ileum mimicking persistent acute cellular rejection (ACR). Concurrent biopsies of the patient's native colon showed similar changes to those from the donor small bowel, suggesting a non-graft specific process, raising suspicion for MMF toxicity. The MMF was discontinued and complete resolution of these changes occurred over three weeks. MMF toxicity should therefore be considered as a differential diagnosis for ACR and graft-versus-host disease in SITs.

Key words: Small intestinal transplantation; Drug toxicity; Mycophenolate mofetil; Acute cellular rejection; Immunosuppression

Core tip: Mycophenolate mofetil (MMF) is a commonly used medication for maintenance immunosuppression in small intestine transplant (SIT) recipients. Enterocolitis is a known side effect of MMF therapy, but there is little literature describing its histological manifestations in SIT recipients. Our case shows that MMF enterocolitis can mimic acute cellular rejection (ACR) and highlights the importance of attempting to biopsy the native gastrointestinal tract in SIT recipients if possible. If the native biopsy is abnormal, drug toxicity should be considered as a differential diagnosis as it may show overlapping features with ACR.

Apostolov R, Asadi K, Lokan J, Kam N, Testro A. Mycophenolate mofetil toxicity mimicking acute cellular rejection in a small intestinal transplant. *World J Transplant* 2017; 7(1): 98-102 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/98.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.98

INTRODUCTION

Mycophenolate mofetil (MMF) acts by inhibiting inosine-5'-monophosphate dehydrogenase, leading to decreased purine synthesis in T and B lymphocytes. This inhibits lymphocyte proliferation and as a result suppresses cell mediated immunity and antibody formation, which are important factors in acute graft rejection[1].

Small intestine transplants (SITs) have a high risk of developing acute graft rejection, with nearly 50% of recipients developing at least one episode of rejection within one year of transplantation[2]. Prevention and early treatment of acute rejection is important in SITs due to its significant consequences. In a large single centre review of 500 small intestine and multi-visceral transplants persistent rejection was the leading cause of graft failure[3]. Current immunosuppression regimens to prevent rejection include induction therapy with antilymphocyte or anti-IL2 antibodies, followed by maintenance therapy with corticosteroids and tacrolimus[3,4]. MMF added to tacrolimus and corticosteroids may further reduce the risk of rejection in SIT recipients[5]. Our centre utilises MMF in addition to tacrolimus and corticosteroids for maintenance therapy in SITs.

A common side effect of MMF is enterocolitis, which clinically presents with non-specific symptoms of increased stomal output and abdominal distension. These same symptoms may also occur in SIT rejection. Biopsies must be obtained for histology to differentiate these potential complications in SIT recipients. Histological patterns of injury related to MMF toxicity have been described in the literature in both the upper and lower gastrointestinal (GI) tracts[6-13]. Most of the existing literature describes histological features of MMF injury in native small and large intestine samples rather than in SITs, making it difficult to diagnose MMF injury in a SIT recipient. We describe a case of a SIT recipient who histologically appeared to have persistent acute cellular rejection (ACR). The patient had similar histological findings in his native colon, implicating MMF toxicity as the cause for the persistent changes.

CASE REPORT

A 47-year-old man underwent a combined SIT and renal transplant. He had short-gut syndrome with 35 cm of small bowel remaining after multiple resections for spontaneous volvulus. The native colon remained intact and functioning. He had end-stage renal failure due to oxalosis which had been demonstrated on pre-transplant renal biopsy. He received induction immunosuppression with pre-operative basiliximab 20 mg, with a second dose given on post-operative day 4. Early maintenance immunosuppression consisted of intravenous methyl-prednisolone, MMF 1000 mg BID, and tacrolimus titrated to a trough level of 10-12 ng/mL.

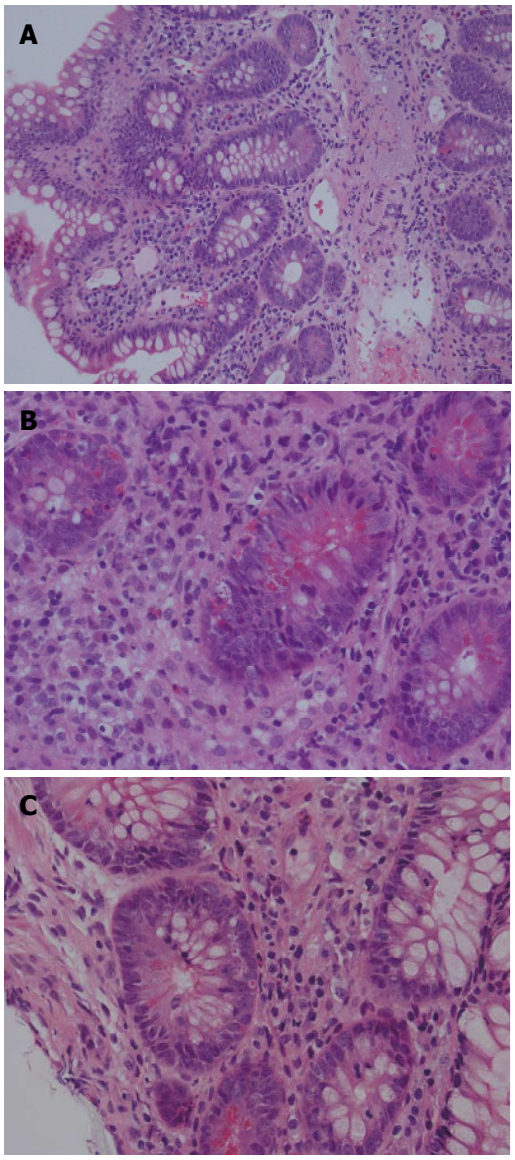

Figure 1 Small bowel allograft biopsy - day 13. A: Increased lamina propria inflammatory infiltrate, including activated cells, regenerative basophilia of crypt epithelium and increased epithelial apoptosis; B: High power view increased crypt apoptosis and rejection type inflammatory infiltrate within the lamina propria; C: Focal confluent apoptosis in a single crypt.

Protocol endoscopy and biopsy of the SIT and native colon, accessed *via* a chimney ileostomy, were performed on day 13 post-transplant. As per our institutional protocol, the biopsies were interpreted independently by two experienced transplant pathologists. The donor ileum and native colon appeared macroscopically normal. Donor ileal biopsy showed a mixed inflammatory infiltrate with activated lymphocytes, eosinophils and plasma cells and evidence of crypt epithelial injury associated with > 6 apoptotic bodies per 10 consecutive crypts (Figure 1). Native colonic biopsies were unremarkable at this time (Figure 2). A diagnosis of mild ACR was made. This

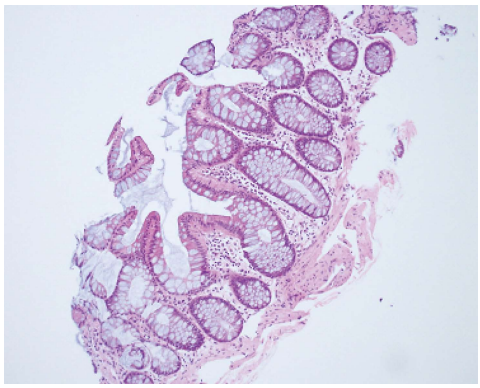

Figure 2 Native colonic biopsy - day 13. Unremarkable mucosa with preserved surface and crypt architecture with no significant inflammation and no crypt apoptosis.

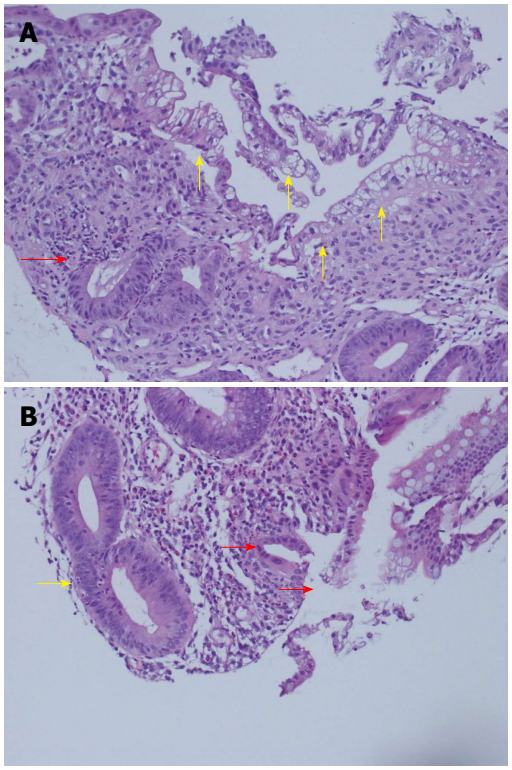

Figure 3 Small bowel allograft biopsy - day 23. A: Mucosal erosion with marked surface enterocyte degeneration and cytoplasmic vacuolation, sloughing (yellow arrows), inflamed granulation-like tissue within the lamina propria, prominent crypt injury (red arrow) and focal drop out; B: Cryptitis with increased epithelial apoptosis (yellow arrow), mixed lamina propria inflammatory infiltrate and surface epithelial erosion (red arrows).

was treated with pulsed methylprednisolone, as per our hospital's protocol. A subsequent biopsy performed 3 d later demonstrated resolution of the ACR, again with normal colonic biopsies.

Further protocol endoscopy and biopsy of the SIT and native colon was performed on day 23. The donor ileum had macroscopically flattened villi and the native colon appeared normal. Biopsy of the donor ileum, from both the chimney and the graft proximal to the colonic anastomosis, demonstrated focal villous blunting and

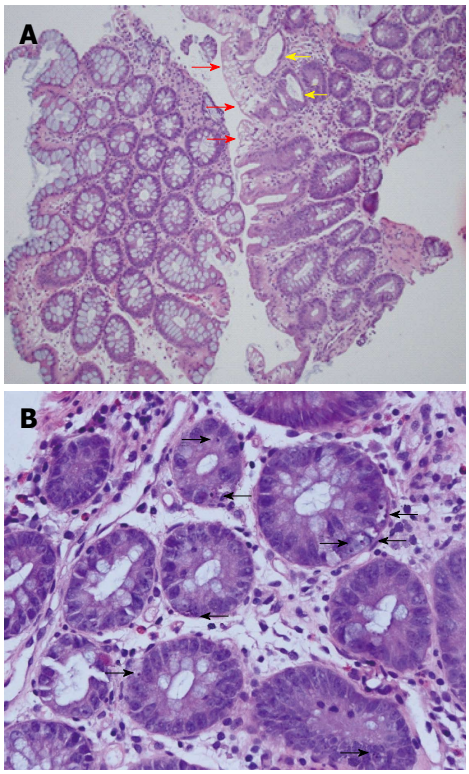

Figure 4 Native colonic biopsy - day 23. A: Striking focal surface epithelial vacuolation/degeneration (red arrows), associated with crypt epithelial injury, crypt withering and goblet cell reduction (yellow arrows); B: High power view - basal crypts with mucin reduction, increased basophilia and several apoptotic bodies (black arrows).

flattening, with multifocal erosion, superficial ulceration with neutrophil clusters and inflamed granulation tissue. In areas there was marked degeneration and vacuolation of the surface epithelium with sloughing, but no viral inclusions were identified on immunohistochemistry. There was mixed mononuclear inflammation with foci of crypt degeneration, neutrophilic cryptitis, areas of crypt drop-out and up to 10 apoptotic bodies per 10 crypts, without confluent apoptosis (Figure 3). In isolation these findings were concerning for at least moderately severe ACR, particularly in the setting of ACR only 10 d prior. An opinion was also sought from an international expert, who reviewed the biopsies, and felt that the changes in the small bowel were suspicious for moderate-severe ACR.

Importantly however, the native colonic biopsies also demonstrated surface epithelial vacuolation associated with crypt injury with dilatation, goblet cell depletion, focal attenuation of the epithelium and focally increased basal apoptosis (Figure 4). These new findings in the previously normal native colon suggested a non-graft specific pathological process and hence, in the absence of viral infection, or clinical features of graft-*vs*-host disease (GVHD), raised suspicion for MMF GI toxicity. We therefore chose to discontinue the MMF (substituted with azathioprine) and not give any specific treatment for rejection, pending an early repeat biopsy.

Further endoscopy and biopsy 4 d later (post-operative

day 27) revealed significant improvement in histologic appearance with only low grade apoptosis, and by post-operative day 34 the endoscopic appearance was normal and histologic examination demonstrated normal villous architecture, regenerative crypts and 3-4 apoptotic bodies per 10 crypts. Native colonic biopsy showed evidence of healing injury and reduced apoptosis. Repeat biopsy on day 41 showed similar findings in the SIT and entirely resolved changes in the native colon. Viral inclusions were absent in all biopsy specimens.

The patient is now one year post transplant and has remained on azathioprine, tacrolimus and prednisolone. He currently has intestinal autonomy and a well-functioning renal graft and has had no further episodes of acute rejection.

DISCUSSION

Distinguishing ACR in a SIT from MMF toxicity presents a challenge for clinicians. This is due to the overlap of endoscopic and histopathologic findings in both conditions and the limited published literature describing histological changes related to MMF use in SITs.

ACR in a SIT can be suspected on endoscopic visualisation and diagnosed histologically. Endoscopic visualisation for detecting ACR was shown to have a sensitivity of 50% and specificity of 91% in SIT recipients undergoing surveillance endoscopy[14]. Abnormalities seen included erythema, friability, bleeding and ulceration of the mucosa as well as shortening, blunting and congestion of villi. MMF enterocolitis can present with similar findings on endoscopic visualisation, including erythema in one third of cases and erosions and ulcers less commonly[7,9]. No endoscopic abnormality is seen in approximately half of the histologically confirmed cases of GI injury attributable to MMF. Our patient had normal endoscopic appearances at the time that ACR was diagnosed. The subsequent endoscopy one week later showed flat villi, a finding that may have suggested ongoing ACR.

Histological features of ACR in SIT recipients include lymphocytic infiltration of the lamina propria, increased number of apoptotic bodies (typically > 6 apoptotic bodies per 10 consecutive crypts), crypt injury and dropout, and ulceration[4].

Recognition and early treatment of ACR in SIT recipients is important, as severe ACR of intestinal grafts has a 50% mortality rate[15]. The treatment of ACR involves high dose steroids or anti-lymphocyte therapy, with an aim to decrease the T-cell mediated immune response towards the graft[2]. In contrast, the treatment of MMF toxicity involves cessation or switching to an alternative agent. Our patient has an intestine-kidney transplant, and had also experienced mild ACR of his intestinal graft. Both of these reasons indicate the need for another immunosuppressant in place of MMF. We used azathioprine in this case, but rapamycin is an alternative agent that may be used[3].

Most of the studies describing histological features of GI mucosal injury from MMF excluded SIT recipients. To our knowledge, only one study of 15 biopsy specimens from four paediatric patients describes the histological changes of MMF injury in SIT recipients[16]. Lymphoplasmacytic inflammatory infiltrate, villous blunting, vascular congestion and apoptotic bodies were the major histological changes described. Only one of 15 specimens in the study had > 6 apoptotic bodies per 10 crypts, and this biopsy was reported as mild ACR. Some of these features were seen on our patient's day 23 biopsy, at which time the differential diagnoses of ACR and MMF mucosal injury were considered. Our patient's day 23 biopsy showed higher crypt apoptotic counts than have been previously attributed to MMF in SITs. Further, and perhaps most importantly, the value of biopsying the remaining native bowel was highlighted by the fact that there was similar pathology evident, suggesting that the pathological process was non-graft specific and hence broadened the differential diagnosis to drug toxicity, GVHD and viral infection.

The histological features of MMF colitis have been described in a number of studies. These changes include acute colitis-like findings, inflammatory bowel disease (IBD) like findings, crypt architectural disarray, erosive colitis and GVHD like features[7-13]. GVHD like features have also been described in ileal biopsies of patients on MMF and include crypt architectural disarray, villous blunting, oedema and crypt epithelial apoptosis[7]. Our patient's day 23 ileal and colonic biopsies showed features of crypt apoptosis with associated active crypt epithelium injury, mucosal erosion and architectural disarray.

MMF-induced enterocolitis presented with similar clinical and histological findings to ACR in our case. Rapid resolution of clinical and histological abnormalities occurred after switching MMF to azathioprine. MMF enterocolitis should be considered as a differential diagnosis for SIT recipients with persistent ACR who are taking MMF. If at all possible, attempts should be made to concurrently biopsy the remnant native GI tract at the time of routine graft surveillance biopsies in order to determine whether observed histologic changes are graft specific.

COMMENTS

Case characteristics

A 47-year-old male small intestinal transplant (SIT) recipient recovering post-operatively with no specific symptoms.

Clinical diagnosis

The patient's clinical examination was unremarkable during the case.

Differential diagnosis

The major differential diagnoses for mycophenolate mofetil (MMF) toxicity are acute cellular rejection (ACR) and graft-versus-host disease.

Imaging diagnosis

Endoscopy revealed flattened villi in the donor ileum and a macroscopically normal native colon in patient.

Pathological diagnosis

Serial biopsies of the patient's SIT and native colon initially showed features of ACR in the SIT and no abnormalities in the native colon, but subsequently showed pathological features in both the SIT and native colon which suggested a non-graft specific pathology.

Treatment

MMF was switched to azathioprine, leading to resolution of the histopathological changes.

Related reports

The case report is a unique case and there is very little data describing the histological effects of MMF in SIT recipients.

Term explanation

MMF enterocolitis is a common side effect of MMF therapy and histological changes associated with MMF use have been described in all sections of the gastrointestinal tract.

Experiences and lessons

By performing concurrent biopsies of the SIT and native colon of patient, the authors identified MMF toxicity, a non-graft specific pathology, as the cause for patient's persistent abnormal histological changes in the SIT.

Peer-review

It is an interesting work that describes a relevant drug toxicity.

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