World Journal of

Transplantation

World Journal of
Transplantation

World J Transplant 2014 March 24; 4(1): 1-39

Baishideng Publishing Group
www.wjgnet.com

World Journal of
Transplantation

World J Transplant 2014 June 24; 4(2): 40-152

Published by Baishideng Publishing Group Inc

World Journal of
Transplantation

World J Transplant 2014 September 24; 4(3): 153-215

Published by Baishideng Publishing Group Inc

World Journal of
Transplantation

World J Transplant 2014 December 24; 4(4): 216-305
Volume End

Published by Baishideng Publishing Group Inc

World Journal of *Transplantation*

A peer-reviewed, online, open-access journal of transplantation

World Journal of

Transplantation

World Journal of *Transplantation*

Contents

Contents

ABOUT COVER

World Journal of Transplantation Editorial Board, Maurizio Salvadori, MD, Professor Maurizio Salvadori, Renal Unit, Careggi University Hospital, V iale Pieraccini 18, Florence 50139, Italy

AIM AND SCOPE

World Journal of Transplantation (*World J Transplant*, WJT, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

AIM AND SCOPE

World Journal of Transplantation is now indexed in PubMed Central, PubMed, Digital Object Identifier.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*   **Responsible Science Editor:** *Ling-Ling Wen*
Responsible Electronic Editor: *Huan-Liang Wu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Impact of donor-specific antibodies on the outcomes of kidney graft: Pathophysiology, clinical, therapy

Maurizio Salvadori, Elisabetta Bertoni

Maurizio Salvadori, Elisabetta Bertoni, Department of Renal Transplantation, Careggi University Hospital, 50139 Florence, Italy

Author contributions: Salvadori M and Bertoni E contributed equally to the concept, acquisition of data, drafting of the article, revisions and final approvals.

Correspondence to: Maurizio Salvadori, MD, Department of Transplantation, Careggi University Hospital, viale Pieraccini 18, 50139 Florence, Italy. maurizio.salvadori1@gmail.com

Telephone: +39-55-597151   Fax: +39-55-597151

Received: November 1, 2013 Revised: January 23, 2014
Accepted: March 3, 2014
Published online: March 24, 2014

Abstract

Allo-antibodies, particularly when donor specific, are one of the most important factors that cause both early and late graft dysfunction. The authors review the current state of the art concerning this important issue in renal transplantation. Many antibodies have been recognized as mediators of renal injury. In particular donor-specific-Human Leukocyte Antigens antibodies appear to play a major role. New techniques, such as solid phase techniques and Luminex, have revealed these antibodies from patient sera. Other new techniques have uncovered alloantibodies and signs of complement activation in renal biopsy specimens. It has been acknowledged that the old concept of chronic renal injury caused by calcineurine inhibitors toxicity should be replaced in many cases by alloantibodies acting against the graft. In addition, the number of patients on waiting lists with preformed anti-human leukocyte antigens (HLA) antibodies is increasing, primarily from patients with a history of renal transplant failure already been sensitized. We should distinguish early and late acute antibody-mediated rejection from chronic antibody-mediated rejection. The latter often manifets late during the course of the post-transplant period and may be difficult to recognize if specific techniques are not applied. Different therapeutic strategies are used to control antibody-induced damage.

These strategies may be applied prior to transplantation or, in the case of acute antibody-mediated rejection, after transplantation. Many new drugs are appearing at the horizon; however, these drugs are far from the clinic because they are in phase I - II of clinical trials. Thus the pipeline for the near future appears almost empty.

Core tip: Clear evidence exists that shows that donor-specific-HLA antibodies (DSAs) are the primary players in the acute and chronic deterioration of graft. The emergence of sensitive techniques that detect DSAs, together with advances in the assessment of graft pathology, has enabled an improved understanding of antibody-mediated graft injury. Acute and chronic antibody-mediated rejection conditions have changed the nomenclature during recent Banff conferences and have enabled the dismissal of older terminologies, such as chronic allograft nephropathy. Therapies aimed at B cells and plasma cells and that control complement activation will be extremely important for improving long-term outcomes in kidney transplantations.

Salvadori M, Bertoni E. Impact of donor-specific antibodies on the outcomes of kidney graft: Pathophysiology, clinical, therapy. *World J Transplant* 2014; 4(1): 1-17 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/i1/1.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i1.1

INTRODUCTION

Despite improvements in renal transplantation outcomes,

kidney allograft loss remains substantial and is associated with increased morbidity, mortality and costs[1,2]. Clearly, the identification of the critical pathologic pathways underlying allograft loss and the development of therapeutic interventions that improve the duration and quality of allograft function are among the most important targets for transplant medicine. One of the most important advances of the past decade has been the realization that the insufficient control of the humoral arm of a recipient's immune system by current immunosuppressive regimens[3] is the factor primarily responsible for allograft dysfunction and loss[4-6].

ALLOGRAFT ANTIBODY EVOLUTION IN TRANSPLANTATION

The induction of allograft injury alloantibodies induced has now superseded the historical dogma that allograft losses were caused by the toxicity of calcineurin inhibitors (CNIs) and by chronic allograft nephropathy (CAN). Indeed, nephrotoxicity and CAN as causes of late graft failure are being challenged by the findings of the Long-Term Deterioration of Kidney Allograft Function (DeKAF)[6-8] and other studies[9,10].

In addition, recent therapeutic strategies that have permitted the human leukocyte antigens (HLA) to be crossed have created a new population at risk of antibody-mediated rejection (ABMR), which has enabled these patients to be studied over an extended time period.

The emergence of sensitive techniques that detect donor-specific anti-HLA antibodies (DSAs) and other HLA and non-HLA antibodies together with advances in the assessment of graft pathology have expanded the spectrum of ABMR.

The different technologies used by researchers and the significance of alloantibodies found by these technologies led recently to a consensus conference that elaborated upon consensus guidelines for testing and clinical management issues associated with HLA and non-HLA antibodies in transplant recipients[11] .

As a consequence of this increase in knowledge, the term CAN was deleted in the Banff'05 meeting report[12]. In the Banff'07 and Banff'09 conferences[13,14], the concept of ABMR was further evaluated, and ABMR was definitively included in the Banff classifications.

The Banff'11 meeting report[15] and the recent Banff' 13 conference (unpublished data) further elaborated upon new concepts in ABMR, which included the significance of C4d-negative and C1q-positive ABMR.

DETECTION OF ANTIBODIES AND THEIR SIGNIFICANCE

Preformed antibodies targeted against HLAs or antibodies formed *de novo* after transplantation predispose to either acute or chronic ABMR. These antibodies can be detected using several techniques.

A complement-dependent lymphocytotoxicity (CDC)

cross-match is typically performed to detect cytotoxic DSAs. The main disadvantages of the CDC assay are that it is subjective and cumbersome and will only detect complement-fixing antibodies[16]. Indeed, ABMR has occurred in patients with a negative cross-match. This observation may indicate that the CDC lacks the sensitivity required to detect some clinically significant antibodies; moreover, acute ABMR can occur in recipients with immunological memory and undetectable levels of circulating HLA antibodies at the time of transplant[17]. Cross-match (XM) and antibody detection techniques have improved with time and show increased sensitivity and specificity[18,19].

Flow cytometry (FC) is another cell-based technique that was introduced more than 20 years ago to improve sensitivity. This test also lacks specificity, and with the introduction of solid-phase assays (SPA), the use of FC has been superseded. The introduction of SPA detection, while providing greater sensitivity than CDC assays, has resulted in a new paradigm with respect to the interpretation of DSAs. Although SPA using the Luminex instrument has permitted the detection of antibodies not detectable by CDC, the clinical significance of these antibodies is not fully understood. In addition, SPA testing raises technical issues that require resolution and careful consideration when interpreting antibody results. SPA, such as flow cytometry using antigen-coated micro particles, enzyme-linked immunosorbent assays (ELISAs) and Luminex, are now used to determine the specificity of anti-HLA antibodies and to better interpret positive CDC-XM results.

ELISA has an advantage over the CDC because is more sensitive and detects antibodies that not fix complement. However, non-specific binding to other immunoglobulins may occur in patients with autoimmune disorders. When detecting antibodies using flow panel-reactive antibody (PRA) beads, micro-particles are coated with purified HLA molecules[19]. The fluorescence is then measured using flow cytometry and the level of fluorescence is indicative of the level of antibody binding.

Luminex technology also uses pools of HLA class Ⅰ or Ⅱ antigen-coated micro-particles. These beads are colored with a combination of two dyes. Serum reactivity is assessed based on the fluorescent signal of each HLA-coated micro particle[19]. The Luminex platform enables the determination of DSAs specificity by using single HLA-coated beads and provides a relative indication of the antibody strength and level in the circulation by returning results to the user in the form of mean fluorescence intensity (MFI)[20]. However, MFI is not standardized across labs, and there is some arbitrariness in determining the MFI thresholds. Molecules with equivalent soluble fluorochrome (MESF) and maximum fluorescence values, obtained using the Luminex machine, enable more standardized measures of antibody strength[21,22].

According to the consensus publication by the National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplantation[23], a current positive CDC or anti-human immunoglobulin-CDC (CDC-AHG)

Table 1 Technological advantages and limitations of luminex human leukocyte antigens single antigen bead

Technological advantages	Technological limitations
Qualitative: enables precise identification of all antibody specificities in complex sera (DSA)	Some positive results can be caused by antibodies to denatured HLA
Comprehensive: distinguishes antibodies to all common alleles for HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3/4/5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1	Occasional high background binding requiring repeat testing and absorption protocols
Semiquantitative: enables determination of antibody levels (high, intermediate, and low)	Variable HLA protein density on beads. Blocking factors may cause false-negative or misleading low assessment of antibody levels (prozone?); IgM and C1 can block IgG binding
Sensitive: enables detection of weak antibody testing	
Rapid: enables real-time antibody monitoring for DSA. Pre-transplantation and post-transplantation antibody monitoring (assist diagnosis of ABMR). Virtual XM	Lot-to-lot variation requiring validation. Vendor-specific variation
Enables detection of non-HLA-specific antibodies (e.g., MICA)	
Detection and differentiation between immunoglobulin class and isotype (e.g., complement fixing and non-complement fixing C4d and C1q	Reagents not standardized

ABMR: Antibody mediated rejection; DSA: Donor specific HLA antibodies; HLA: Human leukocyte antigens; MICA: Major histocompatibility complex class I-related chain A; SAB: Single-antigen beads; XM: Cross-match.

cytotoxicity cross-match (CXM) predisposes to a high risk of ABMR or early graft loss. A current positive CDC or CDC-AHG CXM is a contraindication for transplantation unless DSAs can be reduced using desensitization protocols. A positive flow CXM or a remote (historic) positive CDC or CDC-AHG CXM poses an intermediate risk for early acute rejection and may require augmented immunosuppression.

The wide use of the Luminex technique with its increased specificity and sensitivity did uncover a new paradigm. Using the Luminex technique DSAs have been found in patients who show a negative classic CDC. Several studies have shown that these results represent a risk factor, but not a formal contraindication for transplantation[24-26].

These facts lead to workshops and Consensus Guidelines to further understand these technologies[11,27].

The recent consensus guidelines highlighted the technological advantages and limitations of Luminex as shown in Table 1.

In addition, the consensus guidelines[11] considered the following modifications to SPA for detecting new antibodies and assessing their functionality.

C4d assay
The C4d assay[28,29] shows superior specificity compared with the CDC. The C4d assay requires complement activation to occur and is influenced by complement regulatory factors. Clinical data obtained using various modifications of the C4d assay have shown that the presence of C4d+ antibodies correlates with graft survival in the kidney and hearts[28,29].

C1q assay
The C1q assay was designed to distinguish complement-fixing from non-complement-fixing antibodies and does not require complement activation other than the binding of C1q to the antibody[30]. It detects antibodies capable of binding complement and initiating the classical path-

way.

The results of this technique still remain under debate. Although some authors[31] have reported no correlation with the clinical course in kidney transplant patients, others[32] have reported that both C1q and C4d Luminex assays show increased sensitivity and specificity and that they can be useful for both pre-transplant risk assessment and post-transplant monitoring.

Detection of antibodies targeted to non-HLA antigens
The endothelial cell is the principal target used to detect non-HLA antibodies involved in ABMR. Historically, different assay systems have been used to identify and characterize AECA including CDC[33], flow cytometry[34] and immunofluorescence[35].

The primary limitation is that the endothelial cells used for the detection and characterization of AECA have been derived from third-party donors, and that the cells used show different protein expressions and distinct phenotypes[36]. Surrogates of endothelial cells, such as MICA may be useful. However, MICA is not expressed constitutively on the endothelium; rather, its expression is induced under conditions of cellular stress.

Lymphocyte XM tests fail to detect AECA. The cross-match ONE assay is a Food and Drug Administration (FDA)-approved endothelial ECXM technique that uses endothelial cell precursor cells found in the peripheral blood at a frequency of 1%-2%[37]. An advantage of this test is that it detects DSAs and can be used to test for antibodies targeted to T lymphocytes, B lymphocytes and endothelial cells in the same assay[38].

Proteomics approaches using protein extracts from different sources, including cell lysates and protein microarrays are being used for antibody screening and identification of specificities[39,40].

A variety of non-HLA targets have been identified including MICA, vimentin, angiotensin II type 1 receptor, tubulin, myosin and collagen V. In general, single antigen bead (SAB) testing permits reassessment of the im-

munologic risk for kidney transplantation. Traditionally, high panel-reactive antibody, re-transplant and deceased donor grafts have been associated with increased risk. However, the risk factors for ABMR are DSAs, reduced HLA matching and evaluation of DSAs using different techniques[41].

PATHOPHYSIOLOGY

An increasing body of evidence suggests that patients with high titers of anti-HLA antibodies (particularly if they are donor-specific) that develop either pre-transplant or post-transplant, show a worse outcome. At any given time, approximately 25% of transplant recipients show antibodies against HLA antigens when evaluated using the newest, highly sensitive and specific techniques for DSAs monitoring[42,43]. Moreover, antibodies against non-HLA have also been implicated in ABMR[44]. Antibodies may mediate endothelial injury *via* complement-dependent or independent mechanisms by transducing signals that are pro-inflammatory and proliferative[45].

Preformed or *de novo* DSAs clearly cause acute and chronic ABMR; however the role and scope of non-HLA antibodies in mediating graft injury and loss remains less certain[46].

One hypothesis is that alloantigen sensitization occurs based on non-HLA polymorphic differences between the donor and the recipient [*e.g.*, major-histocompatibility-complex (MHC) class Ⅰ-related chains A and B (MICA and MICB, respectively)]. Unfortunately progress in this area has been limited by a lack of validated clinical assays for non-HLA alloantibodies, the confounding presence of HLA-DSAs and, in the case of MICA antibodies, a lack of proof of specificity[47].

A second hypothesis is that auto antigen sensitization occurs due to exposure of cryptic epitopes after tissue injury or inflammation (including vimentin, K-α Ⅰ tubulin, collagen V and agrin).

Although anti-HLA antibodies are responsible for the majority of antibody-mediated injuries, they do not underlie all ABMRs. In addition, as discussed above, the major histocompatibility antigens and a large number of minor antigens have been recognized as possible antibody targets[48-50].

Endothelial cells are targets for immune-mediated assaults *via* anti-endothelial cell antibodies (AECAs). The *de novo* development of circulating anti-endothelial cell antibodies, rather than pre-existing antibodies, is associated with post-transplant allograft rejection[51].

Apoptotic endothelial cells (ECs) release a bioactive C-terminal fragment of perlecan called laminin G-like 3 (LG3)[52]. LG3 behaves as a neo-antigen and induces the production of anti-LG-3 antibodies. Recently, these anti LG-3 antibodies have been documented to be novel accelerators of immune-mediated vascular injury and to obliterate remodeling[53].

Vimentin[54], collagen V[55] and K-α 1 tubulin[56] are involved in the ABMR of organ other than kidney as neo-antigens. The apoptosis of ECs and subsequent exposure of neo-antigens may induce an autoimmune response.

An autoantibody specific for angiotensin Ⅱ receptor type 1 has been associated with the development of hypertensive vasculopathy and acute renal allograft dysfunction[57]. Antibodies directed towards MHC class Ⅰ polypeptide-related sequences A (MICA) and B (MICB), and not classical HLA molecules, have been implicated in transplant rejection in recipients who were otherwise well-matched for HLA due to the contribution of MICA antigens towards the activation of cellular and humoral immune responses[58].

The HLA complex encodes molecules crucial for the initiation and proliferation of the immune response. It is highly polymorphic and polygenic and its proteins are co-dominantly expressed. The *HLA* genes that are involved in the immune response belong to classes Ⅰ and Ⅱ, which are structurally and functionally different. Recently, DSAs have been reported to activate endothelial cells, thereby increasing their potential to recruit and bind recipient leukocytes and increasing the potential for allograft inflammation[59,60].

Approximately 30% of patients on waiting list show detectable levels of HLA antibodies[61]. After transplantation, 25% of non-sensitized patients develop *de novo* HLA-DSAs.

In both groups of patients, the presence of these antibodies increases the risk of subsequent ABMR[9]. The development of a histological test to identify antibody-mediated complement activation on transplant biopsies (C4d staining) has provided a method for flagging potentially deleterious interactions between antibodies and the graft endothelium. In addition, molecular techniques, such as gene expression profiling, have enabled the identification of subclinical endothelial cell damage that can be present even in the absence of complement activation or detectable DSA[62]. Recent studies have documented the role of B cells and antibodies in transplantation. A study by Lynch *et al*[63] described a technique that may enable a more global assessment of B-cell reactivity to the allograft. Their results suggest that humoral responses to the allograft may be more common than previously appreciated. Antibodies reactive to donor human leukocyte antigen molecules, minor histocompatibility antigens, endothelial cells, red blood cells or auto antigens may trigger or contribute to rejection at both early and late time points after transplantation[64]. Often, the immune system shows an integrated response that results in allograft rejection involving parallel or simultaneous T cell mediated rejection (TCMR) and ABMR[65]. Antibody-mediated injury to the allograft is initiated by DSAs binding to HLA antigens or to other targets on the allograft endothelium. If DSAs are complement-activating, the classic complement pathway is rapidly activated *via* IgG binding and C1q activation[66]. This process typically results in the rapid loss of the allograft. Alternatively, DSAs can bind endothelial cell targets and stimulate cell proliferation or induce antibody-dependent cell-mediated cyto-toxicity with interferon γ release[45]. These processes appear to be more important for the development of the chronic

antibody-mediated injury that is more dependent on natural killer (NK) cells than the complement[67]. Antibodies may also bind HLA and other targets and incompletely activate the complement system without causing apparent injury. This process is referred to as accommodation[68].

ABMR is a continuous process, and its oscillation is characterized by fluctuations in DSAs, C4d deposition and dynamic and multidirectional glomerulitis and/or capillaritis scores[69]. The time to diagnosis of ABMR is highly dependent on the population studied. Early-onset ABMR (typically occurring within the first months after transplantation) is observed predominantly in patients with preformed DSAs, whereas late acute ABMR occurs primarily in patients who develop *de novo* DSAs after transplantation. Indeed, the pathologic and clinical manifestations may vary, including hyper-acute humoral rejection, acute humoral rejection, indolent or subclinical humoral rejection, "C4d"-negative humoral rejection and late acute humoral rejection.

ACUTE ABMR

Hyper-acute ABMR
The pathology of hyper-acute rejection overlaps completely with acute ABMR. It arises within minutes or a few hours after transplantation in pre-sensitized patients who have circulating HLA, AB0, or other alloantibodies to the donor endothelial surface antigens[70]. The outcome is always poor.

Acute ABMR
The diagnosis of acute ABMR relies upon the criteria shown: (1) morphologic evidence of acute tissue injury: acute tubular injury, neutrophils and/or mononuclear cells in PTC and/or glomeruli and/or capillary thrombosis, fibrinoid necrosis/intramural or trans-mural inflammation in arteries; (2) immuno-pathologic evidence for antibody action: C4d and/or (rarely) immunoglobulin in PTC; Ig and complement in arterial fibrinoid necrosis; and (3) serologic evidence of circulating antibodies to donor HLA or other anti-donor endothelial antigen. The endothelial injury has been recently reviewed completely by Drachenberg and colleagues[71]. Although acute ABMR generally occurs within the first year after transplantation in pre-sensitized patients[72], it may also develop years after transplantation and is often triggered by a decrease in immunosuppression (iatrogenic, non-compliance or malabsorption)[5,73-75].

Several patients with acute ABMR show a negative cross-match, which may be due to low level DSAs that are undetectable[76] or to *de novo* DSAs[77].

Recently, an increased risk of acute ABMR has been associated to elevated pre-transplantation soluble B-cell activating factor (BAFF)[78], whose neutralization may be an interesting therapeutic strategy.

Recently, Orandi *et al*[79] examined the long-term effect of early acute ABMR on kidney allograft and patient survival in 201 adult kidney transplant recipients who developed acute ABMR within the first year after trans-

plantation. Each recipient was matched with 5 control patients. The majority of recipients were sensitized. Allograft survival rates at 1, 5 and 10 years in the group that developed acute ABMR were significantly lower than in the control group.

In another study[60] of a cohort of 355 adult kidney transplant recipients, all with a negative CDC-XM, C1q-fixing DSAs did not predict acute ABMR or allograft loss; however, the presence of class I DSAs (versus class II donor specific antigens) predicted acute ABMR and allograft loss.

Indolent or subclinical acute ABMR
Chronic rejection is often preceded by the occurrence of an acute ABMR due to the fact that modern therapeutic strategies fail to deplete antibody secreting plasma cells from the spleen and bone marrow of patients[80].

In addition, kidney transplant recipients who develop *de novo* DSAs are now recognized to often show pathologic features of indolent and slowly progressive microvascular abnormalities, which are referred referred to occasionally as subclinical acute ABMR[16,77,81]. The appearance of *de novo* DSAs likely results from inadequate immunosuppression and represents a dynamic process that begins early after transplantation and continues at varying levels thereafter.

C4d negative acute ABMR
Initial evidence for C4d-negative acute ABMR emerged in 2009 based on the work of the teams in Paris[69] and Edmonton[62]. The latter study demonstrated high endothelial-specific gene expression in kidney transplant biopsy samples with DSAs but without C4d. In this study, C4d-negative acute ABMR was characterized by the high intragraft endothelial gene expression of allo-antibodies, by histology typical of chronic or acute ABMR and by poor outcomes. Several hypotheses have been generated to explain the lack of complement deposition despite the evidence of micro-vascular inflammation and persistence of DSAs in the circulation. The low sensitivity of C4d[13,82] could be due to technical issues including the type of fixative used and the different methods used to detect C4d (immunofluorescence versus immunochemistry). Moreover, as documented by the Edmonton study, some DSAs, although showing poor complement-fixing ability, may nonetheless activate endothelial cells[62]. Another possibility is that the various prophylactic strategies used to prevent ABMR may decrease the burden of complement activation within capillaries[80].

Given the concerns over the lack of sensitivity of C4d for kidney transplantations, a working group was established at the 2011 Banff conference to refine the criteria used for diagnosis of ABMR in the kidney[15]. Although the 2013 Banff Conference, held in Fortaleza (Brazil) in August 2013, has ended, to the best of our knowledge, this work remains in progress.

Late acute ABMR
If the majority of early-acute ABMR depends upon pre-
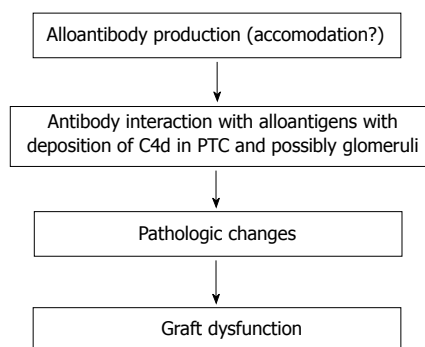
Figure 1 Stages of chronic antibody-mediated chronic rejection. PTC: Peritubular capillaries.

Figure 2 Proposed pathogenetic mechanisms for transplant glomerulopathy.

formed DSAs and primarily occurs in sensitized patients, late-acute ABMR often depends upon *de novo* DSAs.

De novo DSAs appear in 25% of non-sensitized patients[77]. *De novo* DSAs are often linked to late-acute ABMR and are characterized as occurring in patients who are young, with frequent non-adherence or suboptimal immunosuppression[74]. The observation that many cases of *de novo* DSAs are associated with prior therapy non-adherence or with a history of a clinical acute cellular rejection episode, suggests that immunosuppression is a potent inhibitor of the activation of mature, naïve B cells[83]. However, the observation that some cases of *de novo* DSAs formation appear in compliant patients suggests that either T cells capable of helping naïve B cells emerge despite immunosuppression or that some allo-reactive B cells may differentiate into antibody-secreting cells in the absence of T cell assistance. The antibody-producing cells may also originate from an existing population of memory B cells that do not require T-cell mediated activation[84].

CHRONIC ABMR

The clinical significance of chronic ABMR has been increasingly documented in recent years with some data suggesting that it may represent the leading cause of late allograft loss[4]. In contrast to acute ABMR, chronic ABMR is a long-term process that develops in sequential steps over a period of months to years[85]. Chronic ABMR has been proposed to arise over a series of stages or states[86]. The first common event is the production of alloantibodies followed by antibody interaction with alloantigens, resulting in the deposition of C4d in peritubular capillaries (PTC) and possibly glomeruli, followed by pathologic changes and graft dysfunction (Figure 1). Diagnostic features of chronic ABMR may include the presence of DSAs, transplant glomerulopathy (TG), peritubular capillary basement multilayering and the presence of C4d[27].

TG and PTC basement multilayering represent the histological hallmark of chronic ABMR. Transplant glomerulopathy is a morphological pattern of chronic kidney injury that lacks detectable immune-complex deposits and is associated with poor kidney transplant outcomes. It is primarily an endothelial pathology that affects kidney microcirculation endothelium, which is observed as a duplication (double contours) and/or multilamination of capillary basement membranes together with the substantial replacement of endothelial fenestrations with a continuous endothelial lining[87]. DSAs, particularly HLA antigen class II antibodies may cause insidious graft injury and therefore constitute a central causative factor of transplant glomerulopathy (Figure 2). Although the international Banff consensus criteria classify transplant glomerulopathy as chronic ABMR if the pattern is accompanied by detectable DSAs and diffuse or focal linear C4d positivity in peritubular capillaries[4-6], Mauiyyedi *et al*[88] detected the deposition of C4d in peritubular capillaries in 61% of biopsies from patients showing chronic rejection with transplant glomerulopathy. In addition, a study by Regele *et al*[89] reported the presence of C4d in peritubular capillaries in 34% of patients with transplant glomerulopathy and this staining presaged the later development of transplant glomerulopathy.
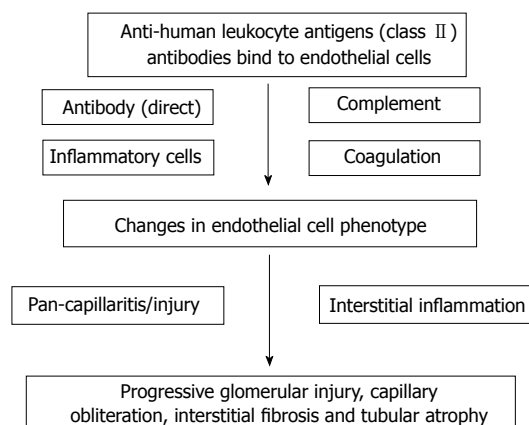
Pathologic patterns of chronic ABMR are observed in renal biopsies performed either for clinical indications or for protocol at a much later date after kidney transplantation[5-83]. In addition to reduced immunosuppression and non-adherence, early acute rejection appears to play a relevant role during late chronic ABMR. Indeed, several years ago, Cosio *et al*[90] documented that in 1-year surveillance biopsies, the degree of inflammation at 1-year post-transplant predicts the loss of graft function and graft failure independently of function and other variables (Figure 3).

Recently El Ters *et al*[91] reported that early acute rejection, even in the absence of pre-transplant DSAs, increases the risk of alloimmune allograft loss late after transplantation and that the phenotype of this late loss is chronic ABMR. The hypothesis of this study was that the formation of new DSAs, particularly class II DSAs, may be a consequence of early acute rejection[92]. El Ters *et al*[91] noted that the presence of inflammation in 1-year protocol biopsies correlated with early acute rejection, presensitization, re-transplantation and HLA mismatch. He also observed that chronic ABMR was responsible for 43% of allograft loss.

Table 2 Anti-antibodies main drugs to date in use and mechanism of action

Steps	Cells or mechanisms involved	Drugs	Mechanism of action
Exposure to antigen	B Cells	Rituximab	Binds CD20 on B cells and mediates cell lysis
		ivig	Multiple B cell apoptosis, decrease in B-cell proliferation
Secretion of alloantibodies	Plasma cells; antibodies	Bortezomib	Decrease donor-specific alloantibody production
			Mechanical removal of alloantibodies
		Plasma- exchange ivig	Multiple B cell apoptosis, decrease in B-cell proliferation
Binding of antibodies to the graft	Complement activation	Eculizumab	Blocks cleavage of terminal complement C5 and halts the process of complement-mediated cell destruction
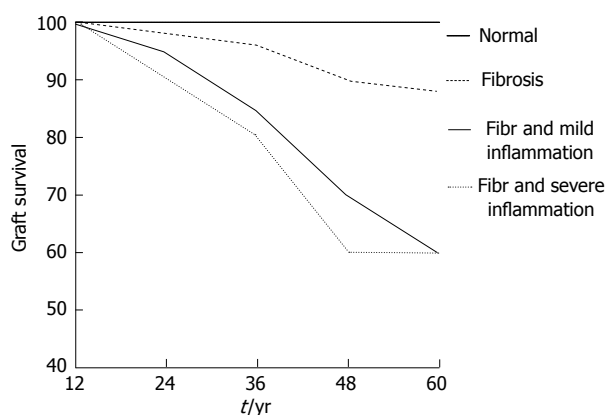
Figure 3 Five year post-transplant graft survival according 1-year post-transplant surveillance biopsy.
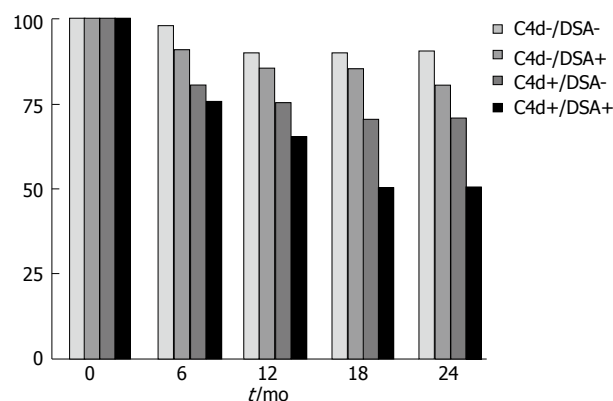
Figure 4 Deterioration of kidney allograft function study. Graft survival at 2-year according presence of donor-specific-HLA antibodies (DSAs) and/or C4d. HLA: Human leukocyte antigens.

In surveillance biopsies performed at 3 years after transplantation, Willicombe[93] reported that, despite excellent serum creatinine values, only one-third of biopsies were normal and that lesions appeared to correlate with the risks of immunological injury.

The 5-year follow-up data of the patient cohort from the DeKAF study[94,95] documented the role of antibodies in late graft dysfunction. Indeed, these studies showed a great number of patients with inflammation accompanying fibrosis or scarring, and their graft survival correlated with the presence of DSAs and/or C4d (Figure 4).

The therapeutic approach to these conditions is one of the major challenges to date in the treatment of transplanted patients.

Finally, recent studies[96,97] examining BAFF, a B-cell stimulating molecule, showed that the appearance of soluble BAFF levels early after transplantation correlated with the *de novo* development of DSAs and, ultimately, with the progression to chronic active ABMR in pediatric and adult first kidney transplant recipients who were highly desensitized prior to transplantation.

Hill *et al*[98] described a new insight into the pathogenesis of chronic ABMR. DSAs-positive patients showed a striking acceleration of arteriosclerosis. Pathologic examination revealed that the inner intima is hypercellular with actively proliferating myofibroblasts that lay down collagen that often overlies older, condensed collagen of pre-transplantation donor origin.

THERAPY

The primary drugs or systems used are shown in Table 2 and are divided based on their action on the different maturation steps of B cells. The primary therapeutic strategies used are the following: (1) removal of antibodies; (2) inhibition of antibody production; (3) complement inhibitors; (4) intravenous immunoglobulins; and (5) splenectomy.

Removal of antibodies by plasmapheresis or immunoabsorption

Plasmapheresis (PP) and immunoabsorption (IA) techniques have been used to remove alloantibodies. PP is not specific for immunoglobulins (Ig) removal and requires replacement with fresh frozen plasma and albumin. IA shows high affinity for binding Igs and has the advantage of specificity over PP.

However, due to the tendency of DSAs to rebound and return to baseline levels, several repeated treatments are required[99] or an additional inhibitor of antibody production is required.

Inhibition of antibody production

Rituximab (anti-CD20): Rituximab is a chimeric murine/human monoclonal antibody that binds CD20 on pre B and mature B lymphocytes[100,101]. Recently rituximab has been documented to also prevent an anamnestic

response in patients with cryptic sensitization to HLA[102].

BAFF blockade: BAFF, also known as B lymphocyte stimulator (Blys), is a member of the tumor necrosis factor cytokine family and is expressed primarily on T cells and dendritic cells for B-cell co-stimulation. BAFF binds to the receptor B-cell maturation antigen (BCMA), to the transmembrane activator (TACI) and to BAFF-receptor (BAFF-R) for B cell survival, proliferation and maturation[103].

BAFF blockade is a possible future therapy for renal transplantation. These drugs are highly promising because they selectively target B cells. Nevertheless no clinical trial is active in the field of transplantation although, these drugs have either been approved or are being examined for other diseases in large studies.

The best BAFF blockade drug is belimumab, which is a fully human recombinant IgG monoclonal antibody targeted against BAFF[104].

Bortezomib: Bortezomib is a proteasome inhibitor that is primarily used to treat acute ABMR or to decrease *de novo* DSA levels post-transplantation[105,106]. In further pilot studies, the authors used bortezomib in desensitization protocols with encouraging results[107,108].

Complement inhibitors

Eculizumab: Eculizumab is a humanized monoclonal antibody targeted against complement protein C5 that binds the C5 protein with high affinity and inhibits its cleavage to C5a and C5b, thereby preventing the generation of the terminal complement complex C5b-9. Eculizumab is used for the treatment of paroxysmal nocturnal hemoglobinuria and for atypical hemolytic uremic syndrome. Stegall *et al*[109] documented a decrease in post-transplant acute ABMR in sensitized renal transplant recipients, indicating its usefulness for desensitization protocols. Case reports have documented the effective rescue treatment of severe complement activation and reversal of acute ABMR by eculizumab in AB0-incompatible kidney-pancreas transplants and re-transplanted kidney recipients[110,111].

Intravenous immunoglobulins

Intravenous immunoglobulins show pleiotropic effects: They neutralize circulating anti-HLA antibodies *via* anti-idiotypic antibodies, inhibit complement activation by binding C3b and C4b and neutralizing C3a and C5a[112]. They also inhibit the expression of CD19 on activated B cells and induce the apoptosis of B cells[113]. Intravenous immunoglobulins (IVIGs) also show inhibitory effects on cellular immune responses with no specific inhibitory effects on the immune system by binding to Fcy receptors on macrophages, neutrophils, platelets, mast cells and NK cells.

IVIGs are used to decrease PRA levels in highly sensitized patients, in desensitization protocols of AB0-incompatible and XM-positive patients and in the treatment of ABMR.

Splenectomy: Splenectomy has been used in desensitization protocols and in the treatment of refractory acute ABMR[114,115]. Splenectomy removes a major source of lymphocytes, but the effect on the immune system is permanent and places the patients at risk for the development of sepsis.

As discussed in the pathophysiology chapter, we should distinguish the following: (1) acute ABMR; and (2) chronic ABMR.

Acute ABMR

Early acute ABMR often occurs in patients with DSAs prior to transplantation with a CDC-XM-positive with the donor. Even after successful desensitization strategies and successful kidney transplantations acute ABMR occurs in up to 40% of recipients. A later occurrence of acute ABMR is typically noted in patients with *de novo* DSAs and often after the reduction of immunosuppression or non-adherence[116,117].

We should now distinguish between the prevention and the treatment of acute ABMR.

Prevention of acute ABMR: Patients waiting for a transplant may be highly immunized and many show detectable DSAs in their serum. Sensitized patients who are DSAs-negative with negative XM-CDC may be transplanted safely. They will likely require more immunosuppressive therapy and an induction therapy[118-120].

The different desensitization protocols apply primarily to DSA-positive patients who are XM-CDC positive. The majority of the current protocols are modified version of the high-dose IVIG initiated at the Cedars-Sinai Medical Center or of the PP with low-dose IVIG initiated at John Hopkins Hospital[121].

Jordan initially provided[122] high dose IVIGs (2 g/kg) to cross-match-positive recipients, and the patients received a kidney transplant when their CDC T cell XM became negative. Due to the high rate of acute ABMR, Jordan[123] decided to use alemtuzumab induction treatment and added rituximab to the protocol to decrease the acute rejection rate.

More recently, Vo *et al*[124] at the Cedars-Sinai reported on the 24-mo outcomes of the aforementioned desensitization protocol and showed a 2-years graft survival of 84% in 76 hyper immune XM-positive recipients.

The other approach to desensitization comprises the use of PP and low-dose anti cytomegalovirus IVIG (CMV Ig). This approach was first adopted in 1998 at John Hopkins Hospital in XM-incompatible living donor kidney transplant candidates[125]. Patients received PP and CMV Ig at 100 mg/kg after each PP, combined with tacrolimus and mycophenolate mofetil. In a recent study, Montgomery *et al*[126] successfully desensitized 211 DSA-positive recipients of living donor kidneys with PP and low-dose IVIG.

A differing approach is the use of peri-transplant

immunoabsorption rather than plasmapheresis. In 68 patients with deceased donors, Bartel and colleagues used peri-transplant IA followed by post-transplant IA and obtained excellent transplant outcomes[127].

Overall, over the last 13 years, almost 1000 patients with DSAs underwent kidney transplants and used varying desensitization protocols. The patient and graft survival rates are 95% and 86%, respectively, at the 2-year median follow-up. The primary issue is the high rates of acute rejection and of ABMR in particular (28%)[128]. New drugs are being developed to reduce this high rate of ABMR.

Stegall *et al*[109] added eculizumab during the pre-post-transplant period in DSAs-positive patients and obtained 7.7% post-transplant acute ABMR compared with 41.2% in the control group. However, at 2-years after transplantation the incidence of chronic ABMR was similar between the two groups. Chronic ABMR remains a major issue when transplanting hyper-immune patients.

A different option is to use the proteasome inhibitor bortezomib. In pilot studies, bortezomib has been used in desensitization protocols with encouraging results[107,108]. It is being used in a current ongoing a prospective iterative trial of proteasome inhibitor-based desensitization[129]. The trial has been approved by the International Review Board (IRB) and is being conducted under the auspices of FDA. Preliminary data suggest that bortezomib-based desensitization regimens comprising only two cycles (8 doses) may consistently reduce immunodominant HLA antibody levels and that multiple treatments with bortezomib (two-cycle regimen) may enable highly sensitized patients to undergo transplantation without IVIGs.

Treatment of acute ABMR: Acute ABMR in kidney recipients responds poorly to corticosteroids and anti-thymocyte agents alone, which are the standard treatment for acute cellular rejection.

International guidelines do not define an evidence-based treatment for acute ABMR. The kidney disease improving global outcomes guidelines (KDIGO) recommend the use of one or more of the following: corticosteroids, PP, IVIG, anti-CD20 antibodies or lymphocyte-depleting antibodies[130].

Two studies have individually reviewed the current approach to the treatment of acute ABMR[46] and the randomized controlled trials treating acute ABMR[131].

Although the literature suggests that plasmapheresis with or without low-dose IVIG or high-dose IVIG alone shows evidence of efficacy against acute ABMR and that they may be considered for the standard of care (SOC), these treatment regimens have not been standardized or optimized.

Approaches vary based on the amount of replacement volume, type of replacement fluids, number of PP sessions and the dose, timing and formulation of IVIGs used.

Other agents, such as rituximab, bortezomib and eculizumab have been used occasionally in conjunction with the above-mentioned therapies.

Of these treatments, rituximab has been used most frequently, and two studies in particular have evaluated rituximab as part of a combination treatment approach[132,133]. The latter study included 54 patients and compared a historical group treated with plasma exchange and IVIGs with a later group receiving a single dose of 500 mg/m^2 rituximab in addition. The use of rituximab was associated with a 90% 2-year graft survival compared with 60% in the control group. Nevertheless, the benefit of adding rituximab remains in question when examining all published patient series.

Several case reports and series have been published on the use of bortezomib in the treatment for acute AMBR.

The largest series of 20 patients treated with bortezomib was reported by Flechner[134]. Using this treatment regimen, a graft survival rate of 85% at 10 mo post-transplant was achieved. The mean decrease in the dominant DSA in MFI values was 50%. However, the side effects of the treatment were considerable. One of the most recent studies compared 10 bortezomib-treated patients with a historical group of 9 rituximab-treated patients and achieved a graft survival of 60% with bortezomib compared with only 11% with rituximab at 18 mo[135].

Taken together, these preliminary results on bortezomib in acute ABMR are promising; however carefully performed, controlled studies are required to prove its benefits.

In the setting of kidney transplantation, there is emerging but limited evidence that eculizumab is efficient in treating acute ABMR[136]. Thus far, only a few reports exist in the literature on the use of eculizumab in refractory acute ABMR[110,111]. Stegall *et al*[109] reported the largest study of eculizumab in renal transplantation in a desensitization strategy. In this study, eculizumab appeared to show no impact on DSAs production after transplantation. In addition, the incidence of chronic ABMR appeared unchanged either by the prevention of early ABMR or by the prolonged complement blockade.

Splenectomy: One last option to salvage a graft with acute therapy-resistant ABMR is rescue splenectomy and its use has been reported by at least three groups[137-139]. The majority of patients underwent this surgery prior to the advent of eculizumab, and in the future, splenectomy may be avoided by using eculizumab instead. Splenectomy is recommended only in resistant cases of acute ABMR where bortezomib or eculizumab have already failed.

In summary, the first step for managing acute ABMR includes steroid pulses and/or antibody removal with PP or IA and IVIGs. The second step in patients with persistent allograft dysfunction includes the use of bortezomib and/or rituximab. The third step in resistant acute ABMR includes eculizumab and rescue splenectomy.

Chronic ABMR: In contrast to acute ABMR, chronic ABMR is a long-term process that develops in sequential steps over months to years[84].
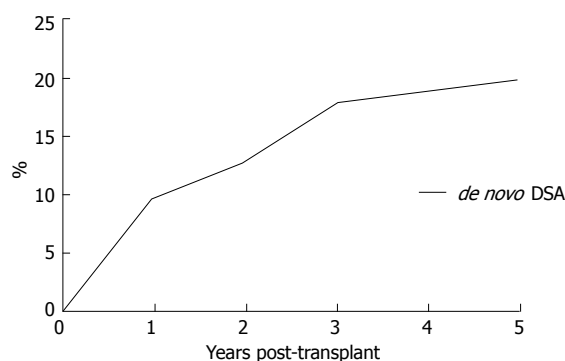
Figure 5 Actual 5-year post-transplantation cumulative incidence of de novo donor-specific-human leukocyte antigens antibodies (DSA). DSA: Donor-specific-human leukocyte antigens antibodies.

Figure 6 Probability of graft loss within 3 years after *de novo* donor-specific-human leukocyte antigens antibodies appearance. DSA:Donor-specific-human leukocyte antigens antibodies.

In theory, every option available to treat acute ABMR may also be applied to chronic ABMR. However, there are no controlled trials in the literature regarding the treatment of chronic ABMR. The only treatment option with some reported benefit is a combination of rituximab and IVIGs[140].

With respect to established chronic ABMR, there have only been three case series treated with this combination therapy[141-143]. DSAs decreased only in some patients, and the therapy showed limited effects in cases with massive proteinuria, more severe peritubular capillaritis and previous acute rejection.

Very few patients have received bortezomib as a rescue treatment for chronic ABMR and proteinuria, and they have shown mixed results[144,145].
An interim analysis of a very recent study[146] of eculizumab therapy in chronic ABMR documented an apparent stabilization of renal function.

Taken together, these results indicate that any treatment for chronic ABMR using drugs with potentially high toxicity should only be performed in the context of a randomized controlled trial.

A recent recognized context that should be distinguished from acute or chronic ABMR is the negative impact of *de novo* DSAs after transplantation on the transplant outcome.

Several authors have reviewed the incidence and impact of *de novo* donor DSAs, in both adult[77] and pediatric recipients[147].

The actual 5-year post-transplantation cumulative incidence of *de novo* DSAs in a low-risk population is 20% (Figure 5). Once DSAs appear, the probability of graft loss within the 3 years of the appearance of DSAs is 24% (Figure 6). In patients without DSAs, the relative risk of graft loss is 9-fold higher at 1 year after the appearance of DSAs. In a multivariate analysis[77], the primary causes of *de novo* DSAs were DQ locus mismatches, a younger age at transplantation and transplants from deceased donors. Others claim prior non-adherence or a history of a clinical acute cellular rejection as being causes of *de novo* DSAs[82].

If the appearance of DSAs is associated with the clinical signs of acute ABMR, the treatments used have already been discussed. The primary issue is how to treat when the appearance of DSAs is not associated with acute rejection.
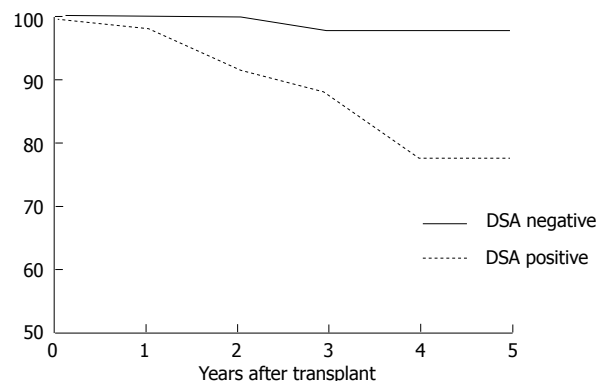
To date, prophylactic treatments, such as rituximab and splenectomy[148] or eculizumab[109], do not appear to induce any effect on the appearance of DSAs.

Monitoring DSAs after transplantation appears to be essential because the appearance of DSAs is associated with a poor prognosis. Because procedures, such as antibody removal by PP or IA and the down regulation of antibody production by B cell- or plasma cell-targeting or complement cascade inhibition show very limited success when employed during the advanced phase of chronic ABMR[142,149,150], the prompt removal of *de novo* DSAs appears to be essential. However, no SOC exist for this issue. To date, only a multicenter antibody removal trial study in Italy is ongoing; it is using a randomized, prospective PP and low-dose CMV-IVIGs[151].

NEW AND FUTURE THERAPIES

Some of the drugs mentioned above that have been used to prevent or treat acute ABMR remain in pre-marketing clinical trials or have been approved for other diseases.

Drugs already known to control T cells also appear to be active in the long-term control of B cells.

Belatacept, a fusion receptor protein that blocks the co-stimulation pathway CD80/CD86-CD28, was recently approved for the prevention of acute rejection. Belatacept inhibited DSAs in phase 3 trials[152].

Another co-stimulation pathway is the CD40/CD40L pathway. Previous studies with antibodies directed against CD40L failed due to severe episodes of thrombosis. Indeed, CD40L is also expressed on the platelet surface, and its inhibition may induce thrombosis. More recently, the inhibition of the CD40/CD40L pathway by directly targeting CD40 has drawn interest from investigators particularly because CD40 is not expressed on the platelet surface. Humanized anti-CD40 antibodies prevented acute rejection and prolonged renal graft in non-human primates. In addition, these anti-CD40 antibodies appear safe and effective as maintenance immunosuppressive therapies[153-155]. To date, five monoclonal antibodies di-

rected against CD40 have been studied for different diseases including kidney transplantation (Clinical Trial.gov NCT01780844).

Newer drugs that target B cell have been described. The most exciting are likely those that target survival factors and are part of the tumor necrosis factor super family: BAFF, Blys and the proliferation-inducing ligand (APRIL)[103].

Belimumab has already been discussed: it is a fully human antibody that neutralizes BAFF and deprives B cells of this important survival factor. The FDA approved belimumab in March 2011 for systemic lupus erythematosus (SLE). A group from Pennsylvania has enrolled patients in a phase II clinical trial of desensitization in sensitized patients awaiting clinical transplantation (clinicaltrials.gov NCT01025193). In this context, the study was unable to demonstrate the efficacy of belimumab.

Atacicept is a fusion receptor protein that neutralizes both BAFF or Blys and APRIL. In allo-sensitized nonhuman primates, atacicept reduced T-cell and B-cell alloantibodies by 36% and 24%, respectively[156].

A further possibility is complement inhibition by C1 esterase inhibitors, a plasma-derived human C1 esterase inhibitor. Initially used in allotransplantation to protect against ischemia/reperfusion injury[157], it is now under investigation for solid organ transplantations and approved by the FDA for use in other disease states. A trial studying the safety and tolerability of C1 inhibitor therapy in the context of the prevention of acute rejection (clinicaltrial.gov NCT01134510) is now ongoing. However, thus far no patients have been recruited.

CONCLUSION

The relevant graft injury is now well recognized to be caused by alloantibodies. Both acute and chronic graft injury may be caused by alloantibodies, and the most recent Banff classifications have been modified to introduce acute and chronic ABMR. The latter appears to be the most relevant cause of long-term graft injury rather than CNIs nephrotoxicity and "chronic allograft nephropathy".

In addition to the major histocompatibility antigens, a large number of minor antigens have been recognized as possible antibody targets. The most important and the most widely studied antibodies responsible for graft injury are the HLA-DSAs.

The availability of new techniques for detecting circulating antibodies has enabled better understanding in recent years of the presence and role of antibodies in determining graft injury.

From a clinical point of view, we must distinguish between acute ABMR and chronic ABMR. In addition, we now recognize indolent ABMR and C4d-negative acute ABMR. Indolent ABMR develops sub-clinically. It often manifests in patients with *de novo* DSAs and causes slowly progressive microvascular abnormalities that lead to chronic ABMR. C4d-negative ABMR is cause for great discussion among scholars. It may be caused by an injury

that is non-complement-mediated; however it may also be due to defective techniques. The Banff group is still working to improve understanding of this entity.

Recently, evidence has accumulated on the significant role of HLA-DSAs in the pathogenesis of slowly progressive graft injury and dysfunction. Several studies have shown that circulating DSAs (class I or class II) are found in a substantial fraction of renal allograft recipients and are associated with long-term graft loss.

The primary therapeutic approach comprises antibody removal, B-cells and plasma cells- targeting and inhibition of the complement pathway. The therapeutic approach used is based on the clinical conditions.

In patients waiting for transplantation who show positive XM-CDC, the removal of antibodies with or without B- or plasma cell-inhibition remains the best approach.

Patients with acute ABMR should be treated with a heavy regimen of T/B cell-targeting drugs (pulse corticosteroids and ATG), by removing antibodies, and using specific B- or plasma cell-inhibition or by complement inhibition.

No SOC exists for chronic ABMR, and only randomized controlled trials will indicate the best therapeutic option.

What may we hope for in the future? Unfortunately, the pipeline is almost empty.

Essentially, we may consider two types of drugs that are either already on the market or remain in premarket trials: (1) drugs targeting both T and B cells; Belatacept has already been approved by the FDA for the prevention of acute rejection. In a 3-year follow-up study[152], it proved to be effective on DSAs also in CNIs free protocols. The blockade of CD40-CD154 with humanized anti-CD 40 antibodies has prevented acute rejection[154]. In addition, these antibodies appear safe and effective in maintenance therapy; and (2) drugs targeting B cells or the complement pathway.

BAFF-blocking drugs: Represent new interesting drugs that target B lymphocyte stimulators. Belimumab, a fully human recombinant IgG monoclonal antibody to BAFF, was approved in 2011 for the treatment of SLE; however the above-mentioned phase II trial for desensitization failed. Atacicept was evaluated in diseases including rheumatoid arthritis, SLE, multiple sclerosis and B-cell malignancies. It awaits evaluation in human transplant patients.

While waiting for the approval of eculizumab, the C1 esterase inhibitor is being studied. This drug has been FDA-approved for treating hereditary angioedema; however it appears to be far from approval for use in transplantation.

REFERENCES

1   **L'Agence de la biomédecine**. Agence de la Biomédecine Annual Report. Available from: URL: http://www. agence-biomedecine.fr/article/111 (2011)
2   Organ Procurement and Transplantation Network. Available from: URL: http://optn.transplant.hrsa.gov/ar2009/default. htm (2012).

3   **Morais OO**, Costa IM, Gomes CM, Shinzato DH, Ayres GM, Cardoso RM. The use of the Er: YAG 2940nm laser associated with amorolfine lacquer in the treatment of onychomycosis. *An Bras Dermatol* 2013; **88**: 847-849 [PMID: 24173203 DOI: 10.5500/wjt.v3.i2.7]

4   **Einecke G**, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, Kaplan B, Halloran PF. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520-2531 [PMID: 19843030 DOI: 10.1111/j.1600-6143.2009.02799.x]

5   **Sellarés J**, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, Hidalgo LG, Famulski K, Matas A, Halloran PF. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388-399 [PMID: 22081892 DOI: 10.1111/j.1600-6143.2011.03840.x]

6   **Gaston RS**, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, Gourishankar S, Grande J, Halloran P, Hunsicker L, Mannon R, Rush D, Matas AJ. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation* 2010; **90**: 68-74 [PMID: 20463643 DOI: 10.1097/TP.0b013e3181e065de]

7   **Gourishankar S**, Leduc R, Connett J, Cecka JM, Cosio F, Fieberg A, Gaston R, Halloran P, Hunsicker L, Kasiske B, Rush D, Grande J, Mannon R, Matas A. Pathological and clinical characterization of the 'troubled transplant': data from the DeKAF study. *Am J Transplant* 2010; **10**: 324-330 [PMID: 20055809 DOI: 10.1111/j.1600-6143.2009.02954.x]

8   **Matas AJ**, Leduc R, Rush D, Cecka JM, Connett J, Fieberg A, Halloran P, Hunsicker L, Cosio F, Grande J, Mannon R, Gourishankar S, Gaston R, Kasiske B. Histopathologic clusters differentiate subgroups within the nonspecific diagnoses of CAN or CR: preliminary data from the DeKAF study. *Am J Transplant* 2010; **10**: 315-323 [PMID: 20041864 DOI: 10.1111/j.1600-6143.2009.02943.x]

9   **Loupy A**, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol* 2012; **8**: 348-357 [PMID: 22508180 DOI: 10.1038/nrneph.2012.81]

10   **Hidalgo LG**, Campbell PM, Sis B, Einecke G, Mengel M, Chang J, Sellares J, Reeve J, Halloran PF. De novo donor-specific antibody at the time of kidney transplant biopsy associates with microvascular pathology and late graft failure. *Am J Transplant* 2009; **9**: 2532-2541 [PMID: 19843031 DOI: 10.1111/j.1600-6143.2009.02800.x]

11   **Tait BD**, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, Reed EF, Bray RA, Campbell P, Chapman JR, Coates PT, Colvin RB, Cozzi E, Doxiadis II, Fuggle SV, Gill J, Glotz D, Lachmann N, Mohanakumar T, Suciu-Foca N, Sumitran-Holgersson S, Tanabe K, Taylor CJ, Tyan DB, Webster A, Zeevi A, Opelz G. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation* 2013; **95**: 19-47 [PMID: 23238534 DOI: 10.1097/TP.0b013e31827a19cc]

12   **Solez K**, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ. Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; **7**: 518-526 [PMID: 17352710 DOI: 10.1111/j.1600-6143.2006.01688.x]

13   **Solez K**, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M,

Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753-760 [PMID: 18294345 DOI: 10.1111/j.1600-6143.2008.02159.x]

14   **Sis B**, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen LC, Solez K, Baldwin WM, Bracamonte ER, Broecker V, Cosio F, Demetris AJ, Drachenberg C, Einecke G, Gloor J, Glotz D, Kraus E, Legendre C, Liapis H, Mannon RB, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Rodriguez ER, Seron D, Seshan S, Suthanthiran M, Wasowska BA, Zachary A, Zeevi A. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant* 2010; **10**: 464-471 [PMID: 20121738 DOI: 10.1111/j.1600-6143.2009.02987.x]

15   **Mengel M**, Sis B, Haas M, Colvin RB, Halloran PF, Racusen LC, Solez K, Cendales L, Demetris AJ, Drachenberg CB, Farver CF, Rodriguez ER, Wallace WD, Glotz D. Banff 2011 Meeting report: new concepts in antibody-mediated rejection. *Am J Transplant* 2012; **12**: 563-570 [PMID: 22300494 DOI: 10.1111/j.1600-6143.2011.03926.x]

16   **Martin S**, Claas F. Antibodies and crossmatching for transplantation. In: Dyer PA, Middleton D, eds. Histocompatibility testing: a practical approach. Oxford: IRL Press, 1993: 97-105

17   **Salvadori M**, Bertoni E. Renal transplant allocation criteria, desensitization strategies and immunosuppressive therapy in retransplant renal patients. *J Nephrol* 2012; **25**: 890-899 [PMID: 22972671 DOI: 10.5301/jn.5000207]

18   **Eng HS**, Bennett G, Bardy P, Coghlan P, Russ GR, Coates PT. Clinical significance of anti-HLA antibodies detected by Luminex: enhancing the interpretation of CDC-BXM and important post-transplantation monitoring tools. *Hum Immunol* 2009; **70**: 595-599 [PMID: 19527759 DOI: 10.1016/j.humimm.2009.06.010]

19   **Fuggle SV**, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. *Transplantation* 2008; **86**: 384-390 [PMID: 18698240 DOI: 10.1097/TP.0b013e31817c90f5]

20   **Vlad G**, Ho EK, Vasilescu ER, Colovai AI, Stokes MB, Markowitz GS, D'Agati VD, Cohen DJ, Ratner LE, Suciu-Foca N. Relevance of different antibody detection methods for the prediction of antibody-mediated rejection and deceased-donor kidney allograft survival. *Hum Immunol* 2009; **70**: 589-594 [PMID: 19375470 DOI: 10.1016/j.humimm.2009.04.018]

21   **Mizutani K**, Terasaki P, Hamdani E, Esquenazi V, Rosen A, Miller J, Ozawa M. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. *Am J Transplant* 2007; **7**: 1027-1031 [PMID: 17391143 DOI: 10.1111/j.1600-6143.2006.01721.x]

22   **Schwartz A**, Gaigalas AK, Wang L, Marti GE, Vogt RF, Fernandez-Repollet E. Formalization of the MESF unit of fluorescence intensity. *Cytometry B Clin Cytom* 2004; **57**: 1-6 [PMID: 14696057 DOI: 10.1002/cyto.b.10066]

23   **Takemoto SK**, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, Kupiec-Weglinski J, Matas A, Montgomery RA, Nickerson P, Platt JL, Rabb H, Thistlethwaite R, Tyan D, Delmonico FL. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant* 2004; **4**: 1033-1041 [PMID: 15196059 DOI: 10.1111/j.1600-6143.2004.00500.x]

24   **Gupta A**, Sinnott P. Clinical relevance of pretransplant human leukocyte antigen donor-specific antibodies in renal patients waiting for a transplant: a risk factor. *Hum Immunol* 2009; **70**: 618-622 [PMID: 19374932 DOI: 10.1016/j.humimm.2009.04.020]

25   **Gupta A**, Iveson V, Varagunam M, Bodger S, Sinnott P, Thuraisingham RC. Pretransplant donor-specific antibodies in cytotoxic negative crossmatch kidney transplants: are they relevant? *Transplantation* 2008; **85**: 1200-1204 [PMID:

18431242 DOI: 10.1097/TP.0b013e31816b1c37]

26 **Riethmüller S**, Ferrari-Lacraz S, Müller MK, Raptis DA, Hadaya K, Rüsi B, Laube G, Schneiter G, Fehr T, Villard J. Donor-specific antibody levels and three generations of crossmatches to predict antibody-mediated rejection in kidney transplantation. *Transplantation* 2010; **90**: 160-167 [PMID: 20658760 DOI: 10.1097/TP.0b013e3181e36e08]

27 **Archdeacon P**, Chan M, Neuland C, Velidedeoglu E, Meyer J, Tracy L, Cavaille-Coll M, Bala S, Hernandez A, Albrecht R. Summary of FDA antibody-mediated rejection workshop. *Am J Transplant* 2011; **11**: 896-906 [PMID: 21521465 DOI: 10.1111/j.1600-6143.2011.03525.x]

28 **Bartel G**, Wahrmann M, Exner M, Regele H, Huttary N, Schillinger M, Körmöczi GF, Hörl WH, Böhmig GA. In vitro detection of C4d-fixing HLA alloantibodies: associations with capillary C4d deposition in kidney allografts. *Am J Transplant* 2008; **8**: 41-49 [PMID: 17924995 DOI: 10.1111/j.1600-6143.2007.01998.x]

29 **Smith JD**, Hamour IM, Banner NR, Rose ML. C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. *Am J Transplant* 2007; **7**: 2809-2815 [PMID: 17908268 DOI: 10.1111/j.1600-6143.2007.01991.x]

30 **Chen G**, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. *Hum Immunol* 2011; **72**: 849-858 [PMID: 21791230 DOI: 10.1016/j.humimm.2011.07.001]

31 **Otten HG**, Verhaar MC, Borst HP, Hené RJ, van Zuilen AD. Pretransplant donor-specific HLA class-I and -II antibodies are associated with an increased risk for kidney graft failure. *Am J Transplant* 2012; **12**: 1618-1623 [PMID: 22404993 DOI: 10.1111/j.1600-6143.2011.03985.x]

32 **Lachmann N**, Todorova K, Schulze H, Schönemann C. Systematic comparison of four cell- and Luminex-based methods for assessment of complement-activating HLA antibodies. *Transplantation* 2013; **95**: 694-700 [PMID: 23354296 DOI: 10.1097/TP.0b013e31827b3dc3]

33 **Glotz D**, Lucchiari N, Pegaz-Fiornet B, Suberbielle-Boissel C. Endothelial cells as targets of allograft rejection. *Transplantation* 2006; **82**: S19-S21 [PMID: 16829788 DOI: 10.1097/01.tp.0000231348.55262.5a]

34 **Ronda C**, Borba SC, Ferreira SC, Glotz D, Ianhez LE, Rodrigues H, Viggiani CS, Nahas W, David-Neto E, Castro MC, David Daisa SR, Kalil J, Panajotopoulos N. Non-human leukocyte antigen antibodies reactive with endothelial cells could be involved in early loss of renal allografts. *Transplant Proc* 2011; **43**: 1345-1348 [PMID: 21620126 DOI: 10.1016/j.transproceed.2011.03.059]

35 **Pontes LF**, Carvalho L, Stumbo AC, Porto LC. Detection and localization of non-HLA-ABC antigenic sites relevant to kidney rejection on endothelial cells. *J Immunol Methods* 2001; **251**: 73-80 [PMID: 11292483 DOI: 10.1016/S0022-1759(01)00309-X]

36 **Hendrickx J**, Doggen K, Weinberg EO, Van Tongelen P, Fransen P, De Keulenaer GW. Molecular diversity of cardiac endothelial cells in vitro and in vivo. *Physiol Genomics* 2004; **19**: 198-206 [PMID: 15304623]

37 **Vermehren D**, Sumitran-Holgersson S. Isolation of precursor endothelial cells from peripheral blood for donor-specific crossmatching before organ transplantation. *Transplantation* 2002; **74**: 1479-1486 [PMID: 12490778]

38 **Alheim M**, Johansson SM, Hauzenberger D, Grufman P, Holgersson J. A flow cytometric crossmatch test for simultaneous detection of antibodies against donor lymphocytes and endothelial precursor cells. *Tissue Antigens* 2010; **75**: 269-277 [PMID: 20070600 DOI: 10.1111/j.1399-0039.2009.01439.x]

39 **Dinavahi R**, George A, Tretin A, Akalin E, Ames S, Bromberg JS, Deboccardo G, Dipaola N, Lerner SM, Mehrotra A, Murphy BT, Nadasdy T, Paz-Artal E, Salomon DR, Schröp-pel B, Sehgal V, Sachidanandam R, Heeger PS. Antibodies reactive to non-HLA antigens in transplant glomerulopathy. *J Am Soc Nephrol* 2011; **22**: 1168-1178 [PMID: 21566057 DOI: 10.1681/ASN.2010111183]

40 **Sigdel TK**, Li L, Tran TQ, Khatri P, Naesens M, Sansanwal P, Dai H, Hsieh SC, Sarwal MM. Non-HLA antibodies to immunogenic epitopes predict the evolution of chronic renal allograft injury. *J Am Soc Nephrol* 2012; **23**: 750-763 [PMID: 22302197 DOI: 10.1681/ASN.2011060596]

41 **Dunn TB**, Noreen H, Gillingham K, Maurer D, Ozturk OG, Pruett TL, Bray RA, Gebel HM, Matas AJ. Revisiting traditional risk factors for rejection and graft loss after kidney transplantation. *Am J Transplant* 2011; **11**: 2132-2143 [PMID: 21812918 DOI: 10.1111/j.1600-6143.2011.03640.x]

42 **Zito A**, Schena A, Grandaliano G, Gesualdo L, Schena FP. Increasing relevance of donor-specific antibodies in antibody-mediated rejection. *J Nephrol* 2013; **26**: 237-242 [PMID: 23475460 DOI: 10.5301/jn.5000240]

43 **Terasaki PI**, Ozawa M, Castro R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. *Am J Transplant* 2007; **7**: 408-415 [PMID: 17229080 DOI: 10.1111/j.1600-6143.2006.01644.x]

44 **Rodríguez PC**, Arroyave IH, Mejía G, García LF. Detection of alloantibodies against non-HLA antigens in kidney transplantation by flow cytometry. *Clin Transplant* 2000; **14**: 472-478 [PMID: 11048992 DOI: 10.1034/j.1399-0012.2000.140505.x]

45 **Colvin RB**. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007; **18**: 1046-1056 [PMID: 17360947]

46 **Nickerson PW**, Rush DN. Antibodies beyond HLA. *Am J Transplant* 2013; **13**: 831-832 [PMID: 23406413 DOI: 10.1111/ajt.12160]

47 **Breimer ME**, Rydberg L, Jackson AM, Lucas DP, Zachary AA, Melancon JK, Von Visger J, Pelletier R, Saidman SL, Williams WW, Holgersson J, Tydén G, Klintmalm GK, Coultrup S, Sumitran-Holgersson S, Grufman P. Multicenter evaluation of a novel endothelial cell crossmatch test in kidney transplantation. *Transplantation* 2009; **87**: 549-556 [PMID: 19307793 DOI: 10.1097/TP.0b013e3181949d4e]

48 **Dragun D**. Humoral responses directed against non-human leukocyte antigens in solid-organ transplantation. *Transplantation* 2008; **86**: 1019-1025 [PMID: 18946337 DOI: 10.1097/TP.0b013e3181889748]

49 **Regele H**. Non-HLA antibodies in kidney allograft rejection: convincing concept in need of further evidence. *Kidney Int* 2011; **79**: 583-586 [PMID: 21358654 DOI: 10.1038/ki.2010.517]

50 **Zhang Q**, Reed EF. Non-MHC antigenic targets of the humoral immune response in transplantation. *Curr Opin Immunol* 2010; **22**: 682-688 [PMID: 20833523 DOI: 10.1016/j.coi.2010.08.009]

51 **Sun Q**, Cheng Z, Cheng D, Chen J, Ji S, Wen J, Zheng C, Liu Z. De novo development of circulating anti-endothelial cell antibodies rather than pre-existing antibodies is associated with post-transplant allograft rejection. *Kidney Int* 2011; **79**: 655-662 [PMID: 20980975 DOI: 10.1038/ki.2010.437]

52 **Soulez M**, Pilon EA, Dieudé M, Cardinal H, Brassard N, Qi S, Wu SJ, Durocher Y, Madore F, Perreault C, Hébert MJ. The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection. *Circ Res* 2012; **110**: 94-104 [PMID: 22076637 DOI: 10.1161/CIRCRESAHA.111.250431]

53 **Cardinal H**, Dieudé M, Brassard N, Qi S, Patey N, Soulez M, Beillevaire D, Echeverry F, Daniel C, Durocher Y, Madore F, Hébert MJ. Antiperlecan antibodies are novel accelerators of immune-mediated vascular injury. *Am J Transplant* 2013; **13**: 861-874 [PMID: 23432943 DOI: 10.1111/ajt.12168]

54 **Mahesh B**, Leong HS, McCormack A, Sarathchandra P, Holder A, Rose ML. Autoantibodies to vimentin cause accelerated rejection of cardiac allografts. *Am J Pathol*

2007; **170**: 1415-1427 [PMID: 17392180 DOI: 10.2353/aj-path.2007.060728]

55   **Braun RK**, Molitor-Dart M, Wigfield C, Xiang Z, Fain SB, Jankowska-Gan E, Seroogy CM, Burlingham WJ, Wilkes DS, Brand DD, Torrealba J, Love RB. Transfer of tolerance to collagen type V suppresses T-helper-cell-17 lymphocyte-mediated acute lung transplant rejection. *Transplantation* 2009; **88**: 1341-1348 [PMID: 20029330 DOI: 10.1097/TP.0b013e3181bcde7b]

56   **Ochoa L**, Merck B, Geli S, Díaz de Liaño A. Hepatic trauma (90 consecutive cases treated in 9 years. *Rev Esp Enferm Dig* 1991; **79**: 393-396 [PMID: 1910916]

57   **Reinsmoen NL**, Lai CH, Heidecke H, Haas M, Cao K, Ong G, Naim M, Wang Q, Mirocha J, Kahwaji J, Vo AA, Jordan SC, Dragun D. Anti-angiotensin type 1 receptor antibodies associated with antibody mediated rejection in donor HLA antibody negative patients. *Transplantation* 2010; **90**: 1473-1477 [PMID: 21030904 DOI: 10.1097/TP.0b013e3181fd97f1]

58   **Sánchez-Zapardiel E**, Castro-Panete MJ, Castillo-Rama M, Morales P, Lora-Pablos D, Valero-Hervás D, Ruiz-García R, Apaza J, Talayero P, Andrés A, Morales JM, Paz-Artal E. Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. *Transplantation* 2013; **96**: 70-78 [PMID: 23624543 DOI: 10.1097/TP.0b013e3182943506]

59   **Naemi FM**, Carter V, Kirby JA, Ali S. Anti-donor HLA class I antibodies: pathways to endothelial cell activation and cell-mediated allograft rejection. *Transplantation* 2013; **96**: 258-266 [PMID: 23823649 DOI: 10.1097/TP.0b013e3182985504]

60   **Crespo M**, Torio A, Mas V, Redondo D, Pérez-Sáez MJ, Mir M, Faura A, Guerra R, Montes-Ares O, Checa MD, Pascual J. Clinical relevance of pretransplant anti-HLA donor-specific antibodies: does C1q-fixation matter? *Transpl Immunol* 2013; **29**: 28-33 [PMID: 23907088]

61   **Clatworthy MR**. B cell responses to allograft--more common than we thought? *Am J Transplant* 2013; **13**: 1629-1630 [PMID: 23802722 DOI: 10.1111/ajt.12309]

62   **Sis B**, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant* 2009; **9**: 2312-2323 [PMID: 19681822 DOI: 10.1111/j.1600-6143.2009.02761.x]

63   **Lynch RJ**, Silva IA, Chen BJ, Punch JD, Cascalho M, Platt JL. Cryptic B cell response to renal transplantation. *Am J Transplant* 2013; **13**: 1713-1723 [PMID: 23750851 DOI: 10.1111/ajt.12308]

64   **Wood KJ**, Goto R. Mechanisms of rejection: current perspectives. *Transplantation* 2012; **93**: 1-10 [PMID: 22138818 DOI: 10.1097/TP.0b013e31823cab44]

65   **Nickerson PW**, Rush DN. Rejection: an integrated response. *Am J Transplant* 2013; **13**: 2239-2240 [PMID: 23911011 DOI: 10.1111/ajt.12365]

66   **Smith RN**, Colvin RB. Chronic alloantibody mediated rejection. *Semin Immunol* 2012; **24**: 115-121 [PMID: 22051115 DOI: 10.1016/j.smim.2011.09.002]

67   **Hirohashi T**, Chase CM, Della Pelle P, Sebastian D, Alessandrini A, Madsen JC, Russell PS, Colvin RB. A novel pathway of chronic allograft rejection mediated by NK cells and alloantibody. *Am J Transplant* 2012; **12**: 313-321 [PMID: 22070565 DOI: 10.1111/j.1600-6143.2011.03836.x]

68   **Jordan SC**, Toyoda M, Vo AA. Regulation of immunity and inflammation by intravenous immunoglobulin: relevance to solid organ transplantation. *Expert Rev Clin Immunol* 2011; **7**: 341-348 [PMID: 21595600 DOI: 10.1586/eci.11.10]

69   **Loupy A**, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, Timsit MO, Duong JP, Bruneval P, Vernerey D, Empana JP, Jouven X, Nochy D, Legendre CH. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant* 2011; **11**: 56-65 [PMID: 21199348 DOI: 10.1111/j.1600-6143.2010.03364.x]

70   **Colvin RB**, Nickeleit V. Renal transplant pathology. In: Heptinstall's Pathology of the kidney, 6th Ed, edited by Jennette JC, Olson JL, Scwartz MM, Silva PG, Philadelphia, Lippincott-Raven, 2006 : 1347-1490

71   **Drachenberg CB**, Papadimitriou JC. Endothelial injury in renal antibody-mediated allograft rejection: a schematic view based on pathogenesis. *Transplantation* 2013; **95**: 1073-1083 [PMID: 23370711 DOI: 10.1097/TP.0b013e31827e6b45]

72   **Caro-Oleas JL**, González-Escribano MF, Gentil-Govantes MÁ, Acevedo MJ, González-Roncero FM, Blanco GB, Núñez-Roldán A. Clinical relevance of anti-HLA donor-specific antibodies detected by Luminex assay in the development of rejection after renal transplantation. *Transplantation* 2012; **94**: 338-344 [PMID: 22814330 DOI: 10.1097/TP.0b013e31825ace2c]

73   **Hourmant M**, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, Dantal J, Giral M, Blancho G, Cantarovich D, Karam G, Follea G, Soulillou JP, Bignon JD. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol* 2005; **16**: 2804-2812 [PMID: 16014742 DOI: 10.1681/ASN.2004121130]

74   **Dörje C**, Midtvedt K, Holdaas H, Naper C, Strøm EH, Øyen O, Leivestad T, Aronsen T, Jenssen T, Flaa-Johnsen L, Lindahl JP, Hartmann A, Reisæter AV. Early versus late acute antibody-mediated rejection in renal transplant recipients. *Transplantation* 2013; **96**: 79-84 [PMID: 23632391 DOI: 10.1097/TP.0b013e31829434d4]

75   **Bista B**, Jackson S, Issa N, Matas A, Kukla A. High creatinine and advanced chronic rejection (CR) predicts graft loss in non-adherent kiney transplant recipients. *Am J Transplant* 2013; **13**: 415 [DOI: 10.1111/ajt.12266]

76   **Lawrence C**, Willicombe M, Brookes PA, Santos-Nunez E, Bajaj R, Cook T, Roufosse C, Taube D, Warrens AN. Preformed complement-activating low-level donor-specific antibody predicts early antibody-mediated rejection in renal allografts. *Transplantation* 2013; **95**: 341-346 [PMID: 23197178 DOI: 10.1097/TP.0b013e3182743cfa]

77   **Everly MJ**, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, Catrou PG, Bolin P, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation* 2013; **95**: 410-417 [PMID: 23380861 DOI: 10.1097/TP.0b013e31827d62e3]

78   **Banham G**, Prezzi D, Harford S, Taylor CJ, Hamer R, Higgins R, Bradley JA, Clatworthy MR. Elevated pretransplantation soluble BAFF is associated with an increased risk of acute antibody-mediated rejection. *Transplantation* 2013; **96**: 413-420 [PMID: 23842189 DOI: 10.1097/TP.0b013e318298dd65]

79   **Orandi B**, Chow E, Van Arendonk K, Montgomery J, Gupta N, Montgomery R, Segev D. Early antibody-mediated rejection portends worse long-term renal graft and patient serviva. *Am J Transplant* 2013; **13**: 324

80   **Loupy A**, Suberbielle-Boissel C, Zuber J, Anglicheau D, Timsit MO, Martinez F, Thervet E, Bruneval P, Charron D, Hill GS, Nochy D, Legendre C. Combined posttransplant prophylactic IVIg/anti-CD 20/plasmapheresis in kidney recipients with preformed donor-specific antibodies: a pilot study. *Transplantation* 2010; **89**: 1403-1410 [PMID: 20386362 DOI: 10.1097/TP.0b013e3181da1cc3]

81   **de Kort H**, Willicombe M, Brookes P, Dominy KM, Santos-Nunez E, Galliford JW, Chan K, Taube D, McLean AG, Cook HT, Roufosse C. Microcirculation inflammation associates with outcome in renal transplant patients with de novo donor-specific antibodies. *Am J Transplant* 2013; **13**: 485-492 [PMID: 23167441 DOI: 10.1111/j.1600-6143.2012.04325.x]

82   **Haririan A**, Kiangkitiwan B, Kukuruga D, Cooper M, Hurley H, Drachenberg C, Klassen D. The impact of c4d pattern and donor-specific antibody on graft survival in

recipients requiring indication renal allograft biopsy. *Am J Transplant* 2009; **9**: 2758-2767 [PMID: 19845596 DOI: 10.1111/j.1600-6143.2009.02836.x]

83   **Wiebe C**, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, Goldberg A, Birk PE, Rush DN, Nickerson PW. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant* 2012; **12**: 1157-1167 [PMID: 22429309 DOI: 10.1111/j.1600-6143.2012.04013.x]

84   **Stegall MD**, Moore N, Taner T, Li H, Dean PG. Down-regulating humoral immune responses: implications for organ transplantation. *Transplantation* 2014; **97**: 247-257 [PMID: 24056631 DOI: 10.1097/TP.0b013e3182a72115]

85   **Smith RN**, Kawai T, Boskovic S, Nadazdin O, Sachs DH, Cosimi AB, Colvin RB. Four stages and lack of stable accommodation in chronic alloantibody-mediated renal allograft rejection in Cynomolgus monkeys. *Am J Transplant* 2008; **8**: 1662-1672 [PMID: 18557724 DOI: 10.1111/j.1600-6143.2008.02303.x]

86   **Colvin RB**, Smith RN. Antibody-mediated organ-allograft rejection. *Nat Rev Immunol* 2005; **5**: 807-817 [PMID: 16175181 DOI: 10.1038/nri1702]

87   **Husain S**, Sis B. Advances in the understanding of transplant glomerulopathy. *Am J Kidney Dis* 2013; **62**: 352-363 [PMID: 23313456 DOI: 10.1053/j.ajkd.2012.10.026]

88   **Mauiyyedi S**, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001; **12**: 574-582 [PMID: 11181806]

89   **Regele H**, Böhmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, Watschinger B, Kerjaschki D, Exner M. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 2002; **13**: 2371-2380 [PMID: 12191982 DOI: 10.1097/01.ASN.0000025780.03790]

90   **Cosio FG**, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; **5**: 2464-2472 [PMID: 16162196 DOI: 10.1111/j.1600-6143.2005.01050.x]

91   **El Ters M**, Grande JP, Keddis MT, Rodrigo E, Chopra B, Dean PG, Stegall MD, Cosio FG. Kidney allograft survival after acute rejection, the value of follow-up biopsies. *Am J Transplant* 2013; **13**: 2334-2341 [PMID: 23865852 DOI: 10.1111/ajt.12370]

92   **Casey E**, Gandhi M, Dean P, Stegall M, Cosio F Class I HLA mismatches increase risk of acute rejection, graft loss and formation of de novo lass II antibody. *Am J Transplant* 2013; **19**: 42 [DOI: 10.1111/ajt.12265]

93   **Willicombe M**, Roufosse C, Brookes P, Galliford J, McLean A, Cook T, Taube D. Potential importance of 3 year surveillance biopsies in renal transplantation. *Am J Transplant* 2013; **19**: 41

94   **Gaston RS**. Our evolving understanding of late kidney allograft failure. *Curr Opin Organ Transplant* 2011; **16**: 594-599 [PMID: 21971511 DOI: 10.1097/MOT.0b0132834c23a7]

95   **Rush D**, Leduc R, Connett J, Cosio F, Gaston R, Mannon R, Hunsicker L, Gourishankar S, Halloran P, Cecka M, Grande J, Kasiske B. DeKAF pathology clusters: follow up at 5 years. *Am J Transplant* 2013; **19**: 417

96   **Comoli P**, Quartuccio G, Cioni M, Parodi A, Fontana I, Basso S, Magnasco A, Tagliamacco A, Nocera A, Cardillo M, Ginevri F. Soluble BAFF levels correlate with de novo DSA development and progression to chronic active antibody-mediated rejection in pediatric recipients of first kidney transplants. *Am J Transplant* 2013; **19**: 113

97   **Thibault-Espitia A**, Foucher Y, Danger R, Migone T, Pallier A, Castagnet S, G-Gueguen C, Devys A, C-Gautier A, Giral M, Soulillou JP, Brouard S. BAFF and BAFF-R levels are associated with risk of long-term kidney graft dysfunction and development of donor-specific antibodies. *Am J Transplant* 2012; **12**: 2754-2762 [PMID: 22883025 DOI: 10.1111/j.1600-6143.2012.04194.x]

98   **Hill GS**, Nochy D, Bruneval P, Duong van Huyen JP, Glotz D, Suberbielle C, Zuber J, Anglicheau D, Empana JP, Legendre C, Loupy A. Donor-specific antibodies accelerate arteriosclerosis after kidney transplantation. *J Am Soc Nephrol* 2011; **22**: 975-983 [PMID: 21493773 DOI: 10.1681/ASN.2010070777]

99   **Hakim RM**, Milford E, Himmelfarb J, Wingard R, Lazarus JM, Watt RM. Extracorporeal removal of anti-HLA antibodies in transplant candidates. *Am J Kidney Dis* 1990; **16**: 423-431 [PMID: 2239932]

100 **Pescovitz MD**. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. *Am J Transplant* 2006; **6**: 859-866 [PMID: 16611321 DOI: 10.1111/j.1600-6143.2006.01288.x]

101 **van den Hoogen MW**, Hilbrands LB. Use of monoclonal antibodies in renal transplantation. *Immunotherapy* 2011; **3**: 871-880 [PMID: 21751955 DOI: 10.2217/imt.11.72]

102 **Zachary AA**, Lucas DP, Montgomery RA, Leffell MS. Rituximab prevents an anamnestic response in patients with cryptic sensitization to HLA. *Transplantation* 2013; **95**: 701-704 [PMID: 23503502 DOI: 10.1097/TP.0b013e31827be3c1]

103 **Page EK**, Dar WA, Knechtle SJ. Biologics in organ transplantation. *Transpl Int* 2012; **25**: 707-719 [PMID: 22420711 DOI: 10.1111/j.1432-2277.2012.01456.x]

104 **Navarra SV**, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, Li EK, Thomas M, Kim HY, León MG, Tanasescu C, Nasonov E, Lan JL, Pineda L, Zhong ZJ, Freimuth W, Petri MA. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 2011; **377**: 721-731 [PMID: 21296403 DOI: 10.1016/S0140-6736(10)61354-2]

105 **Everly MJ**, Everly JJ, Susskind B, Brailey P, Arend LJ, Alloway RR, Roy-Chaudhury P, Govil A, Mogilishetty G, Rike AH, Cardi M, Wadih G, Tevar A, Woodle ES. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 2008; **86**: 1754-1761 [PMID: 19104417 DOI: 10.1097/TP.0b013e318190af83]

106 **Everly MJ**, Terasaki PI, Trivedi HL. Durability of antibody removal following proteasome inhibitor-based therapy. *Transplantation* 2012; **93**: 572-577 [PMID: 22262128 DOI: 10.1097/TP.0b013e31824612df]

107 **Everly MJ**, Everly JJ, Terasaki PI. Role of proteasome inhibition in sensitized transplant candidates. *Chin Med J* (Engl) 2011; **124**: 771-774 [PMID: 21518574]

108 **Kute VB**, Vanikar AV, Trivedi HL, Shah PR, Goplani KR, Patel HV, Gumber MR, Patel RD, Kanodia KV, Suthar KS, Trivedi VB, Modi PR. Desensitization protocol for highly sensitized renal transplant patients: a single-center experience. *Saudi J Kidney Dis Transpl* 2011; **22**: 662-669 [PMID: 21743208]

109 **Stegall MD**, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405-2413 [PMID: 21942930 DOI: 10.1111/j.1600-6143.2011.03757.x]

110 **Biglarnia AR**, Nilsson B, Nilsson T, von Zur-Mühlen B, Wagner M, Berne C, Wanders A, Magnusson A, Tufveson G. Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. *Transpl Int* 2011; **24**: e61-e66 [PMID: 21696455]

111 **Locke JE**, Magro CM, Singer AL, Segev DL, Haas M, Hillel AT, King KE, Kraus E, Lees LM, Melancon JK, Stewart ZA, Warren DS, Zachary AA, Montgomery RA. The

use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant* 2009; **9**: 231-235 [PMID: 18976298 DOI: 10.1111/j.1600-6143.2008.02451.x]

112 **Watanabe J**, Scornik JC. IVIG and HLA antibodies. Evidence for inhibition of complement activation but not for anti-idiotypic activity. *Am J Transplant* 2005; **5**: 2786-2790 [PMID: 16212641 DOI: 10.1111/j.1600-6143.2005.01056.x]

113 **Samuelsson A**, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* 2001; **291**: 484-486 [PMID: 11161202 DOI: 10.1126/science.291.5503.484]

114 **Locke JE**, Zachary AA, Mohammed BS, Warren DS, Montgomery RA. Rescue splenectomy for severe acute antibody-mediated rejection. *Clin Transpl* 2006; : 518-520 [PMID: 18365416]

115 **Locke JE**, Zachary AA, Haas M, Melancon JK, Warren DS, Simpkins CE, Segev DL, Montgomery RA. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant* 2007; **7**: 842-846 [PMID: 17391127 DOI: 10.1111/j.1600-6143.2006.01709.x]

116 **Lefaucheur C**, Suberbielle-Boissel C, Hill GS, Nochy D, Andrade J, Antoine C, Gautreau C, Charron D, Glotz D. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Am J Transplant* 2008; **8**: 324-331 [PMID: 18162086 DOI: 10.1111/j.1600-6143.2007.02072.x]

117 **Kayler LK**, Kiss L, Sharma V, Mohanka R, Zeevi A, Girnita A, Shapiro R, Randhawa PS. Acute renal allograft rejection: diagnostic significance of focal peritubular capillary C4d. *Transplantation* 2008; **85**: 813-820 [PMID: 18360261 DOI: 10.1097/TP.0b013e3181669194]

118 **Noël C**, Abramowicz D, Durand D, Mourad G, Lang P, Kessler M, Charpentier B, Touchard G, Berthoux F, Merville P, Ouali N, Squifflet JP, Bayle F, Wissing KM, Hazzan M. Daclizumab versus antithymocyte globulin in high-immunological-risk renal transplant recipients. *J Am Soc Nephrol* 2009; **20**: 1385-1392 [PMID: 19470677 DOI: 10.1681/ASN.2008101037]

119 **Gurk-Turner C**, Airee R, Philosophe B, Kukuruga D, Drachenberg C, Haririan A. Thymoglobulin dose optimization for induction therapy in high risk kidney transplant recipients. *Transplantation* 2008; **85**: 1425-1430 [PMID: 18497682 DOI: 10.1097/TP.0b013e31816dd596]

120 **Cai J**, Terasaki PI. Current trend of induction and maintenance treatment in positive panel-reactive antibody patients: a report on OPTN/UNOS kidney transplant registry data. *Chin Med J* (Engl) 2011; **124**: 649-654 [PMID: 21518552]

121 **Montgomery RA**, Zachary AA, Racusen LC, Leffell MS, King KE, Burdick J, Maley WR, Ratner LE. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation* 2000; **70**: 887-895 [PMID: 11014642]

122 **Jordan SC**, Vo A, Bunnapradist S, Toyoda M, Peng A, Puliyanda D, Kamil E, Tyan D. Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* 2003; **76**: 631-636 [PMID: 12973100]

123 **Vo AA**, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, Peng A, Villicana R, Jordan SC. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242-251 [PMID: 18635429 DOI: 10.1056/NEJMoa0707894]

124 **Vo AA**, Peng A, Toyoda M, Kahwaji J, Cao K, Lai CH, Reinsmoen NL, Villicana R, Jordan SC. Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. *Transplantation* 2010; **89**: 1095-1102 [PMID: 20110854 DOI: 10.1097/TP.0b013e3181d21e7f]

125 **Montgomery RA**, Zachary AA. Transplanting patients with a positive donor-specific crossmatch: a single center's perspective. *Pediatr Transplant* 2004; **8**: 535-542 [PMID: 15598320 DOI: 10.1111/j.1399-3046.2004.00214.x]

126 **Montgomery RA**, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, Warren DS, Simpkins CE, Dagher NN, Singer AL, Zachary AA, Segev DL. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; **365**: 318-326 [PMID: 21793744 DOI: 10.1056/NEJMoa1012376]

127 **Bartel G**, Wahrmann M, Regele H, Kikić Z, Fischer G, Druml W, Mühlbacher F, Böhmig GA. Peritransplant immunoadsorption for positive crossmatch deceased donor kidney transplantation. *Am J Transplant* 2010; **10**: 2033-2042 [PMID: 20883537 DOI: 10.1111/j.1600-6143.2010.03226.x]

128 **Marfo K**, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. *Clin J Am Soc Nephrol* 2011; **6**: 922-936 [PMID: 21441131 DOI: 10.2215/CJN.08140910]

129 **Woodle ES**, Alloway RR, Girnita A. Proteasome inhibitor treatment of antibody-mediated allograft rejection. *Curr Opin Organ Transplant* 2011; **16**: 434-438 [PMID: 21753709 DOI: 10.1097/MOT.0b013e328348c0e5]

130 **Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group.** KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

131 **Roberts DM**, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. *Transplantation* 2012; **94**: 775-783 [PMID: 23032865 DOI: 10.1097/TP.0b013e31825d1587]

132 **Lefaucheur C**, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, Hill GS, Glotz D, Suberbielle-Boissel C. Comparison of combination Plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am J Transplant* 2009; **9**: 1099-1107 [PMID: 19422335 DOI: 10.1111/j.1600-6143.2009.02591.x]

133 **Kaposztas Z**, Podder H, Mauiyyedi S, Illoh O, Kerman R, Reyes M, Pollard V, Kahan BD. Impact of rituximab therapy for treatment of acute humoral rejection. *Clin Transplant* 2009; **23**: 63-73 [PMID: 19200217 DOI: 10.1111/j.1399-0012.2008.00902.x]

134 **Flechner SM**, Fatica R, Askar M, Stephany BR, Poggio E, Koo A, Banning S, Chiesa-Vottero A, Srinivas T. The role of proteasome inhibition with bortezomib in the treatment of antibody-mediated rejection after kidney-only or kidney-combined organ transplantation. *Transplantation* 2010; **90**: 1486-1492 [PMID: 21042239 DOI: 10.1097/TP.0b013e3181fdd9b0]

135 **Waiser J**, Budde K, Schütz M, Liefeldt L, Rudolph B, Schönemann C, Neumayer HH, Lachmann N. Comparison between bortezomib and rituximab in the treatment of antibody-mediated renal allograft rejection. *Nephrol Dial Transplant* 2012; **27**: 1246-1251 [PMID: 21852274 DOI: 10.1093/ndt/gfr465]

136 **Legendre C**, Sberro-Soussan R, Zuber J, Rabant M, Loupy A, Timsit MO, Anglicheau D. Eculizumab in renal transplantation. *Transplant Rev* (Orlando) 2013; **27**: 90-92 [PMID: 23747093 DOI: 10.1016/j.trre.2013.04.002]

137 **Tzvetanov I**, Spaggiari M, Jeon H, Roca RG, Bhati C, Oberholzer J, Benedetti E. The role of splenectomy in the setting of refractory humoral rejection after kidney transplantation. *Transplant Proc* 2012; **44**: 1254-1258 [PMID: 22663995 DOI: 10.1016/j.transproceed.2012.01.109]

138 **Kaplan B**, Gangemi A, Thielke J, Oberholzer J, Sankary H, Benedetti E. Successful rescue of refractory, severe antibody mediated rejection with splenectomy. *Transplantation* 2007; **83**: 99-100 [PMID: 17220802 DOI: 10.1097/01.tp.0000243739.31440.2b]

139 **Roberti I**, Geffner S, Vyas S. Successful rescue of refractory acute antibody-mediated renal allograft rejection with splenectomy--a case report. *Pediatr Transplant* 2012; **16**: E49-E52 [PMID: 21672105 DOI: 10.1111/j.1399-3046.2011.01518.x]

140 **Fehr T**, Gaspert A. Antibody-mediated kidney allograft re-

jection: therapeutic options and their experimental rationale. *Transpl Int* 2012; **25**: 623-632 [PMID: 22394269 DOI: 10.1111/j.1432-2277.2012.01453.x]

141 **Billing H**, Rieger S, Ovens J, Süsal C, Melk A, Waldherr R, Opelz G, Tönshoff B. Successful treatment of chronic antibody-mediated rejection with IVIG and rituximab in pediatric renal transplant recipients. *Transplantation* 2008; **86**: 1214-1221 [PMID: 19005402 DOI: 10.1097/TP.0b013e3181880b35]

142 **Fehr T**, Rüsi B, Fischer A, Hopfer H, Wüthrich RP, Gaspert A. Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection. *Transplantation* 2009; **87**: 1837-1841 [PMID: 19543061 DOI: 10.1097/TP.0b013e3181a6bac5]

143 **Ahn G**, Chung B, Park C, Choi B, Kim Y, Yang C. Effectiveness of Rituximab and intravenous immunoglobulin for the treatment of chronic antibody mediated rejection in kidney transplant recipients. *Am J Transplant* 2013; **19**: 328

144 **Schwaiger E**, Regele H, Wahrmann M, Werzowa J, Haidbauer B, Schmidt A, Böhmig GA. Bortezomib for the treatment of chronic antibody-mediated kidney allograft rejection: a case report. *Clin Transpl* 2010; : 391-396 [PMID: 21696056]

145 **Lonze BE**, Dagher NN, Simpkins CE, Singer AL, Segev DL, Zachary AA, Montgomery RA. The fate of anti-HLA antibody among renal transplantation recipients treated with bortezomib. *Clin Transpl* 2009; : 377-384 [PMID: 20524301]

146 **Kulkarni S**, Kirkles-Smith, Tomlin R, Formica R, Moeckel G, Pober J. Eculizumab therapy for chronic antibody-mediated injury in kidney transplantation: an interim assessment. *Am J Transplant* 2013; **19**: 86

147 **Ginevri F**, Nocera A, Comoli P, Innocente A, Cioni M, Parodi A, Fontana I, Magnasco A, Nocco A, Tagliamacco A, Sementa A, Ceriolo P, Ghio L, Zecca M, Cardillo M, Garibotto G, Ghiggeri GM, Poli F. Posttransplant de novo donor-specific hla antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant* 2012; **12**: 3355-3362 [PMID: 22959074 DOI: 10.1111/j.1600-6143.2012.04251.x]

148 **Ashimine S**, Watarai Y, Yamamoto T, Hiramitsu T, Tsujita M, Nanmoku K, Goto N, Takeda A, Katayama A, Uchida K, Kobayashi T. Neither pre-transplant rituximab nor splenectomy affects de novo HLA antibody production after renal transplantation. *Kidney Int* 2014; **85**: 425-430 [PMID: 23945498 DOI: 10.1038/ki.2013.291]

149 **Vincenti F**, Cohen SD, Appel G. Novel B cell therapeutic targets in transplantation and immune-mediated glomerular diseases. *Clin J Am Soc Nephrol* 2010; **5**: 142-151 [PMID: 20007678 DOI: 10.2215/CJN.04580709]

150 **Jordan SC**, Reinsmoen N, Peng A, Lai CH, Cao K, Villicana R, Toyoda M, Kahwaji J, Vo AA. Advances in diagnosing and managing antibody-mediated rejection. *Pediatr Nephrol* 2010; **25**: 2035-2045; quiz 2035-2045 [PMID: 20077121 DOI: 10.1007/s00467-009-1386-4]

151 **Seveso M**, Rigotti P, Schena PF, Stefoni S, Sandrini S, Salvadori M, Stratta P, Citterio F, Segoloni GP, Ruffoni E, Furlan L, Grandaliano G, Scolari MP, Bertoni E, Gargiulo A, Biancone L, Cozzi E. Preliminary results from a prospective multi-center study reveal the early appearance of de novo anti-HLA antibodies following renal transplantation. *Transpl Int* 2011; **24**: 38 [DOI: 10.1111/j.1432-2277.2011.01346.x]

152 **Vincenti F**, Larsen CP, Alberu J, Bresnahan B, Garcia VD, Kothari J, Lang P, Urrea EM, Massari P, Mondragon-Ramirez G, Reyes-Acevedo R, Rice K, Rostaing L, Steinberg S, Xing J, Agarwal M, Harler MB, Charpentier B. Three-year outcomes from BENEFIT, a randomized, active-controlled, parallel-group study in adult kidney transplant recipients. *Am J Transplant* 2012; **12**: 210-217 [PMID: 21992533 DOI: 10.1111/j.1600-6143.2011.03785.x]

153 **Badell IR**, Thompson PW, Turner AP, Russell MC, Avila JG, Cano JA, Robertson JM, Leopardi FV, Strobert EA, Iwakoshi NN, Reimann KA, Ford ML, Kirk AD, Larsen CP. Nondepleting anti-CD40-based therapy prolongs allograft survival in nonhuman primates. *Am J Transplant* 2012; **12**: 126-135 [PMID: 21920020 DOI: 10.1111/j.1600-6143.2011.03736.x]

154 **Goldwater R**, Keirns J, Blahunka P, First R, Sawamoto T, Zhang W, Kowalski D, Kaibara A, Holman J. A phase 1, randomized ascending single-dose study of antagonist anti-human CD40 ASKP1240 in healthy subjects. *Am J Transplant* 2013; **13**: 1040-1046 [PMID: 23356210 DOI: 10.1111/ajt.12082]

155 **Watanabe M**, Yamashita K, Suzuki T, Kamachi H, Kuraya D, Koshizuka Y, Ogura M, Yoshida T, Aoyagi T, Fukumori D, Shimamura T, Okimura K, Maeta K, Miura T, Sakai F, Todo S. ASKP1240, a fully human anti-CD40 monoclonal antibody, prolongs pancreatic islet allograft survival in nonhuman primates. *Am J Transplant* 2013; **13**: 1976-1988 [PMID: 23841873 DOI: 10.1111/ajt.12330]

156 **Boskovic S**, Smith RN, Kawai T, Nadazdin, O; Sachs, D; Cosimi, A B.; Colvin, R. Inhibitory effects of atacicept (TACI-Ig) on circulating antibodies, B cells and plasma cells in allosensitized cynomolgus monkeys. *Transplantation* 2008; **86**: 163 [DOI: 10.1097/01.tp.0000332279.02594.98]

157 **Kirschfink M**. C1-inhibitor and transplantation. *Immunobiology* 2002; **205**: 534-541 [PMID: 12396013]

ABO incompatible renal transplants: Good or bad?

Masaki Muramatsu, Hector Daniel Gonzalez, Roberto Cacciola, Atsushi Aikawa, Magdi M Yaqoob,

Carmelo Puliatti

Masaki Muramatsu, Magdi M Yaqoob, Carmelo Puliatti, Department of Translational Medicine and Therapeutics, William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, United Kingdom
Masaki Muramatsu, Atsushi Aikawa, Department of Nephrology, Toho University Faculty of Medicine, Tokyo 143-8541, Japan
Hector Daniel Gonzalez, Roberto Cacciola, Magdi M Yaqoob, Carmelo Puliatti, Transplant Unit, The Royal London Hospital, London E1 1BB, United Kingdom
Author contributions: Muramatsu M, Cacciola R, Aikawa A, Yaqoob MM and Puliatti C contributed to conception and design, acquisition of data, or analysis and interpretation of data; Muramatsu M, Gonzalez HD, Cacciola R, Aikawa A, Yaqoob MM and Puliatti C contributed to drafting the article or revising it critically for important intellectual content; Muramatsu M, Yaqoob MM and Puliatti C contributed to final approval of the version to be published.
Correspondence to: Carmelo Puliatti, MD, Transplant Unit, The Royal London Hospital, Whitechapel Road, London E1 1BB, United Kingdom. Carmelo.Puliatti@bartshealth.nhs.uk
Telephone: +44-20-35942673 Fax: +44-20-35943248
Received: December 7, 2013   Revised: February 12, 2014
Accepted: February 18, 2014
Published online: March 24, 2014

Abstract

ABO incompatible kidney transplantation (ABOi-KT) was previously considered to be an absolute contraindication for patients with end-stage kidney disease (ESKD) due to hyperacute rejection related to blood type barrier. Since the first successful series of ABOi-KT was reported, ABOi-KT is performed increasingly all over the world. ABOi-KT has led to an expanded donor pool and reduced the number of patients with ESKD awaiting deceased kidney transplantation (KT). Intensified immunosuppression and immunological understanding has helped to shape current desensitization protocols. Consequently, in recent years, ABOi-KT outcome is comparable to ABO compatible KT (ABOc-KT). However, many questions still remain unanswered. In ABOi-KT, there is an additional residual immunological risk that may lead to allograft damage, despite using current diverse but usually intensified immunosuppressive protocols at the expense of increasing risk of infection and possibly malignancy. Notably, in ABOi-KT, desensitization and antibody reduction therapies have increased the cost of KT. Reassuringly, there has been an evolution in ABOi-KT leading to a simplification of protocols over the last decade. This review provides an overview of the history, outcome, protocol, advantages and disadvantages in ABOi-KT, and focuses on whether ABOi-KT should be recommended as a therapeutic option of KT in the future.

Core tip: This article demonstrates merits and demerits of ABO incompatible kidney transplantation (ABOi-KT). Although the excellent outcome of ABOi-KT has been achieved, unresolved matters still remain. We review the role of ABOi-KT for patients with end-stage kidney disease and considered validity whether ABOi-KT should be recommended as a therapeutic option of KT in the future.

INTRODUCTION

Kidney transplantation (KT) is known as a standard therapy for patients with end-stage kidney disease (ESKD) and has been adopted widely in the world. However, the

living and deceased kidney donor pool does not resolve the shortage of transplantable organs. Different ways have been proposed to increase the donor pool and ABO incompatible KT (ABOi-KT) represents a valid source of organs to decrease the donor waiting list. ABOi-KT requires extra strategies and suffers extra risks across ABO blood type barrier compared to ABO compatible KT (ABOc-KT). ABOi-KT was previously considered to be contraindicated for many years. Presently, ABOi-KT has been accepted as a valid alternative therapy for ESKD and the outcome of ABOi-KT has become equivalent to ABOc-KT in adult and pediatric recipients[1-4]. When a patient with ESKD requires KT and an acceptable living donor is ABO incompatible with the recipient, the patient can currently chose one of three options: (1) stay on the waiting list for deceased donor KT; (2) have paired kidney donor exchange (PKDE); or (3) undergo ABOi-KT.

According to the Organ Procurement and Transplantation Network (OPTN) report 2011, 86500 patients on the deceased donor waiting list, and almost 28000 were added to the list annually in the United States. Ten thousand patients received deceased donor KT, and 4900 patients received living KT. Almost 5000 patients died while waiting for a kidney. The median waiting time depended on the blood type of patients, but it is reported to be around 4 years for all patients on the OPTN report[5]. Various reports analysing graft and patient survival related to the waiting time showed that 6 mo or more of dialysis negatively affect the outcome[6,7]. PKDE is an innovative method whereby 2 or more incompatible donor-recipient pairs exchange donors to create 2 or more compatible pairs. It is a very reasonable idea for human leukocyte antigen (HLA) sensitized and/or ABO incompatible patients. This primary idea was reported first by Rapaport in 1980s[8]. There are currently several variations of exchange such as three-way, four-way and domino paired donation[9]. PKDE provides a recipient with an incompatible donor the chance to receive a compatible kidney, which is available by expanding the donor source and reducing the waiting time for deceased donor KT. Advantages of PKDE are low immunological risk, avoidance of intensified immunosuppression due to desensitization, and cost effectiveness[10].

Alexandre *et al*[11] demonstrated the ABOi-KT strategy using plasmapheresis and splenectomy to break the ABO barrier. This has been used as a desensitization strategy for ABOi-KT for 20 years. ABOi-KT has become common in Japan due to the lack of deceased donors, and ABOi-KT has accounted for approximately 30% of all living-donor KT in that country.[12]. On the contrary, a tiny proportion, only 738 cases (0.94%) of ABOi-KT were performed between 1995 and 2010 in the United States[4], but this number is increasing annually. The same trend continues in the United Kingdom: over the last decade, there has been an increase of ABOi-KT from less than 10 per year to 100 per year representing 1.0% of living donor transplants performed[13]. This increase is possibly due to the fact that protocols have been simplified

		Donor			
		A	**B**	**O**	**AB**
Recipient	A	-	+	-	+
	B	+	-	-	+
	O	+	+	-	+
	AB	-	-	-	-

Table 1 Combination of blood type and compatibility

+: ABO incompatible transplantation; -: ABO compatible transplantation.

over the years from complex surgical and pharmacological processes that variably may have involved splenectomy, rituximab (RIT), plasmapheresis and antibodies titration.

Although the use of ABOi-KT has increased worldwide, there are arguments against ABOi-KT as a universal treatment. To consider whether ABOi-KT is viable a therapeutic option for patients with ESKD, this review will focus on the transitional outcomes alongside current and future prospects in ABOi-KT.

ABO ANTIGENS AND ANTIBODIES

The concept of blood groups A, B and O (H) was established by Nobel laureate Karl Landsteiner in the early 1900s. These are polysaccharide antigens which are found in red cell, platelets, and other tissues such as endothelium[14]. The antibodies to blood group antigen are isohemagglutinins and can be of either immunoglobulin M (IgM) or immunoglobulin G (IgG) type antibodies. However, in the context of transplantation it is IgG that is functionally significant.Blood type A develops anti-B antibody, and blood type B has anti-A antibody. Blood type AB with A and B antigen has both antibodies, while blood type O with both antibodies does not have any antigen. Blood type incompatibility means the exposure of A or B antigen to a person who has antibodies against these antigens. Therefore, these antigen expressions of an organ have been obstacles for ABOi-KT (Table 1). All blood type recipients accept a blood type O donor as a universal donor, and a blood type AB accepts all blood type donors as a universal recipient. Blood group type A, however, carries A1 or A2. The expression of A2 antigen is weaker than that of A1 antigen[15]. The A2 subtype constitutes approximately 20% of blood type A in white races, while it is only 0.15% in Japanese population[16]. A2 kidney may be less likely to suffer antibody rejection in the presence of anti-A antibody. In fact, non-A recipients receiving kidneys from A2 donors[17], can universally and safely accept the transplantation without preconditioning at times of KT.

HISTORY

Splenectomy, rituximab and no B-cell depletion

Previous clinical studies related to ABOi-KT are summarized in Table 2[1-4,11,18-42]. The first successful report of ABOi-KT is dated back to 1987 when authors achieved long-term allograft survival in a series of 23 patients[11]. Plasmapheresis and splenectomy were performed to re-

Table 2 Historical clinical reports in ABO incompatible kidney transplantation

Ref.	Type of study	Study population	ABOi population	Desensitization	Outcome
Hume *et al*[18]	Observational	9	1	No treatment	Graft nephrectomy day 17
Starzl *et al*[19]	Observational	3	2	SPx (1 case)	Graft survival 74 d (1 case), patient death day 24 (1 case)
Sheil *et al*[20]	Observational	2	2	No treatment	Graft nephrectomy day 14
Alexandre *et al*[11]	Observational	23	23	PE/SPx	2-yr graft survival: 88% (related donor), 50% (unrelated donor)
Ota *et al*[21]	Observational, comparative	51	51	DFPP and/or IAs/SPx	2-yr graft survival: 87% *vs* 84.6% *vs* 50% (A- *vs* B- *vs* ABO-incompatible)
Tanabe *et al*[22]	Observational, comparative	433	67	DFPP and IAs/SPx	8-yr graft survival: 73% *vs* 80 % (ABOi *vs* ABOc)
Ishida *et al*[23]	Observational	93	93	DFPP/SPx	5-yr graft survival: 73%
Ohta *et al*[24]	Observational, pediatric	10	10	DFPP or PE or IAs/SPx	5.4-yr graft survival: 100%
Shishido *et al*[25]	Observational, pediatric	16	16	PE and IAs/SPx	5-yr graft survival: 85%
Takahashi *et al*[2]	Observational, comparative	1496	441	DFPP or PE or IAs/SPx	9-yr graft survival: 59% *vs* 57% (ABOi *vs* ABOc)
Shimmura *et al*[26]	Observational, comparative	167	167	DFPP and/or IAs/SPx	5-yr graft survival: 74.3% *vs* 78.5% (CYA with AZ or MZ *vs* TAC or MMF)
Futagawa *et al*[27]	Observational, comparative	37803	191	NA	5-yr graft survival: 66.2% *vs* 79.5% (ABOi *vs* ABOc)
Ishida *et al*[28]	Observational, comparative	222	222	DFPP/SPx	5-yr graft survival: 73% *vs* 90% (CYA with AZ *vs* TAC with MMF)
Tyden *et al*[29]	Observational, comparative	334	60	IAs/RIT/IVIG	Graft survival: ABOi 97% (1.5-yr) *vs* ABOc 95% (1.8-yr)
Galliford *et al*[30]	Observational	10	10	PE/RIT/IVIG	1-yr graft survival: 100%
Genberg *et al*[31]	Observational, comparative	45	15	IAs/RIT/IVIG	Graft survival: ABOi 86.7% (3.4-yr) *vs* ABOc 86.7% (4.0-yr)
Oettl *et al*[32]	Observational	10	10	IAs/RIT/IVIG	1.3-yr graft survival: 100%
Toki *et al*[33]	Observational, comparative	57	57	DFPP/SPx	8-yr graft survival: 49% *vs* 95% (AAMR *vs* non-AAMR)
Wilpert *et al*[34]	Observational, comparative	83	40	IAs/RIT/IVIG	Graft survival: ABOi 100% (3.3-yr) *vs* ABOc 93% (1.5-yr)
Tyden *et al*[1]	Observational, comparative, pediatric	38	10	IAs/RIT/IVIG	Graft loss within 3 years: ABOi 1 case, ABOc 2 cases
Flint *et al*[35]	Observational, comparative	89	37	PE/IVIG	1-yr graft survival: 100% (ABOi *vs* ABOc)
Fichinoue *et al*[36]	Observational, comparative	393	113	DFPP or PE/SPx or RIT	5-yr graft survival: 88.4% *vs* 90.3% *vs* 100% (ABOc vs ABOi-SPx *vs* ABOi-RIT)
Habicht *et al*[37]	Observational, comparative	68	21	IAs/RIT/IVIG	1-yr graft survival : 100% (ABOi *vs* ABOc)
Lipshutz *et al*[38]	Observational	18	18	PE/RIT/IVIG	1-yr graft survival: 94.4%
Shirakawa *et al*[39]	Observational, comparative	74	74	DFPP/RIT	1-yr graft survival: 95.7% *vs* 98.% (RIT 500mg *vs* RIT 200 mg)
Shishido *et al*[3]	Observational, comparative, pediatric	323	52	PE/SPx or RIT	15-yr graft survival: 86% *vs* 78% (ABOi *vs* ABOc)
Montgomery *et al*[4]	Observational, comparative	78193	738	NA	10-yr cumulative incidence of graft loss: 27.1% *vs* 23.9% (ABOi *vs* ABOc)
Morath *et al*[40]	Observational, comparative	19	19	IAs or IAns/RIT/IVIG	1-yr graft survival: 100% (IAs *vs* IAns)
Uchida *et al*[41]	Observational	25	25	DFPP or PE/SPx or RIT	4.5-yr graft survival: 100%
Ashimine *et al*[42]	Observational, comparative	320	92	DFPP/SPx or RIT or none	5-yr graft survival: 87% *vs* 97.7% (ABOi *vs* ABOc)

ABOi: ABO incompatible; SPx: Splenectomy; PE: Plasma exchange; DFPP: Double-filtration plasmapheresis; IAs: Antigen-specific immunoadsorption; ABOc: ABO compatible; CYA: Cyclosporine; AZ: Azathioprine; MZ: Mizoribine; TAC: Tacrolimus; MMF: Mycophenolate mofetil; NA: Not available; RIT: Rituximab; IVIG: Intravenous immunoglobulin; AAMR: Acute antibody-mediated rejection; IAns: Non-antigen-specific immunoadsorption.

duce anti-blood type A or B (anti-A/B) antibody and to minimize the risk of hyperacute humoral rejection. Most of the modern desensitization protocols of ABOi-KT have been derived from their procedure and have since evolved. Their work was further greatly expanded in Japan due to the shortage of deceased donors with successful outcomes in ABOi-KT[2].

Nowadays, splenectomy has been totally abandoned and the various desensitization protocols in use are combinations of antibody removal by plasmapheresis or immunoadsorption (IA), intravenous immunoglobulin (IVIG) to neutralize preformed antibodies, B lymphocyte depletion by anti-CD20 monoclonal antibody (RIT) and standard triple immunosuppression (calcineurin inhibitor, CNI; mycophenolate mofetil, MMF; and steroid). Recently, some authors reported successful outcomes of

ABOi-KT without RIT and splenectomy[35,42,43].

ABOI-KT PREOPERATIVE MANAGEMENT

Current strategies of ABOi-KT compose three common principles: (1) antibody measurement; (2) B-Cell depletion; and (3) antibody depletion.

Antibody measurement

Assessment of anti-A/B antibody titer is crucial in ABOi-KT. It guides the effectiveness of operative preconditioning and determines the period to permit transplantation. In addition, posttransplant monitoring helps early detection of antibody-mediated rejection (AMR) by antibody rebound.

There are various measurement methods of anti-A/

B titer, the most common used are tube technique, gel technique and flow cytometry[44-48]. Although each center uses their familiar technique, there is a discrepancy of measured titer level. Kobayashi *et al*[46] surveyed the differences of anti-A/B titers from the same blood samples which were measured by tube test in 29 Japanese centers. It was revealed that inter-institutional differences were 1:8 to 1:32 in IgM and 1:16 to 1:256 in IgG, because of low reproducibility by visual observation. Therefore, they concluded standardized measurement should be necessary. Kumlien *et al*[47] analyzed the same blood samples in three centers. They also pointed out an inter-center variation of titer level using tube technique and suggested that gel technique is more reproducible than tube technique. Flow cytometry showed excellent reproducible compared with other techniques and would be suitable for the accurate measurement[48]. However, this technique is not available in all centers due to the expensive equipment required.

High preoperative anti-A/B IgG titers are associated with poor long-term allograft survival in ABOi-KT[49]. Gloor *et al*[50] showed preoperative high anti-A/B IgG titers is a predictor for AMR, and the rapid increasing of titers is also associated with AMR and graft loss. In addition, Tobian *et al*[51] also demonstrated that AMR was also associated with high titer at 1-2 wk posttransplant. Chung *et al*[52] described there was no statistically significant difference between high- (> 1:256) and low-titer (< 1:128) at the baseline in allograft function at 6 mo after transplantation. Therefore, appropriate monitoring of anti-A/B titer is essential before and after ABOi-KT. Although anti-A/B antibody titer has to be measured during the early period after ABOi-KT due to the risk of AMR, but how long the monitoring should be continued remains unclear. Preoperative titer should be low in ABOi-KT, but the acceptable titer of anti-A/B antibody at the time of transplant has varied between 1:4 and 1:32 in line with the protocol of individual centers[1,30-43,53-55]. After the ABO incompatible transplant necessitating initiation of antibody-depletion procedures, the level of anti-ABO antibody titer must be monitored to detect rebound in the serum antibody production.

B-cell depletion

Splenectomy: Splenectomy was considered a prerequisite for desensitization protocol in ABOi-KT after Alexandre *et al*[11] reported that it reduced the risk of AMR. The principle of splenectomy was based on the concept that spleen is reservoir of antibody producing B-cells and antibody-producing plasma cells in the body. However, the efficacy of splenectomy in ABOi-KT is debatable, because severe AMR sometimes still occurs after splenectomy. The effect of splenectomy on the immune system is permanent. Following splenectomy the patients are at risk for the development of life-threatening sepsis, especially from encapsulated bacteria and they require life-long antibiotic prophylaxis. Splenectomy can lead to surgical complications such as hemorrhage, pancreatic

injury, pancreatic leakage , and portal vein thrombosis[56].

A comparative analysis of splenectomized recipients compared with RIT treated but without splenectomy, showed no statistically significant difference in the anti-A/B titer of KT and liver transplantation[57,58]. It was concluded that splenectomy was not an essential prerequisite treatment in ABOi-KT. Although splenectomy has been replaced with RIT, Locke *et al*[59] reported that splenectomy could be useful as salvage treatment for severe AMR secondary to anti-HLA antibody. Current consensus states that splenectomy is not necessary for the induction of ABOi-KT.

Rituximab: Splenectomy has been largely replaced by RIT in ABOi-KT protocols to remove B-cell. RIT is an anti-CD20 monoclonal antibody, which binds to CD20 on immature and mature B-cell resulting in depletion of B-cell. RIT was originally developed for the treatment of non-Hodgkin's lymphoma[60]. RIT has been used extensively in the treatment of patients with autoimmune diseases and KT besides hematological malignancies[61]. Adverse events related to B-cell depletion by RIT include fever, chill, headache, and nausea[60], whilst serious cardiovascular and pulmonary events are rare[61].

In the field of KT, RIT has been used as part of desensitization protocols in ABO- and HLA-incompatible KT, treatment of AMR, post-transplant lymphoproliferative disorder, and recurrent nephrotic syndrome[62]. In the first experience of RIT use in ABOi-KT recipients, Sawada *et al*[63] tried RIT, splenectomy, and double-filtration plasmapheresis (DFPP) for A1 to O ABOi-KT with persistent high anti-A antibody titer. The dosage of RIT was 375 mg/m^2 per week for 4 wk pretransplant and there was no rebound of the titer after transplantation. Tydén *et al*[64] succeeded with 4 ABO incompatible recipients using RIT and antigen-specific IA (IAs) with standard immunosuppression, without splenectomy. In their protocol, RIT (375 mg/m^2) was administered once 10 d prior to transplant which was enough to deplete peripheral B-cell. Moreover, its effect was long-active for at least 12 mo without any serious side effects. After these successful reports were published, RIT has replaced splenectomy in desensitization protocol. Recently, some have tried low dose of RIT or even omitting it in ABOi-KT protocol to avoid over-immunosuppression without compromising excellent outcomes[35,42,43,55].

Twenty-seven recipients who were diagnosed with steroid-resistant cell-mediated rejection or AMR received a single dose of RIT (375 mg/m^2) as a salvage treatment[65]: twenty-four (88.9%) among these demonstrated improved renal function. Serum creatinine decreased from a mean of 5.6 mg/dL before the treatment to a mean of 0.95 mg/dL after the treatment. RIT is useful not only in AMR, but also in chronic antibody-mediated rejection (CAMR) prevention. Kohei *et al*[66] observed that ABOi-KT with RIT had a statistically significant lower rate of CAMR at 2 years posttransplant than living ABOc-KT (3.5% *vs* 28.9%). However, this beneficial effect of RIT

needs independent verification.

Antibody depletion

The antibody depletion treatments are the basis of ABOi-KT. In order to eliminate existing anti-A/B antibody, plasma exchange (PE), DFPP and IA[67] are available. They differ in their mechanisms of action, specificity, efficiency and cost.

In PE, plasma is removed and replaced by human albumin, colloid solutions, and/or fresh frozen plasma (FFP). It has been widely used around the world for antibody removal in ABOi-KT. This method is simple, but it has several disadvantages compared with more specific techniques. Because of non-selective apheresis, PE removes not only anti-A/B antibody, but also coagulation factors and anti-viral/-bacterial immunoglobulin. Consequently, the risk of bleeding and infection is increased. FFP is generally needed for the last session before KT to prevent these complications. Other complications were reported by Tobian *et al*[68]. In all PE sessions (*n* = 512), the total rate of complications was 15.4%. The most common complication was hypocalcemia (6.8%), followed by urticaria or pruritus (4.3%), hypotension (2.9%) and nausea or vomiting (1.2%).

DFPP is designed to remove selectively the immunoglobulin from plasma and requires less substitution fluid compared to PE. When plasma separated by a first filter is passed through a second filter, IgG and IgM are filtered out and discarded. By single DFPP, 70% of IgM and 60% of IgG were removed and a one-fold titer reduction of anti-A/B antibody was observed[69]. This technique also avoids the loss of coagulation factors and albumin unlike PE. However, significant amounts of albumin are lost by DFPP, and almost always albumin is needed as the replacement fluid. DFPP is also removes variable amount of fibrinogen[70], and its measurement is necessary to avoid bleeding complication.

IA can be A/B antigen IAs or A/B non-antigen IAns (non-specific/semi-selective immunoadsorption) respectively if it removes only a specific antibody such as anti-A/B antibody or removes non-antigen-specific immunoglobulin. Between the two techniques IAs is most utilized method in ABO incompatible setting. On the other hand, IAns is suitable for the elimination of HLA antigens and it is most used in HLA incompatible/ABOi KT recipients. In IAs, the plasma is processed through an ABO immunoadsorbent column, which is coated with either blood type A or B antigens and allow selective removal of anti-A or B antibody, and the processed plasma is re-infused into the patient. Volume replacement is not necessary. IAs is selective and free from side effects of PE and DFPP. Single IAs reduces 2- to 4-fold titer between pre- and post-IAs, and at least four preoperative IAs are usually needed to obtain an acceptable titer at the expense of increased cost compared to PE and DFPP[67]. IAs is generally safer and more effective, and therefore normally preferred. However, ultimate choice depends on each center's decision, based on the availability of infrastructure and skill mix of staff.

USE OF IVIG

IVIG's recognized immunomodulatory properties have been employed for the treatment of autoimmune diseases[71]. IVIG is believed to act through various mechanisms: (1) complement down-regulation; (2) interactions with the Fc receptors; (3) inhibit of B/T-cell proliferation; (4) inhibit of CD8 T-cell cytotoxicity; and (5) increased apoptosis of B-cell[71-73]. Mild and early adverse effects of IVIG include headache, chill, nausea, fatigue, myalgia, arthralgia, chest pain, back pain, and elevated blood pressure[74,75]. However, rare but serious delayed adverse effects include renal toxicity, thromboembolic events (cerebrovascular accident and deep venous thrombosis), neurological toxicity (aseptic meningitis), hematological toxicity (neutropenia), and dermatological toxicity[76]. The administration of high dose IVIG can cause hemolysis by anti-A/B antibody within the IVIG[77]. In ABOi-KT, it is preferable if possible to use IVIG with low anti-A/B titer in order to avoid not only hemolysis but also AMR after transplantation due to anti-A/B titer elevation.

There is no uniformity in the dose IVIG used in the desensitization protocols of ABOi-KT[1, 30-32,34,35,37,38,40,43,54,78]. IVIG is usually administered after plasmapheresis, to reconstitute the natural levels of IgG. In the absence of control data, the use of IVIG in ABOi-KT can best be described as empirical.

ACCOMMODATION

Without adequate anti-A/B antibody reduction and desensitization before KT, an incidence of AMR and irreversible damage cannot be avoided. Successful ABOi-KT requires the reduction of anti-A/B antibody titers against ABO antigens on the graft at the time of KT. However, anti-A/B antibody titer returns to the baseline level within almost 1 wk after KT[11,79,80], even if optimal desensitization is performed. Therefore, intense monitoring is necessary during critical first two weeks after ABOi-KT[12]. Paradoxically, a phenomenon of accommodation is acquired in this term.

Accommodation is defined as a phenomenon whereby graft rejection is avoided despite reemergence of incompatible antibody. The mechanism was originally discovered in the field of xenotransplantation[81], whereby endothelial cell posttransplant humoral injury was avoided, possibly due to changes of antibody specificity, avidity, affinity and alteration of the antigen structure. This phenomenon is allegedly responsible for normal graft function and structure despite reemergence of anti-A/B antibody against incompatible A or B antigen in the graft[82]. However, it is fair to accept that mechanism as well as the very existence of accommodation remains speculative.

CURRENT PROTOCOL OF ABOI-KT

In ABOi-KT, intensified immunosuppressive protocol usually starts before KT in order to deplete anti-A/B

antibody. Many centers have modified original successful protocol of ABOi-KT[11]. The splenectomy-free protocols published in the last decade are summarized in Table 3[1,30-32,34-43,53-55,78]. RIT has been adopted in the place of splenectomy by majorities of centers. However, the timing and dose of RIT administrated remains variable. RIT or splenectomy-free protocols have successfully, used low dose IVIG after plasmapheresis. The basis of the North Europe protocol is IAs followed by high dose IVIG. However, postoperative IAs is not performed routinely and its use is determined by antibody titers[83]. Maintenance immunosuppressive agents are mostly triple agents which are CNI, MMF and steroid. Tacrolimus is the CNI of choice in these ABOi-KT protocols. MMF was taken 7-14 d pretransplant in order to inhibit antibody production. Some centers use a protocol without daclizumab, basiliximab or antithymocyte globulin, and report excellent outcomes. Thus it is controversial whether these clonal antibodies should be introduced in ABOi-KT or not. All protocols of ABOi-KT have resulted in satisfactory outcome in the absence of randomized control trials. It is impossible to select an ideal protocol fit for all purpose.

MINIMIZE IMMUNOSUPPRESSION

Efforts have have been made to minimize immunosuppression in order to reduce the long-term risk of over-immunosuppression[84,85]. The long-term effect of steroid use remains unclear in ABOi-KT. Oettl *et al*[86] described 11 ABOi-KT recipients with late steroid withdrawal. Six recipients showed biopsy-proven acute rejection during or soon after steroid cessation. However, Galliford *et al*[30] tried early steroid sparing protocol in 10 recipients. Prednisolone was maintained at 1 mg/kg until 3 d posttransplant. It was reduced to 0.5 mg/kg at 4 d posttransplant, and discontinued after 1 wk posttransplant. In this study, patient and graft survival were 100% at 1 year posttransplant but 3 patients experienced acute rejection within 1 mo after transplantation.

HISTOLOGICAL FINDINGS IN ABOI-KT

In ABOi-KT, acute AMR by anti-A/B antibody is a well-recognized cause of early graft loss. Diagnosis of acute AMR needs C4d staining in the peritubular capillary (PTC) and the presence of anti-donor antibodies[87,88]. Morphologic changes include acute tubular necrosis, capillary and/or glomerular inflammation, and transmural arteritis and/or arterial fibrinoid change. C4d staining is the hallmark of humoral induced complement activation and like ABOc-KT was thought to be a useful indicator of AMR even in the setting of ABOi-KT[89]. However, C4d deposition without AMR was seen in 85.7% of ABOi-KT at 3 mo posttransplant[90]. Setoguchi *et al*[91] analyzed protocol biopsies of ABOc-KT and ABOi-KT. C4d expression of PTC was detected in 94% of ABOi-KT, whereas in only 11% of ABOc-KT. In protocol biopsies during stable allograft function, 80% of ABO incompatible grafts

showed as C4d positive, while 74% of HLA incompatible grafts were C4d negative[92]. These histological studies indicate that the detection of C4d alone in ABO incompatible graft does not indicate AMR and support a concept of accommodation in ABOi-KT. Therefore, AMR after ABOi-KT can only be diagnosed on the basis of morphological evidence, serological evidence and the clinical course.

Morphologically transplant glomerulopathy (TG) at 1 year after transplantation was reported as an indicator of poor outcome[93]. ABOi-KT had more severe TG than ABOc-KT without HLA antibody at 1 year posttransplant[94]. However, there were no differences in interstitial fibrosis, tubular atrophy, chronic vasculopathy and allograft function between both groups. In the absence of prior AMR, histological change at 1 year posttransplant was mild irrespective of ABO compatibility. Moreover, prior AMR in ABOi-KT was associated with TG and interstitial fibrosis and not to arteriolar hyalinosis and chronic vasculopathy[91]. Consequently, ABO incompatible grafts with TG and/or interstitial fibrosis had lower GFR at 1 year after transplantation than those with normal histology.

THE INCIDENCE OF ACUTE CELLULAR AND ANTIBODY MEDIATED REJECTION IN ABOI-KT

As previously described, the outcome of graft survival in ABOi-KT has been similar to ABOc-KT. However, there is an increased risk of AMR in ABOi-KT due to anti-A/B antibody. Protocol biopsies at 3 mo posttransplant in ABOi-KT had a significantly higher incidence of AMR compared to ABOc-KT (17.9% *vs* 1.1%). However, there was no significant difference in the rate of acute cellular rejection between ABOi-KT and ABOc-KT (48.4% *vs* 35.7%)[90]. In the acute lesion score based on Banff classification[95], *t*2-3 and *g*2-3 following ABOi-KT was higher than that of ABOc-KT (*t*2-3: 42.9% *vs* 19.4%, *g*2-3: 28.6% *vs* 6.5%). Gloor *et al*[94] described in the study of protocol biopsies at 1 year posttransplant that there was a significant difference in the incidence of acute rejection between ABOi-KT and ABOc-KT without HLA antibody (50% *vs* 13.6%). Acute rejection in ABOi-KT was mainly AMR (73.3%) as compared to ABOc-KT without HLA antibody (12.5%). Setoguchi *et al*[91] also compared the histologic findings of protocol biopsies in 48 ABO incompatible and 133 compatible grafts. There was no difference in clinical and subclinical rejection between ABO incompatible and compatible grafts (clinical: 37.5% *vs* 25.6%, subclinical: 10.4% *vs* 15%). However, ABO incompatible grafts had a high incidence of AMR compared to ABO compatible grafts (27% *vs* 5.3%). Interestingly, rejection was detected in only 15.0% at 1 mo in ABOi-KT compared to 34.7% in ABOc-KT, but in 30.0% at 6-12 mo compared to 10.5%. Wilpert *et al*[34] demonstrated that the rejection rates in ABOi-KT were similar to that in ABOc-KT. Acute cellular rejection was

Table 3 Current protocols for ABO incompatible kidney transplantation

Author	Country, year	Rituximab dose	Pretransplant IS	Antibody depletion	IVIG	Target titer at the time of transplantation	Induction IS	Maintenance IS	Posttransplant antibody depletion
Adult recipients									
Rituximab protocol									
Saito *et al*[53]	Japan, 2006	375 mg/m² (twice) at -14 and -1 d	MMF/MP at -1 Mo	DFPP or PE	-	< 1:16	BAS (20 mg at 0 and 4 d)	CYA/MMF/MP	-
Tyden *et al*[54]	Sweden, 2006	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -13 d	IAs	0.5 g/kg after last IAs	< 1:8	-	TAC/MMF/Pred	IAs, 3 times
Chikaraishi *et al*[55]	Japan, 2008	100 mg/m² (twice) at -8 and -1 d	MMF/MP at -14 d, TAC at -3 d	DFPP and PE	-	< 1:8	BAS (20 mg at 0 and 4 d)	TAC/MMF/MP	-
Galliford *et al*[30]	United Kingdom, 2008	1000 mg (twice) at first day of PE and at the operative day	TAC/MMF at -14 d	PE	0.1 g/kg after each PE	< 1:4	DAC (2 mg/kg at 0 and 14 d)	TAC/MMF/Pred	PE at 1 and 3 d
Genberg *et al*[31]	Sweden, 2008	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -10 d	IAs	0.5 g/kg at -1 d	< 1:8	-	TAC/MMF/Pred	IAs, 3 times
Oettl *et al*[32]	Switzerland, 2009	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -14 d	IAs	0.5 g/kg after last IAs	< 1:8	BAS (20 mg at 0 and 4 d)	TAC/MMF/Pred	IAs or PE (not routinely)
Sivakumaran *et al*[78]	United States, 2009	375 mg/m² (once) at -3 wk	MMF at -1 mo	PE	2 g/kg after last PE	NA	ALE (1 mg/kg at 0 and 14 d)	TAC/MMF/Pred	-
Wilpert *et al*[34]	Germany, 2010	375 mg/m² (once) at -1 mo	TAC/MMF or MPS/Pred at -7 d	IAs	0.5 g/kg at -1 to -5 d	< 1:4	BAS (20 mg at 0 and 4 d)	TAC/MMF/Pred	IAs (not routinely)
Fuchinoue *et al*[36]	Japan, 2011	100-1000 mg, 1-3 times	CYA or TAC/MMF at -2 d	DFPP or PE	-	< 1:16	BAS (20 mg at 0 and 4 d)	CYA or TAC/MMF/steroid	-
Habicht *et al*[37]	Germany, 2011	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -1 mo	IAs	30 g at -1 to -2 d	< 1:8	-	TAC/MMF/MP	IAs (not routinely)
Lipshutz *et al*[38]	United States, 2011	375 mg/m² (once) at -1 mo	TAC/MMF at the first day of PE	PE	10 g after each PE	< 1:8	ATG (1.5 mg/kg for 4 d)	TAC/MMF/Pred	PE (not routinely)
Shirakawa *et al*[39]	Japan, 2011	500 or 200 mg/m² (once), at -5 to -7 d	TAC/MMF/MP at -7 d	DFPP	-	< 1:32	BAS (20 mg at 0 and 4 d)	TAC/MMF/MP	-
Morath *et al*[40]	Germany, 2012	375 mg/m² (once) at -1 mo	TAC/MMF/MP at the first day of IAs	IAs	0.5 g/kg after last IAs	< 1:16	BAS (20 mg at 0 and 4 d)	TAC/MMF/MP	IAs or PE (not routinely)
Uchida *et al*[41]	Japan, 2012	150 mg/m² (twice) at -14 and 0 d	MMF/MP at -1 Mo, CYA or TAC at -3 d	DFPP or PE	-	< 1:16	BAS (20 mg at 0 and 4 d)	CYA or TAC/MMF/MP	-
Rituximab-free protocol									
Montgomery *et al*[43]	United States, 2009	-	TAC/MMF at the first day of PE	PE	0.1 g/kg after each PE	< 1:16	DAC (2 mg/kg initial dose, 1 mg/kg every 2 wk for total 5 doses)	TAC/MMF/Pred	PE, at least twice (with IVIG 0.1 g/kg)
Flint *et al*[35]	Australia, 2011	-	MMF at -10 to -14 d	PE	0.1 g/kg after each PE	< 1:8	BAS (20 mg at 0 and 4 d)	TAC/MMF/Pred	PE (not routinely)
Ashimine *et al*[42]	Japan, 2013	-	MMF at -14 d	DFPP	-	< 1:8	BAS (20 mg at 0 and 4 d)	CYA or TAC/MMF/Pred	-
Pediatric recipients									
Genberg *et al*[31]	Sweden, 2008	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -10 d	IAs	0.5 g/kg at -1 d	< 1:8	-	TAC/MMF/Pred	IAs, 3 times
Tyden *et al*[1]	Sweden, 2011[1]	375 mg/m² (once) at -1 mo	TAC/MMF/Pred at -13 d	IAs	0.5 g/kg after last IAs	< 1:8	-	TAC/MMF/Pred	IAs, 3 times

IS: Immunosuppression; IVIG: Intravenous immunoglobulin; MMF: Mycophenolate mofetil; MP: Methylprednisolone; DFPP: Double-filtration plasmapheresis; PE: Plasma exchange; BAS: Basiliximab; CYA: Cyclosporine; TAC: Tacrolimus; Pred: Prednisolone; IAs: Antigen-specific immunoadsorption; DAC: Daclizumab; NA: Not available; ALE: Alemtuzamab; MPS: Mycophenolate sodium; ATG: Antithymocyte globlin.

Table 4 Pro and cons for ABO incompatible kidney transplantation

Pro ABOi-KT
  Reducing waiting list and time
  Expanding living donor pool
  Improvement of patient's prognosis
  Excellent graft survival (comparable with ABOc-KT)
Contra ABOi-KT
  Comparative high immunological risk
  Higher incidence of acute AMR
  Intensified immunosuppression
  Antibody depletion therapy
  Increasing expenditure
  Higher incidence of viral infection

ABOi-KT: ABO incompatible kidney transplantation; ABOc-KT: ABO compatible kidney transplantation; AMR: antibody-mediated rejection.

detected in 23.2% of ABOi-KT and in 22.5% of ABOc-KT. Acute AMR was shown in 4.7% of ABOi-KT, which was similar to ABOc-KT (5.0%).

ADVERSE EFFECT OF ABOI-KT

Infection

The improvement in ABOi-KT graft survival rate has come at the expense of increased posttransplant infection. The infection rate in ABOi-KT is significantly higher than in ABOc-KT (60% *vs* 29.8%)[37]. The rates of infection including cytomegalovirus (CMV), herpes simplex virus, varicella zoster virus and BK virus (BKV) in ABOi-KT were also significantly higher than in ABOc-KT. The most common viral infection was BKV in 25% of ABOi-KT compared to only 8.5% of ABOc-KT. However, the incidences of rejection, graft survival rate and function of ABOi-KT patients were compatible with these of ABOc-KT patients. On the contrary, Genberg *et al*[31] showed that there was no statistical difference in overall infection complications between ABOi-KT with RIT and living ABOc-KT (40% *vs* 63.3%). However, ABOi-KT patients who were treated with RIT, may have had different infection profiles. Grim *et al*[96] retrospectively analyzed the incidence of posttransplant infection in HLA sensitized KT or ABOi-KT treated with RIT and compared to HLA sensitized KT without RIT. The acute rejection rate in RIT treated KT was similar to KT without RIT (40% *vs* 33%). However, posttransplant infection rate was 48.0% RIT with KT, but only 11.1% without RIT. Kamar *et al*[97] reported that infection rate was 45.5% in KT with RIT which was similar to KT without RIT (53.9%). Bacterial, viral and fungal infection were observed in 36.3%, 18.2% and 16.9% in KT with RIT, against 31.6%, 34.3% and 5.32% in KT without RIT. Polyoma virus infection rate (64.3%) was relatively high in RIT. Moreover, infection related-death was significantly higher in RIT treated patients. This data ascertained that RIT was associated with severe infection which causes death rather than an increased risk of infection. Other report confirmed earlier observation showing that the incidence of posttransplant infection in RIT-treated recipients was similar to RIT-untreated recipients (52.2% *vs* 40.2%)[98]. However, as in earlier studies the incidences of CMV and BKV infection in RIT-treated recipients were higher than in non RIT-treated recipients (CMV: 16.4% *vs* 5.7%, BKV: 13.4% *vs* 8.0%).

Malignancy

It is generally accepted that immunosuppression is associated with an increased incidence of malignancy in KT recipients compared to the general population[99]. However, several studies have demonstrated that ABOi-KT did not increase the risk of posttransplant malignancy compared with ABOc-KT. Yamamoto *et al*[100] analyzed the risk of ABOi-KT compared to ABOc-KT retrospectively. ABOi-KT recipients were older than ABOc-KT recipients and all ABOi-KT recipients received splenectomy, in this study despite increased age and splenectomy[101,102], there was no significant difference in the incidence of malignancy between ABOi-KT and ABOc-KT (4.8% and 4.2%). Similarly, Hall *et al*[103] showed that 7 of 318 ABOi-KT recipients experienced posttransplant cancer. The incidence rate ratio (IRR) of cancer in ABOi-KT was identical to that in matched control ABOc-KT (IRR: 0.99). This limited data reassuringly indicates that ABOi-KT is not associated with an increasing incidence of malignancy after KT. Thus, a further analysis of long-term observations in ABOi-KT after RIT is needed.

COST OF ABOI-KT

It is recognized that KT is a cost-effective option over dialysis[104-106]. The estimated cost for ABOi-KT over 20 years was $315600, which was approximately 15% lower than dialysis[107]. ABOi-KT is more expensive than ABOc-KT because of requirement for desensitization and removal of anti-A/B antibody. The cost of ABOi-KT in the first 90 d posttransplant is $90300 compared to $52500 for ABOc-KT[108]. The additional cost of ABOi-KT amounts to €31948 for IAs, RIT, IVIG, and prolonged hospital stay[31]. The cost of single IA is approximately €4340-1433[40]. However, despite more expensive, ABOi-KT is still more cost-effective than dialysis in the long-term and delivers a better quality of life.

CONCLUSION

Since first performed over 50 years ago, ABOi-KT has become an accepted source of KT. Reassuringly, despite lack of control trials in ABOi-KT, more than satisfactory outcomes have been observed in adult and pediatric recipients, in many studies equivalent to living ABOc-KT. ABOi-KT also has disadvantages in spite of excellent outcomes (Table 4). Preconditioning treatment of ABOi-KT, such as antibody reduction and desensitization, is more intensified and complicated than that of ABOc-KT. With current protocols, the occurrence of early graft loss and AMR are not completely abolished. Preconditioning strategy in ABOi-KT has evolved over time. RIT has replaced splenectomy which was once thought a cru-

cial procedure for ABOi-KT, although this is increasingly abandoned in favor of IAs and IVIG. Overall, ABOi-KT is more expensive than ABOc-KT which may restrict its adoption in resource poor countries. We believe that a live donor ABOi-KT is a viable alternative to waiting on deceased donor list.

REFERENCES

1   **Tydén G**, Kumlien G, Berg UB. ABO-incompatible kidney transplantation in children. *Pediatr Transplant* 2011; **15**: 502-504 [PMID: 21771231 DOI: 10.1111/j.1399-3046.2011.01480.x]

2   **Takahashi K**, Saito K, Takahara S, Okuyama A, Tanabe K, Toma H, Uchida K, Hasegawa A, Yoshimura N, Kamiryo Y. Excellent long-term outcome of ABO-incompatible living donor kidney transplantation in Japan. *Am J Transplant* 2004; **4**: 1089-1096 [PMID: 15196066 DOI: 10.1111/j.1600-6143.2004.00464.x]

3   **Shishido S**, Hyodo YY, Aoki Y, Takasu J, Kawamura T, Sakai KK, Aikawa AA, Satou H, Muramatsu MM, Matsui Z. Outcomes of pediatric ABO-incompatible kidney transplantations are equivalent to ABO-compatible controls. *Transplant Proc* 2012; **44**: 214-216 [PMID: 22310617 DOI: 10.1016/j.transproceed.2011.12.017]

4   **Montgomery JR**, Berger JC, Warren DS, James NT, Montgomery RA, Segev DL. Outcomes of ABO-incompatible kidney transplantation in the United States. *Transplantation* 2012; **93**: 603-609 [PMID: 22290268 DOI: 10.1097/TP.0b013e318245b2af]

5   **Organ Procurement and Transplantation Network**. OPTN/SRTR 2011 Annual Data Report: kidney. Available from: URL: http://srtr.transplant.hrsa.gov/annual_reports/2011/default.aspx

6   **Goldfarb-Rumyantzev A**, Hurdle JF, Scandling J, Wang Z, Baird B, Barenbaum L, Cheung AK. Duration of end-stage renal disease and kidney transplant outcome. *Nephrol Dial Transplant* 2005; **20**: 167-175 [PMID: 15546892 DOI: 10.1093/ndt/gfh541]

7   **Meier-Kriesche HU**, Kaplan B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes: a paired donor kidney analysis. *Transplantation* 2002; **74**: 1377-1381 [PMID: 12451234 DOI: 10.1097/01.TP.0000034632.77029.91]

8   **Rapaport FT**. The case for a living emotionally related international kidney donor exchange registry. *Transplant Proc* 1986; **18**: 5-9 [PMID: 11649919]

9   **Gentry SE**, Montgomery RA, Segev DL. Kidney paired donation: fundamentals, limitations, and expansions. *Am J Kidney Dis* 2011; **57**: 144-151 [PMID: 21184921 DOI: 10.1053/j.ajkd.2010.10.005]

10 **Segev DL**, Gentry SE, Warren DS, Reeb B, Montgomery RA. Kidney paired donation and optimizing the use of live donor organs. *JAMA* 2005; **293**: 1883-1890 [PMID: 15840863 DOI: 10.1001/jama.293.15.1883]

11 **Alexandre GP**, Squifflet JP, De Bruyère M, Latinne D, Reding R, Gianello P, Carlier M, Pirson Y. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. *Transplant Proc* 1987; **19**: 4538-4542 [PMID: 3321614]

12 **Takahashi K**, Saito K. ABO-incompatible kidney transplantation. *Transplant Rev* (Orlando) 2013; **27**: 1-8 [PMID: 22902167 DOI: 10.1016/j.trre.2012.07.003]

13 **National Health System Blood and Trasnplant**. Activity Report 2012-2013, kidney activity. Available from: URL: http://www.organdonation.nhs.uk/statistics/transplant_activity_report/

14 **Rydberg L**. ABO-incompatibility in solid organ transplantation. *Transfus Med* 2001; **11**: 325-342 [PMID: 11532188]

15 **Economidou J**, Hughes-Jones NC, Gardner B. Quantitative measurements concerning A and B antigen sites. *Vox Sang* 1967; **12**: 321-328 [PMID: 6067900]

16 **Thielke J**, Kaplan B, Benedetti E. The role of ABO-incompatible living donors in kidney transplantation: state of the art. *Semin Nephrol* 2007; **27**: 408-413 [PMID: 17616273 DOI: 10.1016/j.semnephrol.2007.03.003]

17 **Nelson PW**, Helling TS, Pierce GE, Ross G, Shield CF, Beck ML, Blake B, Cross DE. Successful transplantation of blood group A2 kidneys into non-A recipients. *Transplantation* 1988; **45**: 316-319 [PMID: 3278421]

18 **Hume DM**, Merrill JP, Miller BF, Thorn GW. Experiences with renal homotransplantation in the human: report of nine cases. *J Clin Invest* 1955; **34**: 327-382 [PMID: 13233354 DOI: 10.1172/JCI103085]

19 **Starzl TE**, Marchioro TL, Holmes JH, Hermann G, Brittain RS, Stonington OH, Talmage DW, Waddell WR. Renal homografts in patients with major donor-recipient blood group incompatibilities. *Surgery* 1964; **55**: 195-200 [PMID: 14121762]

20 **Sheil AG**, Stewart JH, Tiller DJ, May J. ABO blood group incompatibility in renal transplantation. *Transplantation* 1969; **8**: 299-300 [PMID: 4908385 DOI: 10.1097/00007890-196909000-00028]

21 **Ota K**, Takahashi K, Agishi T, Sonda T, Oka T, Ueda S, Amemiya H, Shiramizu T, Okazaki H, Akiyama N, Hasegawa A, Kawamura T, Takagi H, Ueno A. Multicentre trial of ABO-incompatible kidney transplantation. Japanese Biosynsorb ABO-incompatible kidney transplant study group. *Transpl Int* 1992; **5** Suppl 1: S40-S43 [PMID: 14628736]

22 **Tanabe K**, Takahashi K, Sonda K, Tokumoto T, Ishikawa N, Kawai T, Fuchinoue S, Oshima T, Yagisawa T, Nakazawa H, Goya N, Koga S, Kawaguchi H, Ito K, Toma H, Agishi T, Ota K. Long-term results of ABO-incompatible living kidney transplantation: a single-center experience. *Transplantation* 1998; **65**: 224-228 [PMID: 9458019 DOI: 10.1097/00007890-199801270-00014]

23 **Ishida H**, Koyama I, Sawada T, Utsumi K, Murakami T, Sannomiya A, Tsuji K, Yoshimura N, Tojimbara T, Nakajima I, Tanabe K, Yamaguchi Y, Fuchinoue S, Takahashi K, Teraoka S, Ito K, Toma H, Agishi T. Anti-AB titer changes in patients with ABO incompatibility after living related kidney transplantations: survey of 101 cases to determine whether splenectomies are necessary for successful transplantation. *Transplantation* 2000; **70**: 681-685 [PMID: 10972229 DOI: 10.1097/00007890-200008270-00024]

24 **Ohta T**, Kawaguchi H, Hattori M, Takahashi K, Nagafuchi H, Akioka Y, Mizushima W, Ishikawa N, Tanabe K, Toma H, Takahashi K, Ota K, Ito K. ABO-incompatible pediatric kidney transplantation in a single-center trial. *Pediatr Nephrol* 2000; **14**: 1-5 [PMID: 10654320]

25 **Shishido S**, Asanuma H, Tajima E, Hoshinaga K, Ogawa O, Hasegawa A, Honda M, Nakai H. ABO-incompatible living-donor kidney transplantation in children. *Transplantation* 2001; **72**: 1037-1042 [PMID: 11579297 DOI: 10.1097/00007890-200109270-00010]

26 **Shimmura H**, Tanabe K, Ishida H, Tokumoto T, Ishikawa N, Miyamoto N, Shirakawa H, Setoguchi K, Nakajima I, Fuchinoue S, Teraoka S, Toma H. Lack of correlation between results of ABO-incompatible living kidney transplantation and anti-ABO blood type antibody titers under our current immunosuppression. *Transplantation* 2005; **80**: 985-988 [PMID: 16249740 DOI: 10.1097/01.tp.0000173647.43616.78]

27 **Futagawa Y**, Terasaki PI. ABO incompatible kidney transplantation - an analysis of UNOS Registry data. *Clin Transplant* 2006; **20**: 122-126 [PMID: 16556166 DOI: 10.1111/j.1399-0012.2005.00452.x]

28 **Ishida H**, Miyamoto N, Shirakawa H, Shimizu T, Tokumoto T, Ishikawa N, Shimmura H, Setoguchi K, Toki D, Iida S, Teraoka S, Takahashi K, Toma H, Yamaguchi Y, Tanabe K. Evaluation of immunosuppressive regimens in ABO-incompatible living kidney transplantation--single center

analysis. *Am J Transplant* 2007; **7**: 825-831 [PMID: 17250557 DOI: 10.1111/j.1600-6143.2006.01676.x]

29 **Tydén G**, Donauer J, Wadström J, Kumlien G, Wilpert J, Nilsson T, Genberg H, Pisarski P, Tufveson G. Implementation of a Protocol for ABO-incompatible kidney transplantation--a three-center experience with 60 consecutive transplantations. *Transplantation* 2007; **83**: 1153-1155 [PMID: 17496528 DOI: 10.1097/01.tp.0000262570.18117.55]

30 **Galliford J**, Charif R, Chan KK, Loucaidou M, Cairns T, Cook HT, Dorling A, Hakim N, McLean A, Papalois V, Malde R, Regan F, Redman M, Warrens AN, Taube D. ABO incompatible living renal transplantation with a steroid sparing protocol. *Transplantation* 2008; **86**: 901-906 [PMID: 18852653 DOI: 10.1097/TP.0b013e3181880c0f]

31 **Genberg H**, Kumlien G, Wennberg L, Berg U, Tydén G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. *Transplantation* 2008; **85**: 1745-1754 [PMID: 18580466 DOI: 10.1097/TP.0b013e3181726849]

32 **Oettl T**, Halter J, Bachmann A, Guerke L, Infanti L, Oertli D, Mihatsch M, Gratwohl A, Steiger J, Dickenmann M. ABO blood group-incompatible living donor kidney transplantation: a prospective, single-centre analysis including serial protocol biopsies. *Nephrol Dial Transplant* 2009; **24**: 298-303 [PMID: 18728155 DOI: 10.1093/ndt/gfn478]

33 **Toki D**, Ishida H, Setoguchi K, Shimizu T, Omoto K, Shirakawa H, Iida S, Horita S, Furusawa M, Ishizuka T, Yamaguchi Y, Tanabe K. Acute antibody-mediated rejection in living ABO-incompatible kidney transplantation: long-term impact and risk factors. *Am J Transplant* 2009; **9**: 567-577 [PMID: 19260836 DOI: 10.1111/j.1600-6143.2008.02538.x]

34 **Wilpert J**, Fischer KG, Pisarski P, Wiech T, Daskalakis M, Ziegler A, Neumann-Haefelin E, Drognitz O, Emmerich F, Walz G, Geyer M. Long-term outcome of ABO-incompatible living donor kidney transplantation based on antigen-specific desensitization. An observational comparative analysis. *Nephrol Dial Transplant* 2010; **25**: 3778-3786 [PMID: 20466677 DOI: 10.1093/ndt/gfq229]

35 **Flint SM**, Walker RG, Hogan C, Haeusler MN, Robertson A, Francis DM, Millar R, Finlay M, Landgren A, Cohney SJ. Successful ABO-incompatible kidney transplantation with antibody removal and standard immunosuppression. *Am J Transplant* 2011; **11**: 1016-1024 [PMID: 21449947 DOI: 10.1111/j.1600-6143.2011.03464.x]

36 **Fuchinoue S**, Ishii Y, Sawada T, Murakami T, Iwadoh K, Sannomiya A, Koyama I, Kubota K, Tojimbara T, Nakajima I, Teraoka S. The 5-year outcome of ABO-incompatible kidney transplantation with rituximab induction. *Transplantation* 2011; **91**: 853-857 [PMID: 21297552 DOI: 10.1097/TP.0b013e31820f08e8]

37 **Habicht A**, Bröker V, Blume C, Lorenzen J, Schiffer M, Richter N, Klempnauer J, Haller H, Lehner F, Schwarz A. Increase of infectious complications in ABO-incompatible kidney transplant recipients--a single centre experience. *Nephrol Dial Transplant* 2011; **26**: 4124-4131 [PMID: 21622990 DOI: 10.1093/ndt/gfr215]

38 **Lipshutz GS**, McGuire S, Zhu Q, Ziman A, Davis R, Goldfinger D, Reed EF, Wilkinson AH, Danovitch GM, Pham PT. ABO blood type-incompatible kidney transplantation and access to organs. *Arch Surg* 2011; **146**: 453-458 [PMID: 21502455 DOI: 10.1001/archsurg.2011.40]

39 **Shirakawa H**, Ishida H, Shimizu T, Omoto K, Iida S, Toki D, Tanabe K. The low dose of rituximab in ABO-incompatible kidney transplantation without a splenectomy: a single-center experience. *Clin Transplant* 2011; **25**: 878-884 [PMID: 21175849 DOI: 10.1111/j.1399-0012.2010.01384.x]

40 **Morath C**, Becker LE, Leo A, Beimler J, Klein K, Seckinger J, Kihm LP, Schemmer P, Macher-Goeppinger S, Wahrmann M, Böhmig GA, Opelz G, Süsal C, Zeier M, Schwenger V. ABO-incompatible kidney transplantation enabled by non-antigen-

41 **Uchida J**, Kuwabara N, Machida Y, Iwai T, Naganuma T, Kumada N, Nakatani T. Excellent outcomes of ABO-incompatible kidney transplantation: a single-center experience. *Transplant Proc* 2012; **44**: 204-209 [PMID: 22310615 DOI: 10.1016/j.transproceed.2011.11.006]

42 **Ashimine S**, Watarai Y, Yamamoto T, Hiramitsu T, Tsujita M, Nanmoku K, Goto N, Takeda A, Katayama A, Uchida K, Kobayashi T. Neither pre-transplant rituximab nor splenectomy affects de novo HLA antibody production after renal transplantation. *Kidney Int* 2014; **85**: 425-430 [PMID: 23945498 DOI: 10.1038/ki.2013.291]

43 **Montgomery RA**, Locke JE, King KE, Segev DL, Warren DS, Kraus ES, Cooper M, Simpkins CE, Singer AL, Stewart ZA, Melancon JK, Ratner L, Zachary AA, Haas M. ABO incompatible renal transplantation: a paradigm ready for broad implementation. *Transplantation* 2009; **87**: 1246-1255 [PMID: 19384174 DOI: 10.1097/TP.0b013e31819f2024]

44 **Stussi G**, Huggel K, Lutz HU, Schanz U, Rieben R, Seebach JD. Isotype-specific detection of ABO blood group antibodies using a novel flow cytometric method. *Br J Haematol* 2005; **130**: 954-963 [PMID: 16156865 DOI: 10.1111/j.1365-2141.2005.05705.x]

45 **Lindberg L**, Johansson SM, Liu J, Grufman P, Holgersson J. Is there a clinical need for a diagnostic test allowing detection of chain type-specific anti-A and anti-B? *Transfusion* 2011; **51**: 494-503 [PMID: 20849404 DOI: 10.1111/j.1537-2995.2010.02870.x]

46 **Kobayashi T**, Saito K. A series of surveys on assay for anti-A/B antibody by Japanese ABO-incompatible Transplantation Committee. *Xenotransplantation* 2006; **13**: 136-140 [PMID: 16623808 DOI: 10.1111/j.1399-3089.2006.00296.x]

47 **Kumlien G**, Wilpert J, Säfwenberg J, Tydén G. Comparing the tube and gel techniques for ABO antibody titration, as performed in three European centers. *Transplantation* 2007; **84**: S17-S19 [PMID: 18162980 DOI: 10.1097/01.tp.0000296019.85986.af]

48 **Tanabe K**. Interinstitutional variation in the measurement of anti-A/B antibodies: the Japanese ABO-Incompatible Transplantation Committee survey. *Transplantation* 2007; **84**: S13-S16 [PMID: 18162979 DOI: 10.1097/01.tp.0000296018.82857.ef]

49 **Shimmura H**, Tanabe K, Ishikawa N, Tokumoto T, Takahashi K, Toma H. Role of anti-A/B antibody titers in results of ABO-incompatible kidney transplantation. *Transplantation* 2000; **70**: 1331-1335 [PMID: 11087148]

50 **Gloor JM**, Lager DJ, Moore SB, Pineda AA, Fidler ME, Larson TS, Grande JP, Schwab TR, Griffin MD, Prieto M, Nyberg SL, Velosa JA, Textor SC, Platt JL, Stegall MD. ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. *Transplantation* 2003; **75**: 971-977 [PMID: 12698082 DOI: 10.1097/01.TP.0000058226.39732.32]

51 **Tobian AA**, Shirey RS, Montgomery RA, Cai W, Haas M, Ness PM, King KE. ABO antibody titer and risk of antibody-mediated rejection in ABO-incompatible renal transplantation. *Am J Transplant* 2010; **10**: 1247-1253 [PMID: 20420632 DOI: 10.1111/j.1600-6143.2010.03103.x]

52 **Chung BH**, Lee JY, Kang SH, Sun IO, Choi SR, Park HS, Kim JI, Moon IS, Choi BS, Park CW, Kim YS, Yang CW. Comparison of clinical outcome between high and low baseline anti-ABO antibody titers in ABO-incompatible kidney transplantation. *Ren Fail* 2011; **33**: 150-158 [PMID: 21332336 DOI: 10.3109/0886022x.2011.552149]

53 **Saito K**, Nakagawa Y, Suwa M, Kumagai N, Tanikawa T, Nishiyama T, Ueno M, Gejyo F, Nishi S, Takahashi K. Pinpoint targeted immunosuppression: anti-CD20/MMF desensitization with anti-CD25 in successful ABO-incompatible kidney transplantation without splenectomy. *Xenotransplantation* 2006; **13**: 111-117 [PMID: 16623803 DOI: 10.1111/

j.1399-3089.2006.00277.x]

54 **Tydén G**, Kumlien G, Genberg H, Sandberg J, Sedigh A, Lundgren T, Gjertsen H, Fehrman I. The Stockholm experience with ABO-incompatible kidney transplantations without splenectomy. *Xenotransplantation* 2006; **13**: 105-107 [PMID: 16623801 DOI: 10.1111/j.1399-3089.2006.00292.x]

55 **Chikaraishi T**, Sasaki H, Tsutsumi H, Miyano S, Nakazawa R, Nakano T, Kitajima K, Kudo H, Takahashi T, Sato Y, Kimura K. ABO blood type incompatible kidney transplantation without splenectomy prepared with plasma exchange and rituximab. *Transplant Proc* 2008; **40**: 3445-3447 [PMID: 19100409 DOI: 10.1016/j.transproceed.2008.06.110]

56 **Cadili A**, de Gara C. Complications of splenectomy. *Am J Med* 2008; **121**: 371-375 [PMID: 18456028 DOI: 10.1016/j.amjmed.2008.02.014]

57 **Gloor JM**, Lager DJ, Fidler ME, Grande JP, Moore SB, Winters JL, Kremers WK, Stegall MD. A Comparison of splenectomy versus intensive posttransplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation. *Transplantation* 2005; **80**: 1572-1577 [PMID: 16371928]

58 **Raut V**, Mori A, Kaido T, Ogura Y, Taku I, Nagai K, Sasaki N, Endo K, Hata T, Yagi S, Egawa H, Uemoto S. Splenectomy does not offer immunological benefits in ABO-incompatible liver transplantation with a preoperative rituximab. *Transplantation* 2012; **93**: 99-105 [PMID: 22094955 DOI: 10.1097/TP.0b013e318239e8e4]

59 **Locke JE**, Zachary AA, Haas M, Melancon JK, Warren DS, Simpkins CE, Segev DL, Montgomery RA. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant* 2007; **7**: 842-846 [PMID: 17391127 DOI: 10.1111/j.1600-6143.2006.01709.x]

60 **Maloney DG**, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-Lopez A, Levy R. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. *Blood* 1994; **84**: 2457-2466 [PMID: 7522629]

61 **Pescovitz MD**. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. *Am J Transplant* 2006; **6**: 859-866 [PMID: 16611321 DOI: 10.1111/j.1600-6143.2006.01288.x]

62 **Becker YT**, Samaniego-Picota M, Sollinger HW. The emerging role of rituximab in organ transplantation. *Transpl Int* 2006; **19**: 621-628 [PMID: 16827678 DOI: 10.1111/j.1432-2277.2006.00345.x]

63 **Sawada T**, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation* 2002; **74**: 1207-1210 [PMID: 12451255 DOI: 10.1097/01.tp.0000040990.15896.76]

64 **Tydén G**, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. *Transplantation* 2003; **76**: 730-731 [PMID: 12973118 DOI: 10.1097/01.tp.0000078622.43689.d4]

65 **Becker YT**, Becker BN, Pirsch JD, Sollinger HW. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplant* 2004; **4**: 996-1001 [PMID: 15147435 DOI: 10.1111/j.1600-6143.2004.00454.x]

66 **Kohei N**, Hirai T, Omoto K, Ishida H, Tanabe K. Chronic antibody-mediated rejection is reduced by targeting B-cell immunity during an introductory period. *Am J Transplant* 2012; **12**: 469-476 [PMID: 22054413 DOI: 10.1111/j.1600-6143.2011.03830.x]

67 **Tydén G**, Kumlien G, Efvergren M. Present techniques for antibody removal. *Transplantation* 2007; **84**: S27-S29 [PMID: 18162984 DOI: 10.1097/01.tp.0000296102.94695.c0]

68 **Tobian AA**, Shirey RS, Montgomery RA, Tisch DJ, Ness PM, King KE. Therapeutic plasma exchange reduces ABO titers

to permit ABO-incompatible renal transplantation. *Transfusion* 2009; **49**: 1248-1254 [PMID: 19210321 DOI: 10.1111/j.1537-2995.2008.02085.x]

69 **Tanabe K**. Double-filtration plasmapheresis. *Transplantation* 2007; **84**: S30-S32 [PMID: 18162985 DOI: 10.1097/01.tp.0000296103.34735.b8]

70 **Nakanishi T**, Suzuki N, Kuragano T, Nagasawa Y, Hasuike Y. Current topics in therapeutic plasmapheresis. *Clin Exp Nephrol* 2014; **18**: 41-49 [PMID: 23887747 DOI: 10.1007/s10157-013-0838-0]

71 **Kazatchkine MD**, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med* 2001; **345**: 747-755 [PMID: 11547745 DOI: 10.1056/NEJMra993360]

72 **Glotz D**, Antoine C, Julia P, Pegaz-Fiornet B, Duboust A, Boudjeltia S, Fraoui R, Combes M, Bariety J. Intravenous immunoglobulins and transplantation for patients with anti-HLA antibodies. *Transpl Int* 2004; **17**: 1-8 [PMID: 14685653 DOI: 10.1007/s00147-003-0674-3]

73 **Jordan S**, Cunningham-Rundles C, McEwan R. Utility of intravenous immune globulin in kidney transplantation: efficacy, safety, and cost implications. *Am J Transplant* 2003; **3**: 653-664 [PMID: 12780556]

74 **Ryan ME**, Webster ML, Statler JD. Adverse effects of intravenous immunoglobulin therapy. *Clin Pediatr* (Phila) 1996; **35**: 23-31 [PMID: 8825847]

75 **Stangel M**, Hartung HP, Marx P, Gold R. Side effects of high-dose intravenous immunoglobulins. *Clin Neuropharmacol* 1997; **20**: 385-393 [PMID: 9331514]

76 **Orbach H**, Katz U, Sherer Y, Shoenfeld Y. Intravenous immunoglobulin: adverse effects and safe administration. *Clin Rev Allergy Immunol* 2005; **29**: 173-184 [PMID: 16391392]

77 **Kahwaji J**, Barker E, Pepkowitz S, Klapper E, Villicana R, Peng A, Chang R, Jordan SC, Vo AA. Acute hemolysis after high-dose intravenous immunoglobulin therapy in highly HLA sensitized patients. *Clin J Am Soc Nephrol* 2009; **4**: 1993-1997 [PMID: 19833910 DOI: 10.2215/CJN.04540709]

78 **Sivakumaran P**, Vo AA, Villicana R, Peng A, Jordan SC, Pepkowitz SH, Klapper EB. Therapeutic plasma exchange for desensitization prior to transplantation in ABO-incompatible renal allografts. *J Clin Apher* 2009; **24**: 155-160 [PMID: 19658162 DOI: 10.1002/jca.20206]

79 **Reding R**, Squifflet JP, Pirson Y, Jamart J, De Bruyère M, Moriau M, Latinne D, Carlier M, Alexandre GP. Living-related and unrelated donor kidney transplantation: comparison between ABO-compatible and incompatible grafts. *Transplant Proc* 1987; **19**: 1511-1513 [PMID: 3274365]

80 **Aikawa A**, Hadano T, Ohara T, Hasegawa A, Yamashita M, Shindo M, Mizuiri T, Fushimi T, Sakai K, Hirayama N, Arai K, Tajima E. Relation between ABO blood type antigen and antibody and acute vascular rejection in ABO incompatible kidney transplantation. *Transplant Proc* 1998; **30**: 3507-3509 [PMID: 9838538]

81 **Platt JL**, Vercellotti GM, Dalmasso AP, Matas AJ, Bolman RM, Najarian JS, Bach FH. Transplantation of discordant xenografts: a review of progress. *Immunol Today* 1990; **11**: 450-456; discussion 456-457 [PMID: 2073317]

82 **Park WD**, Grande JP, Ninova D, Nath KA, Platt JL, Gloor JM, Stegall MD. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant* 2003; **3**: 952-960 [PMID: 12859529]

83 **Geyer M**, Donauer J, Pisarski P, Drognitz O, Schulz-Huotari C, Wisniewski U, Gropp A, Göbel H, Gerke P, Teschner S, Walz G, Wilpert J. Preemptive postoperative antigen-specific immunoadsorption in ABO-incompatible kidney transplantation: necessary or not? *Transplantation* 2007; **84**: S40-S43 [PMID: 18162989 DOI: 10.1097/01.tp.0000296021.72977.3b]

84 **Lightner A**, Concepcion W, Grimm P. Steroid avoidance in renal transplantation. *Curr Opin Organ Transplant* 2011; **16**: 477-482

85   **Flechner SM**, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. *Clin Transplant* 2008; **22**: 1-15 [PMID: 18217899 DOI: 10.1111/j.1399-0012.2007.00739.x]

86   **Oettl T**, Zuliani E, Gaspert A, Hopfer H, Dickenmann M, Fehr T. Late steroid withdrawal after ABO blood group-incompatible living donor kidney transplantation: high rate of mild cellular rejection. *Transplantation* 2010; **89**: 702-706 [PMID: 20010328 DOI: 10.1097/TP.0b013e3181c9cc67]

87   **Racusen LC**, Haas M. Antibody-mediated rejection in renal allografts: lessons from pathology. *Clin J Am Soc Nephrol* 2006; **1**: 415-420 [PMID: 17699240 DOI: 10.2215/CJN.01881105]

88   **Sis B**, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen LC, Solez K, Baldwin WM, Bracamonte ER, Broecker V, Cosio F, Demetris AJ, Drachenberg C, Einecke G, Gloor J, Glotz D, Kraus E, Legendre C, Liapis H, Mannon RB, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Rodriguez ER, Seron D, Seshan S, Suthanthiran M, Wasowska BA, Zachary A, Zeevi A. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant* 2010; **10**: 464-471 [PMID: 20121738 DOI: 10.1111/j.1600-6143.2009.02987.x]

89   **Fidler ME**, Gloor JM, Lager DJ, Larson TS, Griffin MD, Textor SC, Schwab TR, Prieto M, Nyberg SL, Ishitani MB, Grande JP, Kay PA, Stegall MD. Histologic findings of antibody-mediated rejection in ABO blood-group-incompatible living-donor kidney transplantation. *Am J Transplant* 2004; **4**: 101-107 [PMID: 14678040]

90   **Ushigome H**, Okamoto M, Koshino K, Nobori S, Okajima H, Masuzawa N, Urasaki K, Yoshimura N. Findings of graft biopsy specimens within 90 days after ABO blood group incompatible living donor kidney transplantation compared with ABO-identical and non-identical transplantation. *Clin Transplant* 2010; **24** Suppl 22: 16-21 [PMID: 20590688 DOI: 10.1111/j.1399-0012.2010.01278.x]

91   **Setoguchi K**, Ishida H, Shimmura H, Shimizu T, Shirakawa H, Omoto K, Toki D, Iida S, Setoguchi S, Tokumoto T, Horita S, Nakayama H, Yamaguchi Y, Tanabe K. Analysis of renal transplant protocol biopsies in ABO-incompatible kidney transplantation. *Am J Transplant* 2008; **8**: 86-94 [PMID: 18021283 DOI: 10.1111/j.1600-6143.2007.02036.x]

92   **Haas M**, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, Segev DL, Simpkins CE, Warren DS, King KE, Zachary AA, Montgomery RA. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant* 2006; **6**: 1829-1840 [PMID: 16889542 DOI: 10.1111/j.1600-6143.2006.01356.x]

93   **Cosio FG**, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; **5**: 2464-2472 [PMID: 16162196 DOI: 10.1111/j.1600-6143.2005.01050.x]

94   **Gloor JM**, Cosio FG, Rea DJ, Wadei HM, Winters JL, Moore SB, DeGoey SR, Lager DJ, Grande JP, Stegall MD. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. *Am J Transplant* 2006; **6**: 1841-1847 [PMID: 16780546 DOI: 10.1111/j.1600-6143.2006.01416.x]

95   **Racusen LC**, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, Furness P, Gaber LW, Gibson IW, Glotz D, Goldberg JC,

Grande J, Halloran PF, Hansen HE, Hartley B, Hayry PJ, Hill CM, Hoffman EO, Hunsicker LG, Lindblad AS, Yamaguchi Y. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713-723 [PMID: 9987096 DOI: 10.1046/j.1523-1755.1999.00299.x]

96   **Grim SA**, Pham T, Thielke J, Sankary H, Oberholzer J, Benedetti E, Clark NM. Infectious complications associated with the use of rituximab for ABO-incompatible and positive cross-match renal transplant recipients. *Clin Transplant* 2007; **21**: 628-632 [PMID: 17845637 DOI: 10.1111/j.1399-0012.2007.00700.x]

97   **Kamar N**, Milioto O, Puissant-Lubrano B, Esposito L, Pierre MC, Mohamed AO, Lavayssière L, Cointault O, Ribes D, Cardeau I, Nogier MB, Durand D, Abbal M, Blancher A, Rostaing L. Incidence and predictive factors for infectious disease after rituximab therapy in kidney-transplant patients. *Am J Transplant* 2010; **10**: 89-98 [PMID: 19656128 DOI: 10.1111/j.1600-6143.2009.02785.x]

98   **Baek CH**, Yang WS, Park KS, Han DJ, Park JB, Park SK. Infectious risks and optimal strength of maintenance immunosuppressants in rituximab-treated kidney transplantation. *Nephron Extra* 2012; **2**: 66-75 [PMID: 22619669 DOI: 10.1159/000337339]

99   **Kasiske BL**, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. *Am J Transplant* 2004; **4**: 905-913 [PMID: 15147424 DOI: 10.1111/j.1600-6143.2004.00450.x]

100   **Yamamoto T**, Kawaguchi T, Watarai Y, Tujita M, Hiramitsu T, Nanmoku K, Goto N, Katayama A, Kobayashi T, Uchida K. Potent immunosuppression for ABO-incompatible renal transplantation may not be a risk factor for malignancy. *Transplant Proc* 2012; **44**: 210-213 [PMID: 22310616 DOI: 10.1016/j.transproceed.2011.11.048]

101   **Danpanich E**, Kasiske BL. Risk factors for cancer in renal transplant recipients. *Transplantation* 1999; **68**: 1859-1864 [PMID: 10628765]

102   **Webster AC**, Wong G, Craig JC, Chapman JR. Managing cancer risk and decision making after kidney transplantation. *Am J Transplant* 2008; **8**: 2185-2191 [PMID: 18782291 DOI: 10.1111/j.1600-6143.2008.02385.x]

103   **Hall EC**, Engels EA, Montgomery RA, Segev DL. Cancer risk after ABO-incompatible living-donor kidney transplantation. *Transplantation* 2013; **96**: 476-479 [PMID: 23799426 DOI: 10.1097/TP.0b013e318299dc0e]

104   **Loubeau PR**, Loubeau JM, Jantzen R. The economics of kidney transplantation versus hemodialysis. *Prog Transplant* 2001; **11**: 291-297 [PMID: 11871278]

105   **Haller M**, Gutjahr G, Kramar R, Harnoncourt F, Oberbauer R. Cost-effectiveness analysis of renal replacement therapy in Austria. *Nephrol Dial Transplant* 2011; **26**: 2988-2995 [PMID: 21310740 DOI: 10.1093/ndt/gfq780]

106   **Matas AJ**, Schnitzler M. Payment for living donor (vendor) kidneys: a cost-effectiveness analysis. *Am J Transplant* 2004; **4**: 216-221 [PMID: 14974942]

107   **Schnitzler M**, Machnicki G. ABO-incompatible living donor transplantation: is it economically "compatible"? *Transplantation* 2006; **82**: 168-169 [PMID: 16858277 DOI: 10.1097/01.tp.0000226242.10027.e7]

108   **Schwartz J**, Stegall MD, Kremers WK, Gloor J. Complications, resource utilization, and cost of ABO-incompatible living donor kidney transplantation. *Transplantation* 2006; **82**: 155-163 [PMID: 16858274 DOI: 10.1097/01.tp.0000226152.13584.ae]

REVIEW

Immune monitoring post liver transplant

Siddharth Sood, Adam G Testro

Siddharth Sood, Adam G Testro, Liver Transplant Unit Victoria, Level 8 HSB, Austin Health, University of Melbourne, Melbourne, Victoria 3084, Australia
Author contributions: Sood S and Testro AG were involved in the writing of this manuscript.
Correspondence to: Adam G Testro, Head of Liver Immunology Research, Liver Transplant Unit Victoria, Level 8 HSB, Austin Health, University of Melbourne, Studley Road, Heidelberg, Melbourne, Victoria 3084,
Australia. adam.testro@austin.org.au
Telephone: +61-3-94965353 Fax: +61-3-94963487
Received: November 27, 2013 Revised: January 14, 2014
Accepted: January 17, 2014
Published online: March 24, 2014

Abstract

Many of the causes of short and late morbidity following liver transplantation are associated with immunosuppression or immunosuppressive medications. Current care often involves close monitoring of liver biochemistry as well as therapeutic drug levels. However, the postoperative course following liver transplantation can often be associated with significant complications including infection and rejection, suggesting an inadequacy in current immune function monitoring. Many assays have been tested in the research setting to identify possible biomarkers that may be used to predict clinical events such as acute cellular rejection, and therefore allow modification of a patient's immunosuppressive regimen prior to a clinical event. However, these generally require significant laboratory processing and have had difficulty becoming established in common clinical use outside the research setting. One assay, Cylex ImmuKnow has been food and drug administration approved but has had variable results. In this review we discuss the assays that have been used to assess monitoring of immune function after liver transplantation and consider possible future directions.

Core tip: Although many research assays have attempted to identify potential biomarkers that may be used to monitor immune function after liver transplantation, most require significant laboratory processing and are not clinically feasible. The rejection cascade is complex and not completely understood, with many likely interactions between innate and adaptive immune processes. Therefore, no single test is likely to provide a fool-proof window to the immune response and a combination of assays may be necessary. However, nothing can replace the clinical judgement of an expert transplant clinician for pooling together data to individualize immunosuppression therapy.

INTRODUCTION

Although the use of modern immunosuppression has greatly increased the life expectancy of organ transplant recipients, they are not without problems. Mortality within the first year following liver transplantation (OLTx) usually occurs within the first three months with causes including infection, primary graft failure, rejection and technical complications[1]. Causes of late mortality include cardiovascular disease (9%-22%), *de novo* malignancy (16%-23%), infections (6%-19%), chronic rejection and graft failure (5%-19%) and chronic renal failure (5%-10%)[2-5]. Many of the causes of short and late mortality following OLTx are related to immunosuppression, with an estimated 40%-70% of all post-transplant mortality attributable to immunosuppression or immunosuppressants[5,6].

Table 1 Clinically available immune monitoring after adult liver transplantation

	Sensitivity	Specificity
Currently available		
Liver biochemistry	High	Low
Therapeutic drug levels	Low	Low
ImmuKnow	Low	High
Liver histology	Gold standard	Gold standard
Future possibilities		
PlexImmune	Only Paedeatric studies published	
? Combination assays		

To minimize side-effects, clinicians often empirically attempt to minimize dosages. Only very few patients are trialed or able to completely withdraw successfully from all immunosuppression. Tailored therapy for each patient, based on a functional measure of their individual immune response, would clearly be preferable to empiric reduction of therapy in all patients[7].

The challenge in balancing the risks of over and under immunosuppression is complicated by the lack of reliable means of predicting patients' immunosuppressive needs. OLTx in particular, presents unique challenges compared with other solid organ transplants. The liver is an immunotolerant organ but rejection rates remain at 30%-40%[8-10]. Despite this, some individuals have the potential for complete withdrawal of immunosuppression. Furthermore, the postoperative course after OLTx is often complicated, with biliary strictures and recurrent diseases shrouding the diagnosis of rejection and confusing the management of a patient's immune function post transplant. Therefore, it has long been suggested that we monitor transplant patients for their functional immunity to optimize therapy[11,12].

An ideal immune function assay would be based on whole blood, require minimal handling, be reproducible and standardized across laboratories, relatively cheap, and offer a rapid turn around that would allow interpretation of results and corresponding adjustments in immunosuppression early enough to prevent complications or drug related side-effects.

Currently available standard of care in most centres to monitor immune function involves liver biochemistry, drug levels and clinical events (Table 1). Several other potential bio-markers and diagnostic parameters have been suggested in order to confront the immune monitoring challenge and are summarized in Table 2. In this review, we examine the current available options for monitoring the immune system after liver transplantation.

LIVER BIOCHEMISTRY

Clinicians have traditionally relied on liver biochemistry (LFT) in making non-invasive assessments regarding graft function after OLTx. An increase in LFTs is seen during rejection but is non-specific and many other important aetiologies need to be considered. These include but are not limited to biliary strictures, hepatic artery thrombosis, cholangitis, recurrent viral hepatitis and drug induced injuries. There is often a delay between the first LFT abnormality being noted, and patients undergoing a liver biopsy for diagnosis of rejection. It is an imprecise and late marker of graft injury.

THERAPEUTIC DRUG MONITORING

Calcineurin inhibitors

Cyclosporine and tacrolimus are the two commonest drugs used in maintenance following OLTx and inhibit the phosphatase activity of calcineurin through binding of cyclosporine-cyclophilin and tacrolimus-FKBP12 complexes. This inhibits T-cell activation, but because calcineurin and the nuclear factor activated T-cell pathway are not T-cell specific, calcineurin inhibitors (CNIs) are often associated with significant toxicity[13]. In particular, tacrolimus has high rates of diabetes, while cyclosporin is associated with increased hypertension and dyslipidemia[14]. Furthermore, both drugs are associated with end-stage renal failure that can complicate up to 20% of patients following OLTx[15].

Tacrolimus (> 90%) and cyclosporine (> 50%) are concentrated in erythrocytes, and therefore whole blood is used to measure the therapeutic drug levels[16]. Most centres use an ELISA to measure trough levels of tacrolimus, while large clinical trials of OLTx patients treated with cyclosporine show lower rates of rejection and nephrotoxicity complications with monitoring based on either AUC_{0-4} or the concentration 2 h following administration[17-19]. Therefore many units (including our own) perform a level 2 h (C2) following the patient's morning dose.

Setting a therapeutic target for the CNIs has been difficult with standard protocols generalized to managing large number of recipients, but not specific to each patient's individual clinical situation[20]. CNIs also have a poor dose-level correlation, an unpredictable level-effect association, individual pharmacokinetic differences, and an unclear level-toxicity relationship[21,22]. Side-effects are seen even with CNI levels below the "therapeutic range"[23]. Further problems arise as the monoclonal antibodies used to detect certain metabolites may not capture all biologically active forms of the CNIs[24,25].

Given the level of drug determined by immunoassay is not correlated with immunosuppressive drug efficacy or the level of immunosuppression[22,26,27] the United States Food and Drug Administration (FDA) has gone so far as to reclassify assays for measuring tacrolimus and cyclosporin blood levels indicating that no suitable therapeutic ranges exist and these tests should not be used alone to adjust drug dosing[28].

Optimising CNI drug dosing

CNI dosing is impacted by the variable metabolism of the drugs. Tacrolimus is metabolised by CYP3A enzymes in the small intestine and the enzymatic activity can vary by a factor of 5 between patients[29]. Genetic polymorphisms of CYP3A have shown higher tacrolimus clearance and lower levels in some kidney transplant re-

Table 2 Summary of assays for immune function monitoring

		Advantages	Disadvantages
Antigen-specific assays:	Limiting dilution aAssays, mixed lymphocyte reactions, ELISPOT	Measure individual antigen specific response	Need donor cells, Laboratory intensive
Antigen non-specific:	ImmuKnow	Available, FDA approved	Inconsistent results
	Cytokine levels/polymorphisms		Inconsistent results
	Immune competence scores	Readily available	Lack of published validation studies
	Regulatory T cells (Tregs)	Associated with rejection	Laboratory intensive. Lack of published validation studies
	Soluble CD30		Lack of association with clinical outcomes in OLTx
Identifying operational tolerant recipients:	Tregs, Gene expression, dendritic cell types, delayed type hypersensitivity	Able to identify recipients in whom immunosuppression could be withdrawn	Laboratory intensive. Only few recipients suitable

FDA: Food and drug administration; ELISPOT: Enzyme-linked immunosorbent spots; OLTx: Liver transplantation.

cipients[30] while attempts to evaluate pharmacodynamics directly through monitoring of CNI biological activity have demonstrated correlation between peak levels of CNIs and residual gene expression (by nuclear factor of activated T-cells), but not clinical events[31].

High-performance liquid chromatography was developed for evaluating four cyclosporine degradation products and two related compounds (CyB and CyG)[32]. Initially developed to test quality control of generic formulations, future studies may consider evaluating whether these could have a closer association with outcomes than the cyclosporin blood level[20].

Other drugs

The CNIs are often used in combination with other immunosuppressants. Steroids and induction agents such as basiliximab (anti-IL2) have no specific monitoring mechanisms apart from side-effects, while the optimal dosing and levels of the mTOR inhibitors remain uncertain.

Even if the biological activity of each individual drug could be accurately determined, this would not provide an objective net biomarker of immune function as the cross-reactive effects of the drugs would remain uncertain. As such, therapeutic drug monitoring may continue to assist clinicians in managing patients, but is unlikely to be the dominant method of future immune system monitoring following OLTx.

Clinical events

One of the major influences on drug dosing and immunosuppression following liver transplantation is the presence of complications. In particular, patients who develop sepsis or malignancy following transplantation often have their immunosuppression empirically reduced. Correspondingly, patients undergoing rejection are treated with increased medication. Clearly this is a crude method of monitoring immunosuppression and the purpose of immune monitoring is to optimise immunosuppression prior to the occurrence of clinical events.

Biopsies

Acute cellular rejection is diagnosed on histology based on the commonly accepted Banff criteria[33]. Sampling graft tissue has the further advantage that it can reveal the local ongoing antidonor immune responses[34] and protocol biopsies provide a more accurate marker of graft function compared to liver biochemistry[13]. Surveillance biopsies of the transplanted organ may represent the gold standard for directly assessing the extent of immune activity within the allograft. However, serial biopsies are invasive and almost impractical outside of a research setting[35].

Immune monitoring assays

Although commonly used, the aforementioned tests have significant disadvantages and do not provide an accurate marker of a patient's immune system following OLTx. As a consequence, clinical events and side-effects remain common causes of morbidity and mortality. Many assays have been developed and evaluated with varying results but are yet to achieve use outside of research settings. In general, these assays can be broadly classified as antigen-specific or non-antigen specific and will be discussed below.

ANTIGEN-SPECIFIC ASSAYS

Donor specific assays

Functional donor specific assays may allow detection of immunological states favouring alloimmune quiescence over reactivity[36]. Functional or cytokine kinetics assays may then be applied to determine preemptively whether immunosuppression dosing should be altered.

Limiting dilution assays (LDA) are an example which can provide more precise quantification of immunity to a given stimulus and allow estimation of frequencies of antigen-specific cells participating in an immune response[37]. It requires recipient peripheral blood mononuclear cells (PBMCs) interacting with donor stimulator cells. This can then be used to determine production of different cytokines in the presence of supernatant cultures such as interferon-gamma, interleukin (IL)-5, IL-4, IL-10, IL-13 or TNF-α present in the well[37]. LDA has been employed to show a highly significant correlation between the donor-specific and third-party stimulated IL-4 and IL-10 produced from recipient PBMCs with

stable liver graft function compared with rejectors, independent to level of immunosuppression[38].

The main limiting step is availability of donor cells that can be difficult to obtain from cadaveric transplants unless cells are harvested at time of surgery from the spleen or lymph nodes and cryopreserved for future donor-specific assays[20]. Furthermore, the assays often require substantial laboratory work and may need significant amounts of blood and cells for repeated stimulations/experiments.

Mixed lymphocyte reaction

Mixed lymphocyte reaction (MLR) assays provide an estimate of the primary *in vitro* response to the direct recognition of allogenic molecules[37]. Their main value is in assessing tolerance - that is MLR responsiveness in the face of clinically evident donor-specific tolerance.

Studies with ^{3}H-thymidine mixed leukocyte responses (MLR) show that enhanced donor-specific alloreactivity persists longer among children with early rejection and is associated with early and late liver rejection[39,40]. To account for the significant variation that is often seen in donor-specific alloresponses, values are often expressed as a ratio to a third-party response known as the immunoreactivity index. A ratio under 1 suggests low rejection risk[40]. However, this assay is non-antigen specific, requires prolonged stimulation and larger amounts of blood than would be routinely feasible in transplant populations[41].

Further enhancements to MLR include combination of results with carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling by flow cytometry[42]. CFSE is an intracellular fluorescent label that divides equally amongst daughter cells and can be used to study cell division[37]. It measures the proliferative response of recipient lymphocytes after culture or stimulation with donor cells. Unlike many other immune monitoring studies, this has been investigated in an interventional study of 51 adult OLTx recipients. Immunosuppression was increased, decreased or maintained depending on results from the MLR compared with 64 OLTx recipients who had standard of care with empirical based management. This showed trends towards improved rates of rejection and survival, but not sufficient to reach significance ($P < 0.05$)[42]. A MLR-CFSE assay has also been used to distinguish between rejection on suspicious biopsies[43].

To overcome the issues of prolonged stimulations and blood sample requirements common in MLR assays, Ashokkumar *et al*[41] evaluated a CD154^{+} (CD40L) T-helper and T-cytotoxic cells MLR as measures of rejection risk[41]. This requires < 24 h of stimulation and only 3 mL of blood. These authors identified pre OLTx CD154^{+} cytotoxic T memory cell responses were associated with significantly increased risk (HR = 7.355, $P = 0.02$) for rejection. This assay can be ordered as PlexImmune™ (Plexison, Pittsburgh, United States) with results in the United States available 2 d after obtaining blood samples. Only small studies have been published to date with PlexImmune in paediatric liver and small intestinal transplant recipients. The assay requires extraction of PBMCs not only from the recipient but also the donor. In some cases when donor cells have been insufficient or unavailable, "surrogate PBMCs" have been used[41] but their validity is uncertain in a clinical population.

Enzyme-linked immunosorbent spots

Enzyme-linked immunosorbent spots (ELISPOT) quantifies the frequency of previously activated (memory) T cells that respond to donor antigens by producing a selected cytokine *in vitro*. Recipient T cells are cultured with donor cells on tissue culture plates coated with a cytokine-specific antibody that is detected using labeled secondary antibodies. Each detected spot represents an effector or memory T cell which has been primed to the stimulating antigens[37].

ELISPOT has been proposed as a surrogate marker of allogenic responsiveness in renal transplantation[44-46]. Pretransplant IFN-γ ELISPOT has been associated with rejection risk following renal transplant[44,45,47] which suggests that IFN-γ-producing cells represent cells that have been sensitized to the graft antigens. Thus providing an *ex vivo* reflection of the evolving *in vivo*, donor-reactive immune response which may allow patients without a positive response to reduce or withdraw their immunosuppression[7]. Apart from IFN-γ, granzyme B (GrB) has been studied in a small number of paediatric OLTx recipients but failed to predict the occurrence of rejection[48].

The labor-intensiveness and time-consuming nature of these assays, the need for donor cells, the questionable reliability for stored cells along with some inconsistent correlations with clinical outcomes have prevented their broad acceptance as reliable immune monitoring tools[7,49].

Chimerism

After OLTx, haematopoietic donor cells are transferred with the graft from donor to recipient. These chimeric cells may persist in the recipient and be detectable even years post-transplant[50]. It has been hypothesised that developing chimerism may be desirable after OLTx and potentially associated with tolerance[51]. This could allow immunosuppression to be reduced in patients who have detectable chimerism. However, a meta-analysis has failed to demonstrate a significant association between microchimerism and rejection, but techniques of varying sensitivity were used to measure the degree of chimerism[52]. The value and role of chimerism after liver transplantation remains uncertain, and may also differ depending on the time post-transplant[53].

ANTIGEN NON-SPECIFIC

ImmuKnow

As immunosuppressive drugs ultimately target T-cell function, it would seem logical that assessing T-cell function would provide a potential biomarker for monitoring immune function after transplantation[54]. ImmuKnow (Cylex Ltd, United States) was developed as a biomarker

to guide immunosuppressant dosing following solid organ transplantation and was approved by the United States FDA in 2002. ImmuKnow measures adenosine triphosphate produced after stimulation of T-cells with plant lectin phytohemagglutinin (PHA) mitogen[54]. Whole blood is used to ensure that CNIs are maintained during incubation. After overnight incubation, CD4 cells are selected using paramagnetic particles coated with a monoclonal antibody to CD4[54]. ImmuKnow does not correlate with CD4 cell numbers, and the assay is theorized to provide an independent variable[54].

Studies in OLTx recipients have reported contradictory results for ImmuKnow in predicting acute rejection and infection[55-62]. Most of these studies are retrospective, have limited follow-up, heterogenous in study design, and often include multiple solid organ transplants in the analysis despite immunosuppression protocols and clinical event risks differing substantially amongst different transplant populations.

Further, many of these studies only employ single time point measurements and risk potential bias and the effect of confounders. For example, one study assessing ImmuKnow and infection risk declared lower values in patients who suffer an infection following transplant. However, one of the triggers to run the assay in this study was an event such as fever or raised liver biochemistry[63]. Furthermore, a single result cannot be expected to predict the long-term immune function of the patient. Ideally serial measures, correlated with changes in immunosuppressant dosing, would be needed to adequately assess the immune response post OLTx.

To coincide with the multiple studies demonstrating conflicting results, there have been two opposing meta-analyses published[64,65]. One recent meta-analysis by Ling *et al*[64] suggests a sensitivity of 0.43 (95%CI: 0.34-0.52) and specificity of 0.75 (95%CI: 0.72-0.78) of ImmuKnow for predicting rejection with a diagnostic odds ratio 1.19 (95%CI: 0.65-2.20). This study incorporated multiple organ transplants and when a sub-analysis of liver transplant patients was conducted, results suggested poor sensitivity but improved specificity (sensitivity 0.11 95%CI: 0.01-0.33, specificity 0.94 95%CI: 0.91-0.95).

A separate meta-analysis in liver transplant recipients identified 4 studies which assessed ImmuKnow for both infection risk and rejection, one further study assessing infection specifically, and a further study examining rejection risk alone. All but one study were retrospective, and in general had small patient numbers with short or undeclared periods of follow-up. In this meta-analysis, the ImmuKnow assay was identified as having a diagnostic odds ratio of 14.7 with sensitivity 83.8% and specificity 75.3% for diagnosing infection. When evaluating rejection, a diagnostic odds ratio of 8.8 (sensitivity 65.6%, specificity 80.4%) was noted alongside significant variation amongst studies included in analysis. In particular, the sensitivity ranged from 9.1%-85.7%[65].

A possible explanation for the perceived poor sensitivity of ImmuKnow in detecting rejection may be that it relies on T cell stimulation with PHA mitogen, which is a non-specific antigen that stimulates the adaptive immune system. With the renewed interest in Toll-like receptors, current evidence suggest that the innate immune system also plays a central role in rejection and allorecognition[66-69]. By only stimulating the adaptive immune system, we postulate that the poor sensitivity may reflect ImmuKnow failing to recognize and therefore measure the contribution made by innate immune mediators to rejection processes.

Clearly there have been issues with several studies that incorporate ImmuKnow. However, the assay is FDA approved and with few other options, the assay is employed in several centres. However, there are often no clear protocols and use varies even amongst individual clinicians in the same centre[35]. A large, formal, multi-centre randomized controlled trial would resolve many questions regarding ImmuKnow in regards to its ability to be an objective biomarker of immune function in OLTx patients.

Cytokine genetic polymorphisms
Productions of cytokines vary amongst individuals, and detecting possible polymorphisms in the responsible genes could help in stratifying patients for risk of clinical outcomes. However, in a meta-analysis studying the impact of cytokine gene polymorphism on graft acceptance in clinical transplantation, the only genetic risk factor associated with acute liver rejection was IL-10 polymorphism at position 1082[70] which is associated with low *in vitro* production of IL-10[71].

Circulating cytokine levels
Circulating cytokine levels have the benefit of being reasonably easy to determine. However, analysis of published clinical studies correlating circulating levels with immunological status after liver transplantation are confusing and often contradictory[49]. This probably reflects the multitude of confounding factors that impact this patient population, including surgical stress, the associated ischaemia-reperfusion injury, blood transfusions, hepatic regeneration and infectious complications[72].

Immune competence scores
Some have evaluated multiple factors such as complement and immunoglobulin levels in an attempt to determine an immune competence score to assist in determining risk of infection[73]. This scoring system assigned two points for each of the following: increased levels of baseline IgG, increased levels of baseline IgA, and decreased levels of pre-OLTx C3. This score was found to have a relative risk of infection of 1.99 (*P* < 0.001) and would be both relatively cheap and employs pathology tests already available in many labs[73]. However, to our knowledge it has not been validated in larger cohorts and would not take into account the multitude of other factors involved in a patient's immune function after the transplant operation.

Regulatory T Cells (Treg)
In adult allograft recipients there is evidence that Tregs

are involved in transplantation tolerance by directly inhibiting the proliferation of effector T cells. A substantial number of donor Tregs detach from the liver graft during perfusion and continue to migrate into the recipient after OLTx. These suppress the direct pathway alloresponses and are theorized to contribute to chimerism-associated tolerance *in vivo* in the early stage after transplantation[74].

Lower levels of these regulatory cells have been identified in patients undergoing acute rejection[75,76] while patients completely weaned off immunosuppression demonstrate higher numbers in their grafts and peripheral circulation[77-81]. Despite this, Treg analysis still requires significant laboratory work to isolate PBMCs and perform laboratory analysis and are not currently marketed or used in clinical settings that we are aware of.

Soluble CD30
Both CD4 and CD8 cells express CD30 after primary alloantigenic stimulation. Although there is some suggestion that soluble CD30 may be a useful marker in kidney transplantation[82,83], studies in adult[84] and paedeatric[85] liver transplantation have failed to reveal a role in predicting rejection outcomes.

Operational tolerance
The liver allograft can often be maintained after transplantation with low levels of immunosuppression and in some cases be withdrawn completely without histological damage from rejection - defined as operational tolerance (OT)[86]. It is estimated that OT rates after OLTx are as high as 20%-25%[87,88]. It appears that OT recipients have different cellular immunophenotypic or peripheral blood transcriptional profiles compared with healthy volunteers, recipients on immunosuppression or those experiencing rejection[80,86]. Several studies have sought to identify which patients are likely to achieve OT which could then facilitate drug withdrawal in this select group.

Gene expression
Martínez-Llordella *et al*[89] identified and validated a "tolerant genetic fingerprint" using transcriptional profiling from transplant PBMCs. This identified a modest number of genes capable of identifying tolerant liver recipients with good accuracy. In particular, NK and $\gamma\delta TCR^+$ T cells were the main PBMC subsets associated with tolerance-associated transcriptional patterns.

Although transcriptional profiling of peripheral blood may allow identification of some patients capable of completely weaning off immunosuppression, data directly supporting these assays and their ability to monitor the net immunosuppressive state are yet to be published and not available in clinical settings[20].

Dendritic cells
In humans, 2 major types of blood dendritic cells have been described[90]. Monocytoid DC (CD11c$^+$) can be derived from circulating monocytes in response to granulocyte-macrophage colony-stimulating factor and IL-4 and induce Th1 cell differentiation *in vitro* and may be specialized for induction of immunity. Plasmacytoid DC (CD123$^+$) develop after stimulation with IL-3 and CD125$^+$ (CD40L) and promote Th2 responses which can be for induction of tolerance[91]. The ratio of these cells may be important, with flow cytometry demonstrating operationally tolerant patients exhibiting higher incidence of plasmacytoid dendritic cells (theorised to induce tolerance) compared with myeloid dendritic cells[92,93].

Delayed-type hypersensitivity
In OLTx patients, the trans vivo delayed-type hypersensitivity (DTH) assay has been shown to be valuable in identifying OT recipients[94]. This technique involves transfer of PBMCs plus donor antigen in the footpads of naive, severe combined immunodeficiency mice and measuring for response[94]. This has the advantage of evaluating *in vivo* cell-mediated allogenic immunity without direct exposure of patients[95]. The logical limitation is the need to have immunodeficient mice available and this makes the assay unfeasible outside research.

Identifying patients who can achieve OT would prove valuable in reducing immunosuppression and related side-effects in these recipients. It would also reduce the ad hoc nature that is sometimes employed to withdraw immunosuppressants following OLTx. However, only a small proportion of patients are likely to have the potential to achieve full operational tolerance and other methods of immune monitoring are therefore needed for the majority of patients.

CONCLUSION
Immune function monitoring following OLTx remains a difficult area, but an area in which even small advances would likely result in significant improvements to morbidity and long-term mortality for patients following liver transplantation today. Many options for immune monitoring have been considered, and vary in methodology from predicting risk of clinical complications, varying dosing of immunosuppressants, and identifying those who may be able to develop operational tolerance.

No single method or assay has been able to meet the diagnostic requirements while answering the basic technical requirements: an assay that is standardized, reproducible, cost-effective, easy and intuitive to perform[55]. Most vary in degree of promise based on ease of execution, precision, specificity, reproducibility and cost, as well as the type of information they provide[96]. It is possible that multiple assays or a combination assay may be needed in the same patient at different times to distinguish an accurate immunological profile in the future[37]. In particular, combining assays from both arms of the immune system (innate and adaptive) may provide clinicians a more comprehensive net immune response of a patient.

Many antigen specific assays also suffer from being based on PBMC which excludes the red cells from. This can pose several issues. Firstly, both the CNIs and mTOR inhibitors are found in whole blood rather than extracted PBMCs, and whole blood has been considered the best

matrix for monitoring immune function[20,97]. Secondly, extraction of PBMCs is often a process that requires significant laboratory effort and its applicability outside research settings in commercial laboratories would likely be personnel and cost-prohibitive.

Without available objective markers of immune function, drug levels, liver biochemistry and clinical events are often used to guide immunotherapy. This approach is crude and drug side-effects and clinical complications remain common[63]. Although the ImmuKnow assay offered early promise and is FDA approved, some conflicting results have limited its widespread acceptance. A formal randomised controlled trial would help in answering many questions regarding the assay given the issues in many of the trials previously undertaken.

The rejection cascade is complex and not firmly understood, with many likely interactions between innate and adaptive immune processes. Therefore no single test is likely to provide a foolproof window to the immune response. As such, nothing can replace the clinical judgement of an expert transplant clinician for pooling together data to individualize immunosuppression therapy[20] but an unmet need exists to measure immune function and assess the risk of clinical complications objectively in OLTx patients[41].

REFERENCES

1   **Abbasoglu O**, Levy MF, Brkic BB, Testa G, Jeyarajah DR, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Ten years of liver transplantation: an evolving understanding of late graft loss. *Transplantation* 1997; **64**: 1801-1807 [PMID: 9422423]

2   **Neuberger J**. Liver transplantation. *J Hepatol* 2000; **32**: 198-207 [PMID: 10728805]

3   **Sudan DL**, Venkataramani A, Lynch J, Lynch JC, Fox IJ, Jr. Shaw BW, Langnas AN. Causes of late mortality in survivors of liver transplantation [abstract]. *Transplantation* 1999; **67**: S564

4   **Rabkin JM**, de La Melena V, Orloff SL, Corless CL, Rosen HR, Olyaei AJ. Late mortality after orthotopic liver transplantation. *Am J Surg* 2001; **181**: 475-479 [PMID: 11448447]

5   **Gelson W**, Hoare M, Dawwas MF, Vowler S, Gibbs P, Alexander G. The pattern of late mortality in liver transplant recipients in the United Kingdom. *Transplantation* 2011; **91**: 1240-1244 [PMID: 21516069 DOI: 10.1097/TP.0b013e31821841ba]

6   **Asfar S**, Metrakos P, Fryer J, Verran D, Ghent C, Grant D, Bloch M, Burns P, Wall W. An analysis of late deaths after liver transplantation. *Transplantation* 1996; **61**: 1377-1381 [PMID: 8629300]

7   **Gebauer BS**, Hricik DE, Atallah A, Bryan K, Riley J, Tary-Lehmann M, Greenspan NS, Dejelo C, Boehm BO, Hering BJ, Heeger PS. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. *Am J Transplant* 2002; **2**: 857-866 [PMID: 12392292]

8   **Wallia A**, Parikh ND, Molitch ME, Mahler E, Tian L, Huang JJ, Levitsky J. Posttransplant hyperglycemia is associated with increased risk of liver allograft rejection. *Transplantation* 2010; **89**: 222-226 [PMID: 20098286 DOI: 10.1097/TP.0b013e3181c3c2ff]

9   **Gómez-Manero N**, Herrero JI, Quiroga J, Sangro B, Pardo F, Cienfuegos JA, Prieto J. Prognostic model for early acute rejection after liver transplantation. *Liver Transpl* 2001; **7**:

246-254 [PMID: 11244167 DOI: 10.1053/jlts.2001.22460]

10 **Ziolkowski J**, Paczek L, Niewczas M, Senatorski G, Oldakowska-Jedynak U, Wyzgal J, Foroncewicz B, Mucha K, Zegarska J, Nyckowski P, Zieniewicz K, Patkowski W, Krawczyk M, Ziarkiewicz-Wroblewska B, Gornicka B. Acute liver transplant rejection: incidence and the role of high-doses steroids. *Transplant Proc* 2003; **35**: 2289-2291 [PMID: 14529918]

11 **Schulick RD**, Weir MB, Miller MW, Cohen DJ, Bermas BL, Shearer GM. Longitudinal study of in vitro CD4+ T helper cell function in recently transplanted renal allograft patients undergoing tapering of their immunosuppressive drugs. *Transplantation* 1993; **56**: 590-596 [PMID: 8105569]

12 **Ahmed M**, Venkataraman R, Logar AJ, Rao AS, Bartley GP, Robert K, Dodson FS, Shapiro R, Fung JJ, Zeevi A. Quantitation of immunosuppression by tacrolimus using flow cytometric analysis of interleukin-2 and interferon-gamma inhibition in CD8(-) and CD8(+) peripheral blood T cells. *Ther Drug Monit* 2001; **23**: 354-362 [PMID: 11477316]

13 **Naesens M**, Sarwal MM. Monitoring calcineurin inhibitor therapy: localizing the moving target. *Transplantation* 2010; **89**: 1308-1309 [PMID: 20458272 DOI: 10.1097/TP.0b013e3181dc13fa]

14 **Post DJ**, Douglas DD, Mulligan DC. Immunosuppression in liver transplantation. *Liver Transpl* 2005; **11**: 1307-1314 [PMID: 16237688 DOI: 10.1002/lt.20614]

15 **Gonwa TA**, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, Klintmalm GB. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934-1939 [PMID: 11773892]

16 **Nagase K**, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol* 1994; **46**: 113-117 [PMID: 7517447]

17 **Thervet E**, Pfeffer P, Scolari MP, Toselli L, Pallardó LM, Chadban S, Pilmore H, Connolly J, Buchler M, Schena FP, Carreño CA, Dandavino R, Cole E. Clinical outcomes during the first three months posttransplant in renal allograft recipients managed by C2 monitoring of cyclosporine microemulsion. *Transplantation* 2003; **76**: 903-908 [PMID: 14508352 DOI: 10.1097/01.TP.0000089006.00653.64]

18 **Tanaka K**, Lake J, Villamil F, Levy G, Marotta P, Mies S, de Hemptinne B, Moench C. Comparison of cyclosporine microemulsion and tacrolimus in 39 recipients of living donor liver transplantation. *Liver Transpl* 2005; **11**: 1395-1402 [PMID: 16237691 DOI: 10.1002/lt.20508]

19 **Levy G**, Grazi GL, Sanjuan F, Wu Y, Mühlbacher F, Samuel D, Friman S, Jones R, Cantisani G, Villamil F, Cillo U, Clavien PA, Klintmalm G, Otto G, Pollard S, McCormick PA. 12-month follow-up analysis of a multicenter, randomized, prospective trial in de novo liver transplant recipients (LIS2T) comparing cyclosporine microemulsion (C2 monitoring) and tacrolimus. *Liver Transpl* 2006; **12**: 1464-1472 [PMID: 17004259 DOI: 10.1002/lt.20802]

20 **Levitsky J**. Next level of immunosuppression: drug/immune monitoring. *Liver Transpl* 2011; **17** Suppl 3: S60-S65 [PMID: 21761551 DOI: 10.1002/lt.22385]

21 **Naesens M**, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009; **4**: 481-508 [PMID: 19218475 DOI: 10.2215/CJN.04800908]

22 **Venkataramanan R**, Shaw LM, Sarkozi L, Mullins R, Pirsch J, MacFarlane G, Scheller D, Ersfeld D, Frick M, Fitzsimmons WE, Virji M, Jain A, Brayman KL, Shaked A. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. *J Clin Pharmacol* 2001; **41**: 542-551 [PMID: 11361051]

23 **Ekberg H**, Tedesco-Silva H, Demirbas A, Vítko S, Nashan B, Gürkan A, Margreiter R, Hugo C, Grinyó JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF. Reduced exposure to

calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562-2575 [PMID: 18094377 DOI: 10.1056/NEJMoa067411]

24 **Jusko WJ**, Thomson AW, Fung J, McMaster P, Wong SH, Zylber-Katz E, Christians U, Winkler M, Fitzsimmons WE, Lieberman R. Consensus document: therapeutic monitoring of tacrolimus (FK-506). *Ther Drug Monit* 1995; **17**: 606-614 [PMID: 8588229]

25 **Yatscoff RW**, Copeland KR, Faraci CJ. Abbott TDx monoclonal antibody assay evaluated for measuring cyclosporine in whole blood. *Clin Chem* 1990; **36**: 1969-1973 [PMID: 2242580]

26 **Rovira P**, Mascarell L, Truffa-Bachi P. The impact of immunosuppressive drugs on the analysis of T cell activation. *Curr Med Chem* 2000; **7**: 673-692 [PMID: 10702633]

27 **Wieland E**, Olbricht CJ, Süsal C, Gurragchaa P, Böhler T, Israeli M, Sommerer C, Budde K, Hartmann B, Shipkova M, Oellerich M. Biomarkers as a tool for management of immunosuppression in transplant patients. *Ther Drug Monit* 2010; **32**: 560-572 [PMID: 20814352 DOI: 10.1097/FTD.0b013e3181efb3d2]

28 **US FDA Centre for Devices and Radiological Health.** Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Draft Guidance for Industry and FDA. In: Devices DoCL. Rockville, USA, 2002

29 **Lampen A**, Christians U, Guengerich FP, Watkins PB, Kolars JC, Bader A, Gonschior AK, Dralle H, Hackbarth I, Sewing KF. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos* 1995; **23**: 1315-1324 [PMID: 8689938]

30 **MacPhee IA**, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004; **4**: 914-919 [PMID: 15147425 DOI: 10.1111/j.1600-6143.2004.00435.x]

31 **Zahn A**, Schott N, Hinz U, Stremmel W, Schmidt J, Ganten T, Gotthardt D, Meuer S, Zeier M, Giese T, Sommerer C. Immunomonitoring of nuclear factor of activated T cells-regulated gene expression: the first clinical trial in liver allograft recipients. *Liver Transpl* 2011; **17**: 466-473 [PMID: 21445930 DOI: 10.1002/lt.22254]

32 **Bonifacio FN**, Giocanti M, Reynier JP, Lacarelle B, Nicolay A. Development and validation of HPLC method for the determination of Cyclosporin A and its impurities in Neoral capsules and its generic versions. *J Pharm Biomed Anal* 2009; **49**: 540-546 [PMID: 19124213 DOI: 10.1016/j.jpba.2008.11.027]

33 **Demetris A**, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. *Hepatology* 2000; **31**: 792-799 [PMID: 10706577 DOI: 10.1002/hep.510310337]

34 **Sawitzki B**, Schlickeiser S, Reinke P, Volk HD. Monitoring tolerance and rejection in organ transplant recipients. *Biomarkers* 2011; **16** Suppl 1: S42-S50 [PMID: 21707443 DOI: 10.3109/1354750X.2011.578754]

35 **Israeli M**, Klein T, Brandhorst G, Oellerich M. Confronting the challenge: individualized immune monitoring after organ transplantation using the cellular immune function assay. *Clin Chim Acta* 2012; **413**: 1374-1378 [PMID: 22333671 DOI: 10.1016/j.cca.2012.01.033]

36 **Levitsky J**. Operational tolerance: past lessons and future prospects. *Liver Transpl* 2011; **17**: 222-232 [PMID: 21384504 DOI: 10.1002/lt.22265]

37 **Truong DQ**, Bourdeaux C, Wieërs G, Saussoy P, Latinne D, Reding R. The immunological monitoring of kidney and liver transplants in adult and pediatric recipients. *Transpl Immunol* 2009; **22**: 18-27 [PMID: 19800003 DOI: 10.1016/j.trim.2009.09.008]

38 **Chung SW**, Yoshida EM, Cattral MS, Hu Y, Gorczynski RM. Donor-specific stimulation of peripheral blood mononuclear cells from recipients of orthotopic liver transplants is associated, in the absence of rejection, with type-2 cytokine production. *Immunol Lett* 1998; **63**: 91-96 [PMID: 9761370]

39 **Sindhi R**, Magill A, Bentlejewski C, Abdullah A, Tresgaskes M, Seward J, Janosky J, Zeevi A. Enhanced donor-specific alloreactivity occurs independently of immunosuppression in children with early liver rejection. *Am J Transplant* 2005; **5**: 96-102 [PMID: 15636616 DOI: 10.1111/j.1600-6143.2004.00639.x]

40 **Khera N**, Janosky J, Zeevi A, Mazariegos G, Marcos A, Sindhi R. Persistent donor-specific alloreactivity may portend delayed liver rejection during drug minimization in children. *Front Biosci* 2007; **12**: 660-663 [PMID: 17127326]

41 **Ashokkumar C**, Talukdar A, Sun Q, Higgs BW, Janosky J, Wilson P, Mazariegos G, Jaffe R, Demetris A, Dobberstein J, Soltys K, Bond G, Thomson AW, Zeevi A, Sindhi R. Allospecific CD154+ T cells associate with rejection risk after pediatric liver transplantation. *Am J Transplant* 2009; **9**: 179-191 [PMID: 18976293 DOI: 10.1111/j.1600-6143.2008.02459.x]

42 **Tanaka Y**, Tashiro H, Onoe T, Ide K, Ishiyama K, Ohdan H. Optimization of immunosuppressive therapy based on a multiparametric mixed lymphocyte reaction assay reduces infectious complications and mortality in living donor liver transplant recipients. *Transplant Proc* 2012; **44**: 555-559 [PMID: 22410068 DOI: 10.1016/j.transproceed.2012.01.038]

43 **Tanaka Y**, Ohdan H, Onoe T, Mitsuta H, Tashiro H, Itamoto T, Asahara T. Low incidence of acute rejection after living-donor liver transplantation: immunologic analyses by mixed lymphocyte reaction using a carboxyfluorescein diacetate succinimidyl ester labeling technique. *Transplantation* 2005; **79**: 1262-1267 [PMID: 15880082]

44 **Augustine JJ**, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. *Am J Transplant* 2005; **5**: 1971-1975 [PMID: 15996247 DOI: 10.1111/j.1600-6143.2005.00958.x]

45 **Nickel P**, Presber F, Bold G, Biti D, Schönemann C, Tullius SG, Volk HD, Reinke P. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 2004; **78**: 1640-1646 [PMID: 15591953]

46 **Hricik DE**, Rodriguez V, Riley J, Bryan K, Tary-Lehmann M, Greenspan N, Dejelo C, Schulak JA, Heeger PS. Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predicts renal function in kidney transplant recipients. *Am J Transplant* 2003; **3**: 878-884 [PMID: 12814480]

47 **Näther BJ**, Nickel P, Bold G, Presber F, Schönemann C, Pratschke J, Volk HD, Reinke P. Modified ELISPOT technique--highly significant inverse correlation of post-Tx donor-reactive IFNgamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transpl Immunol* 2006; **16**: 232-237 [PMID: 17138059 DOI: 10.1016/j.trim.2006.09.026]

48 **Truong DQ**, Cornet A, Wieërs G, Robert A, Reding R, Latinne D. Pre- and post-transplant monitoring of granzyme B enzyme-linked immunosorbent spot assay in pediatric liver recipients. *Transpl Immunol* 2008; **19**: 215-219 [PMID: 18602007 DOI: 10.1016/j.trim.2008.06.001]

49 **Reding R**, Gras J, Truong DQ, Wieërs G, Latinne D. The immunological monitoring of alloreactive responses in liver transplant recipients: a review. *Liver Transpl* 2006; **12**: 373-383

[PMID: 16498661 DOI: 10.1002/lt.20704]

50   **Monaco AP**. Chimerism in organ transplantation: conflicting experiments and clinical observations. *Transplantation* 2003; **75**: 13S-16S [PMID: 12819484 DOI: 10.1097/01.TP.0000067945.90241.F4]

51   **Mineo D**, Ricordi C. Chimerism and liver transplant tolerance. *J Hepatol* 2008; **49**: 478-480 [PMID: 18644653 DOI: 10.1016/j.jhep.2008.06.021]

52   **Sahota A**, Gao S, Hayes J, Jindal RM. Microchimerism and rejection: a meta-analysis. *Clin Transplant* 2000; **14**: 345-350 [PMID: 10945206]

53   **Wood KJ**. Passenger leukocytes and microchimerism: what role in tolerance induction? *Transplantation* 2003; **75**: 17S-20S [PMID: 12819485 DOI: 10.1097/01.TP.0000067946.90241.2F]

54   **Kowalski R**, Post D, Schneider MC, Britz J, Thomas J, Deierhoi M, Lobashevsky A, Redfield R, Schweitzer E, Heredia A, Reardon E, Davis C, Bentlejewski C, Fung J, Shapiro R, Zeevi A. Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. *Clin Transplant* 2003; **17**: 77-88 [PMID: 12709071]

55   **Cabrera R**, Ararat M, Soldevila-Pico C, Dixon L, Pan JJ, Firpi R, Machicao V, Levy C, Nelson D, Morelli G. Using an immune functional assay to differentiate acute cellular rejection from recurrent hepatitis C in liver transplant patients. *Liver Transpl* 2009; **15**: 216-222 [PMID: 19177434 DOI: 10.1002/lt.21666]

56   **Xue F**, Dong H, Wu J, Wu Z, Hu W, Sun A, Troxell B, Yang XF, Yan J. Transcriptional responses of Leptospira interrogans to host innate immunity: significant changes in metabolism, oxygen tolerance, and outer membrane. *PLoS Negl Trop Dis* 2010; **4**: e857 [PMID: 21049008 DOI: 10.1371/journal.pntd.0000857]

57   **Hashimoto K**, Miller C, Hirose K, Diago T, Aucejo F, Quintini C, Eghtesad B, Corey R, Yerian L, Lopez R, Zein N, Fung J. Measurement of CD4+ T-cell function in predicting allograft rejection and recurrent hepatitis C after liver transplantation. *Clin Transplant* 2010; **24**: 701-708 [PMID: 20047619 DOI: 10.1111/j.1399-0012.2009.01169.x]

58   **Mizuno S**, Hamada T, Nakatani K, Kishiwada M, Usui M, Sakurai H, Tabata M, Sakamoto Y, Nishioka J, Muraki Y, Okuda M, Nobori T, Isaji S. Monitoring peripheral blood CD4+ adenosine triphosphate activity after living donor liver transplantation: impact of combination assays of immune function and CYP3A5 genotype. *J Hepatobiliary Pancreat Sci* 2011; **18**: 226-232; discussion 232-234 [PMID: 21165655 DOI: 10.1007/s00534-010-0335-8]

59   **Cheng JW**, Shi YH, Fan J, Huang XW, Qiu SJ, Xiao YS, Wang Z, Dai Z, Tang ZY, Zhou J. An immune function assay predicts post-transplant recurrence in patients with hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2011; **137**: 1445-1453 [PMID: 21809031 DOI: 10.1007/s00432-011-1014-0]

60   **Hwang S**, Kim KH, Song GW, Yu YD, Park GC, Kim KW, Choi NK, Park PJ, Choi YI, Jung DH, Ahn CS, Moon DB, Ha TY, Lee SG. Peritransplant monitoring of immune cell function in adult living donor liver transplantation. *Transplant Proc* 2010; **42**: 2567-2571 [PMID: 20832545 DOI: 10.1016/j.transproceed.2010.04.040]

61   **Zhou T**, Xue F, Han LZ, Xi ZF, Li QG, Xu N, Zhang JJ, Xia Q. Invasive fungal infection after liver transplantation: risk factors and significance of immune cell function monitoring. *J Dig Dis* 2011; **12**: 467-475 [PMID: 22118697 DOI: 10.1111/j.1751-2980.2011.00542.x]

62   **Mendler M**, Kwok H, Franco E, Baron P, Weissman J, Ojogho O. Monitoring peripheral blood CD4+ adenosine triphosphate activity in a liver transplant cohort: insight into the interplay between hepatitis C virus infection and cellular immunity. *Liver Transpl* 2008; **14**: 1313-1322 [PMID: 18756485 DOI: 10.1002/lt.21529]

63   **Xue F**, Zhang J, Han L, Li Q, Xu N, Zhou T, Xi Z, Wu Y, Xia Q. Immune cell functional assay in monitoring of adult liver transplantation recipients with infection. *Transplantation* 2010; **89**: 620-626 [PMID: 20010326 DOI: 10.1097/TP.0b013e3181c690fa]

64   **Ling X**, Xiong J, Liang W, Schroder PM, Wu L, Ju W, Kong Y, Shang Y, Guo Z, He X. Can immune cell function assay identify patients at risk of infection or rejection? A meta-analysis. *Transplantation* 2012; **93**: 737-743 [PMID: 22357178 DOI: 10.1097/TP.0b013e3182466248]

65   **Rodrigo E**, López-Hoyos M, Corral M, Fábrega E, Fernández-Fresnedo G, San Segundo D, Piñera C, Arias M. ImmuKnow as a diagnostic tool for predicting infection and acute rejection in adult liver transplant recipients: a systematic review and meta-analysis. *Liver Transpl* 2012; **18**: 1245-1253 [PMID: 22740321 DOI: 10.1002/lt.23497]

66   **Testro AG**, Visvanathan K, Skinner N, Markovska V, Crowley P, Angus PW, Gow PJ. Acute allograft rejection in human liver transplant recipients is associated with signaling through toll-like receptor 4. *J Gastroenterol Hepatol* 2011; **26**: 155-163 [PMID: 21175809 DOI: 10.1111/j.1440-1746.2010.06324.x]

67   **Land WG**. The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. *Transplantation* 2005; **79**: 505-514 [PMID: 15753838]

68   **Kreisel D**, Goldstein DR. Innate immunity and organ transplantation: focus on lung transplantation. *Transpl Int* 2013; **26**: 2-10 [PMID: 22909350 DOI: 10.1111/j.1432-2277.2012.01549.x]

69   **Leventhal JS**, Schröppel B. Toll-like receptors in transplantation: sensing and reacting to injury. *Kidney Int* 2012; **81**: 826-832 [PMID: 22297675 DOI: 10.1038/ki.2011.498]

70   **Warlé MC**, Metselaar HJ, Hop WC, Tilanus HW. Cytokine gene polymorphisms and acute liver graft rejection: a meta-analysis. *Liver Transpl* 2005; **11**: 19-26 [PMID: 15690532 DOI: 10.1002/lt.20316]

71   **Turner DM**, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; **24**: 1-8 [PMID: 9043871]

72   **Asakura T**, Ohkohchi N, Satomi S. Changes of serum cytokines associated with hepatic regeneration after living-related liver transplantation. *Transplant Proc* 2000; **32**: 2199-2203 [PMID: 11120132]

73   **Carbone J**, Micheloud D, Salcedo M, Rincon D, Bañares R, Clemente G, Jensen J, Sarmiento E, Rodriguez-Molina J, Fernandez-Cruz E. Humoral and cellular immune monitoring might be useful to identify liver transplant recipients at risk for development of infection. *Transpl Infect Dis* 2008; **10**: 396-402 [PMID: 18657086 DOI: 10.1111/j.1399-3062.2008.00329.x]

74   **Demirkiran A**, Bosma BM, Kok A, Baan CC, Metselaar HJ, Ijzermans JN, Tilanus HW, Kwekkeboom J, van der Laan LJ. Allosuppressive donor CD4+CD25+ regulatory T cells detach from the graft and circulate in recipients after liver transplantation. *J Immunol* 2007; **178**: 6066-6072 [PMID: 17475831]

75   **Stenard F**, Nguyen C, Cox K, Kambham N, Umetsu DT, Krams SM, Esquivel CO, Martinez OM. Decreases in circulating CD4+CD25hiFOXP3+ cells and increases in intragraft FOXP3+ cells accompany allograft rejection in pediatric liver allograft recipients. *Pediatr Transplant* 2009; **13**: 70-80 [PMID: 18331536 DOI: 10.1111/j.1399-3046.2008.00917.x]

76   **He Q**, Fan H, Li JQ, Qi HZ. Decreased circulating CD4+CD25highFoxp3+ T cells during acute rejection in liver transplant patients. *Transplant Proc* 2011; **43**: 1696-1700 [PMID: 21693260 DOI: 10.1016/j.transproceed.2011.03.084]

77   **Koshiba T**, Li Y, Takemura M, Wu Y, Sakaguchi S, Minato N, Wood KJ, Haga H, Ueda M, Uemoto S. Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transpl Immunol* 2007; **17**: 94-97 [PMID: 17306739 DOI: 10.1016/

j.trim.2006.10.004]

78   **Martínez-Llordella M**, Puig-Pey I, Orlando G, Ramoni M, Tisone G, Rimola A, Lerut J, Latinne D, Margarit C, Bilbao I, Brouard S, Hernández-Fuentes M, Soulillou JP, Sánchez-Fueyo A. Multiparameter immune profiling of operational tolerance in liver transplantation. *Am J Transplant* 2007; **7**: 309-319 [PMID: 17241111 DOI: 10.1111/j.1600-6143.2006.01621.x]

79   **Li Y**, Koshiba T, Yoshizawa A, Yonekawa Y, Masuda K, Ito A, Ueda M, Mori T, Kawamoto H, Tanaka Y, Sakaguchi S, Minato N, Wood KJ, Tanaka K. Analyses of peripheral blood mononuclear cells in operational tolerance after pediatric living donor liver transplantation. *Am J Transplant* 2004; **4**: 2118-2125 [PMID: 15575917 DOI: 10.1111/j.1600-6143.2004.00611.x]

80   **Pons JA**, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sánchez-Bueno F, Robles R, Rios A, Aparicio P, Parrilla P. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* 2008; **86**: 1370-1378 [PMID: 19034005 DOI: 10.1097/TP.0b013e318188d3e6]

81   **Li Y**, Zhao X, Cheng D, Haga H, Tsuruyama T, Wood K, Sakaguchi S, Tanaka K, Uemoto S, Koshiba T. The presence of Foxp3 expressing T cells within grafts of tolerant human liver transplant recipients. *Transplantation* 2008; **86**: 1837-1843 [PMID: 19104431 DOI: 10.1097/TP.0b013e31818febc4]

82   **Cinti P**, Pretagostini R, Arpino A, Tamburro ML, Mengasini S, Lattanzi R, De Simone P, Berloco P, Molajoni ER. Evaluation of pretransplant immunologic status in kidney-transplant recipients by panel reactive antibody and soluble CD30 determinations. *Transplantation* 2005; **79**: 1154-1156 [PMID: 15880060]

83   **Süsal C**, Pelzl S, Döhler B, Opelz G. Identification of highly responsive kidney transplant recipients using pretransplant soluble CD30. *J Am Soc Nephrol* 2002; **13**: 1650-1656 [PMID: 12039995]

84   **Matinlauri I**, Höckerstedt K, Isoniemi H. High serum soluble CD30 does not predict acute rejection in liver transplant patients. *Transplant Proc* 2006; **38**: 3601-3604 [PMID: 17175343 DOI: 10.1016/j.transproceed.2006.10.063]

85   **Truong DQ**, Darwish AA, Gras J, Wieërs G, Cornet A, Robert A, Mourad M, Malaise J, de Ville de Goyet J, Reding R, Latinne D. Immunological monitoring after organ transplantation: potential role of soluble CD30 blood level measurement. *Transpl Immunol* 2007; **17**: 283-287 [PMID: 17493532 DOI: 10.1016/j.trim.2007.01.007]

86   **Sánchez-Fueyo A**. Hot-topic debate on tolerance: immunosuppression withdrawal. *Liver Transpl* 2011; **17** Suppl 3: S69-S73 [PMID: 21850680 DOI: 10.1002/lt.22421]

87   **Lerut J**, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006; **6**: 1774-1780 [PMID: 16889539 DOI: 10.1111/j.1600-6143.2006.01396.x]

88   **Orlando G**, Soker S, Wood K. Operational tolerance after liver transplantation. *J Hepatol* 2009; **50**: 1247-1257 [PMID: 19394103 DOI: 10.1016/j.jhep.2009.03.006]

89   **Martínez-Llordella M**, Lozano JJ, Puig-Pey I, Orlando G, Tisone G, Lerut J, Benítez C, Pons JA, Parrilla P, Ramírez P, Bruguera M, Rimola A, Sánchez-Fueyo A. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. *J Clin Invest* 2008; **118**: 2845-2857 [PMID: 18654667 DOI: 10.1172/JCI35342]

90   **Robinson SP**, Patterson S, English N, Davies D, Knight SC, Reid CD. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol* 1999; **29**: 2769-2778 [PMID: 10508251 DOI: 10.1002/(SICI)1521-4141(199909)29:]

91   **Rissoan MC**, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999; **283**: 1183-1186 [PMID: 10024247]

92   **Mazariegos GV**, Zahorchak AF, Reyes J, Chapman H, Zeevi A, Thomson AW. Dendritic cell subset ratio in tolerant, weaning and non-tolerant liver recipients is not affected by extent of immunosuppression. *Am J Transplant* 2005; **5**: 314-322 [PMID: 15643991 DOI: 10.1111/j.1600-6143.2004.00672.x]

93   **Tokita D**, Mazariegos GV, Zahorchak AF, Chien N, Abe M, Raimondi G, Thomson AW. High PD-L1/CD86 ratio on plasmacytoid dendritic cells correlates with elevated T-regulatory cells in liver transplant tolerance. *Transplantation* 2008; **85**: 369-377 [PMID: 18301333 DOI: 10.1097/TP.0b013e3181612ded]

94   **VanBuskirk AM**, Burlingham WJ, Jankowska-Gan E, Chin T, Kusaka S, Geissler F, Pelletier RP, Orosz CG. Human allograft acceptance is associated with immune regulation. *J Clin Invest* 2000; **106**: 145-155 [PMID: 10880058 DOI: 10.1172/JCI9171]

95   **Carrodeguas L**, Orosz CG, Waldman WJ, Sedmak DD, Adams PW, VanBuskirk AM. Trans vivo analysis of human delayed-type hypersensitivity reactivity. *Hum Immunol* 1999; **60**: 640-651 [PMID: 10439310]

96   **Castellaneta A**, Thomson AW, Nayyar N, de Vera M, Mazariegos GV. Monitoring the operationally tolerant liver allograft recipient. *Curr Opin Organ Transplant* 2010; **15**: 28-34 [PMID: 19890211 DOI: 10.1097/MOT.0b013e328334269a]

97   **Stenton SB**, Partovi N, Ensom MH. Sirolimus: the evidence for clinical pharmacokinetic monitoring. *Clin Pharmacokinet* 2005; **44**: 769-786 [PMID: 16029064 DOI: 10.2165/00003088-200544080-00001]

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

We encourage authors to submit their manuscripts to *WJT*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJT is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 43 OA clinical medical journals, including 42 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJT* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer

to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in transplantation; (12) Brief Articles: To briefly report the novel and innovative findings in transplantation; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJT*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of transplantation; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including t-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJT* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS
Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified.

A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgoffice@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, Uni-

versity of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present P values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc*. It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^aP <$

0.05, [b]$P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, [c]$P < 0.05$ and [d]$P < 0.01$ are used. A third series of P values can be expressed as [e]$P < 0.05$ and [f]$P < 0.01$. Other notes in tables or under illustrations should be expressed as [1]F, [2]F, [3]F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634   DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format
Journals
English journal article (list all authors and include the PMID where applicable)

1   **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224   DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2   **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3   **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4   **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462   PMCID:2516377   DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5   **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764   DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6   21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303   DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7   **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325   DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8   **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900   DOI:10.1097/00003086-200208000-00026]

No volume or issue

9   Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books
Personal author(s)

10   **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11   **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12   **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13   **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14   **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15   Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16   **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3230/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073445.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

Statement about anonymous publication of the peer reviewers' comments

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJT is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. All invited articles are published free of charge.

World Journal of

Transplantation

World Journal of Transplantation

Contents

Contents

Contents

World Journal of Transplantation
Volume 4 Number 2 June 24, 2014

ABOUT COVER

World Journal of Transplantation Editorial Board, Roberto J Firpi, MD, MS, Associate Professor of Medicine, University of Florida, Department of Medicine, 1600 SW Archer Rd, MSB Room M440, Gainesville, Fl 32610, United States

AIM AND SCOPE

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

   WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

AIM AND SCOPE

World Journal of Transplantation is now indexed in PubMed Central, PubMed, Digital Object Identifier.

EDITORIAL

Organ assessment and repair centers: The future of transplantation is near

Bryan A Whitson, Sylvester M Black

Bryan A Whitson, Division of Cardiac Surgery, The Collaboration for Organ Perfusion, Protection, Engineering and Regeneration (COPPER) Laboratory, The Ohio State University Wexner Medical Center, Columbus, OH 43210, United States

Sylvester M Black, Division of Transplantation, The Collaboration for Organ Perfusion, Protection, Engineering and Regeneration (COPPER) Laboratory, The Ohio State University Wexner Medical Center, Columbus, OH 43210, United States

Author contributions: Whitson BA and Black SM contributed equally to this works authorship and revision.

Correspondence to: Bryan A Whitson, MD, PhD, Division of Cardiac Surgery, The Collaboration for Organ Perfusion, Protection, Engineering and Regeneration (COPPER) Laboratory, The Ohio State University Wexner Medical Center, N-813 Doan Hall, 410 W 10th Ave, Columbus, OH 43210, United States. bryan.whitson@osumc.edu

Telephone: +1-614-3667414 **Fax:** +1-614-2932020

Received: November 28, 2013 **Revised:** April 9, 2014

Accepted: April 16, 2014

Published online: June 24, 2014

Abstract

Solid organ transplantation is limited by suitable donor organ availability and the geographic limitations that lead to prolonged ischemic times. *Ex vivo* organ perfusion is an evolving technology that enables assessment of organ function prior to transplantation. As a byproduct, overall out of body organ times are able to be extended. The future implications organ assessment and repair centers utilizing this technology are discussed.

Core tip: Regional organ assessment and repair centers will build upon normo-thermic *ex vivo* organ perfusion technology, which in turn provides a potential platform to assess, repair and eventually modify donor organs.

Solid organ transplantation is currently limited by an inadequate number of donor organs and the inability to optimally evaluate and assess those organs prior to transplantation. The lack of donor organs has led to many innovative strategies to increase the number of donor organs available for transplantation. Split organ transplantation (liver), living donor transplantation and the use of marginal or extended criteria donor organs has had a modest effect on organ availability. The organ utilization rate for many donor organ types (*i.e.*, lung) remains low and the benefits of increasing the donor pool are not fully realized. From the initial pioneering work in kidney transplantation by Belzer[1], successful outcomes tended to depend on short ischemic time and good organ quality. Techniques in rapid procurement, implantation and advances in organ preservation were crucial to development of the field of transplantation. Out of the pioneering work of F.O. Belzer, *ex vivo* kidney perfusion circuits were conceptualized and then utilized with impressive results. Alexis Carrel and Charles Lindberg had telegraphed this possibility with their prescient work in 1935 "The Culture of Organs"[2]. With current advancements in perfusion technology, molecular biology and biomedical engineering the next evolution in organ transplantation will be regional organ assessment and repair centers (ARCs)[3].

Regional organ ARCs build upon normo-thermic *ex vivo* organ perfusion (EVOP) technology, which in turn provides a potential platform to assess, repair and eventually modify donor organs. Currently, normo-thermic EVOP allows for assessment of organ function prior to transplantation and is largely focused clinically on lung transplantation. Normo-thermic EVOP allows for potential donor organs to have their function evaluated and
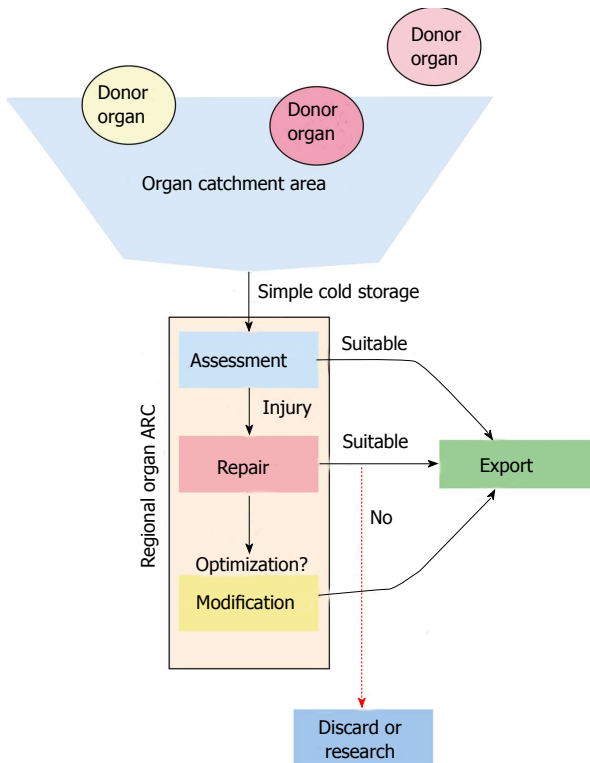
Figure 1 A conceptualized Organ assessment and repair centers would receive donated organs (traditional and marginal donor quality) and assess their function. During the assessment, the quality, viability, and function of the evaluated organ would be determined. There may be subspecialization of assessment and repair center with local expertise in one or two organ types. ARC: Assessment and repair center.

Figure 2 Through the use of *ex vivo* organ perfusion, preservation times would be extended. With this extension of total ischemic times, organs would have the opportunity to truly be matched with the recipient who would most benefit from that particular organ at that particular time. Occasionally, the allocation may be the current, traditional approach, where the allocation directly bypasses the ex-vivo assessment at the assessment and repair center and proceed directly to the recipient. (Adapted from Black and Whitson, 2013)[3]. ARC: Assessment and repair center.

the suitability for transplantation assessed[4,5]. Simple cold storage and hypothermic machine preservation, while appreciably lowering the metabolic rate and increasing preservation times do not allow for optimal assessment of an individual organ. Most of the determinations regarding an organ's function are made pre-procurement and while hypothermia decreases the metabolic rate of the donor organ to 5%-10% of normal, significant anaerobic metabolism continues to take place. Prolonged preservation and marginal donor organ quality can lead to significant delayed graft function or graft non-function in the recipient[3]. At present, there is a multi-institutional clinical trial in the United States to assess the safety and efficacy of *ex vivo* ung perfusion (EVLP) in clinical transplantation. What's more, EVLP is used clinically in Canada[4], Australia, and Europe. *Ex vivo* liver perfusion likewise is in a clinical trial in Europe to assess the feasibility of this approach. A beneficial side effect of EVOP is that organ ischemic times and thus distance between center, donor, and recipient, can be significantly extended.

The potential of *ex vivo* technology was recently illustrated by the work of Wigfield *et al*[6] where a marginal lung donor organ was transported from a donor center internationally to the organ repair center at the University of Toronto Lung Transplant Program and then back internationally to the recipient center[6]. This process extended the ischemic time to 15 h and 20 min. In essence, this is the index case for conceptualizing and operational-

izing a regional organ ARC approach.

One could envision a time where EVOP would be used as a platform for assessment, repair, and modification of all organs. However, the logistic and financial feasibility of such an approach would likely only allow this technology to be employed when the organs are of marginal quality, of extended donor criteria, or when the best tissue match is in a geographically distinct area.

Each specific organ type has a unique function and as such has a unique set of metabolic demands and metrics for assessing viability. With this approach, organs would optimally be assessed and evaluated in a regional ARC (Figure 1). Organs of all types (heart, lung, liver, kidney, intestine and pancreas) that are in geographic proximity to the ARC would be procured and prepared with standard cold static storage. The organs would be transported to the ARC. At the ARC a thorough evaluation of the donor organ takes place, with appropriate repair or regenerative measures employed based on the deficits in function encountered. If the donor organs are deemed to be suitable (or can be made suitable) for transplantation, the organs would then be allocated in a national or international fashion to the most suitable recipient (Figure 2). This more broad, detailed, and individualized matching is only possible with the extended preservation (total ischemic time) that EVOP allows. This ability to match more thoroughly and to repair or resuscitate marginal donor organs would potentially improve graft survival and long-term outcomes.

The impact on organ transplant waitlists will be enormous. Even modest increases in organ availability will markedly increase the total number of transplants performed. For example, in lung transplantation in the United States alone, increasing the overall conversion rate from 17%[7] to 32% (an absolute increase of only 15% more organs transplanted) would practically double

the number of transplants performed and eliminates the waiting list. EVLP will have significant impacts by saving the lives of recipients on the waiting list, extending the opportunity for a life-saving and life-extending transplant to patients who currently are not ill enough, and reduce the mortality in transplant recipients.

As the science and technique of organ perfusion and preservation progress, we have the expectation that in the very near future EVOP and regional organ ARCs will serve as the platform for organ repair and modification[8]. *Ex vivo* perfusion technology has already been used to repair injured livers and lungs prior to transplantation[4-6,8,9]. In livers, bile duct injury can be mitigated[9] and steatotic livers are able to be de-fatted and thus improve overall organ quality and function[9]. In the lung, edema may be removed, infection cleared, and gas exchange improved[4].

By any measure, organ transplantation is one of the success stories of modern medicine. We will look back on EVOP and the development of regional organ ARCs as a similar milestone where the multidisciplinary approach to a complex problem has allowed innovation to propel our science to new frontiers in the treating end-stage organ disease.

REFERENCES

1   **Belzer FO**. Organ preservation: A personal perspective. In: Terasaki PI, ed. History of Transplantation. Thirty-five Recollections: UCLA Tissue Typing Laboratory, 1991: 595-614

2   **Carrel A**, Lindbergh CA. The Culture of Whole Organs. *Science* 1935; **81**: 621-623 [DOI: 10.1126/science.81.2112.621]

3   **Black SM**, Whitson BA. Regional Organ Assessment and Repair Centers (ARC's). *IJMBS* 2013; **5**: 243-246

4   **Cypel M**, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, Sato M, Laratta J, Azad S, Madonik M, Chow CW, Chaparro C, Hutcheon M, Singer LG, Slutsky AS, Yasufuku K, de Perrot M, Pierre AF, Waddell TK, Keshavjee S. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011; **364**: 1431-1440 [PMID: 21488765 DOI: 10.1056/NEJMoa1014597]

5   **Knaak JM**, Spetzler VN, Selzner N, Selzner M. Normothermic machine perfusion of discarded liver grafts-what is viable? *Am J Transplant* 2013; **13**: 2503 [PMID: 23915165]

6   **Wigfield CH**, Cypel M, Yeung J, Waddell T, Alex C, Johnson C, Keshavjee S, Love RB. Successful emergent lung transplantation after remote ex vivo perfusion optimization and transportation of donor lungs. *Am J Transplant* 2012; **12**: 2838-2844 [PMID: 23009140]

7   **Punch JD**, Hayes DH, LaPorte FB, McBride V, Seely MS. Organ donation and utilization in the United States, 1996-2005. *Am J Transplant* 2007; **7**: 1327-1338 [PMID: 17428283]

8   **Yeung JC**, Wagnetz D, Cypel M, Rubacha M, Koike T, Chun YM, Hu J, Waddell TK, Hwang DM, Liu M, Keshavjee S. Ex vivo adenoviral vector gene delivery results in decreased vector-associated inflammation pre- and post-lung transplantation in the pig. *Mol Ther* 2012; **20**: 1204-1211 [PMID: 22453765]

9   **Boehnert MU**, Yeung JC, Bazerbachi F, Knaak JM, Selzner N, McGilvray ID, Rotstein OD, Adeyi OA, Kandel SM, Rogalla P, Yip PM, Levy GA, Keshavjee S, Grant DR, Selzner M. Normothermic acellular ex vivo liver perfusion reduces liver and bile duct injury of pig livers retrieved after cardiac death. *Am J Transplant* 2013; **13**: 1441-1449 [PMID: 23668775]

REVIEW

Selecting suitable solid organ transplant donors: Reducing the risk of donor-transmitted infections

Christopher S Kovacs Jr, Christine E Koval, David van Duin, Amanda Guedes de Morais, Blanca E Gonzalez, Robin K Avery, Steven D Mawhorter, Kyle D Brizendine, Eric D Cober, Cyndee Miranda, Rabin K Shrestha, Lucileia Teixeira, Sherif B Mossad

Christopher S Kovacs Jr, Christine E Koval, Steven D Mawhorter, Kyle D Brizendine, Eric D Cober, Cyndee Miranda, Rabin K Shrestha, Lucileia Teixeira, Sherif B Mossad, Department of Infectious Diseases, Medicine Institute, Cleveland Clinic, Cleveland, OH 44195, United States
David van Duin, Division of Infectious Diseases, Immunocompromised Host Infectious Disease Program, University of North Carolina, Chapel Hill, NC 27599, United States
Amanda Guedes de Morais, Infectious Disease and Internal Medicine, Augusta Health, Fishersville, VA 22939, United States
Blanca E Gonzalez, Department of Pediatric Infectious Diseases, Cleveland Clinic Children's, Cleveland, OH 44195, United States
Robin K Avery, Division of Infectious Disease (Transplant/Oncology), Johns Hopkins, Baltimore, MD 21205, United States
Author contributions: Avery RK created the idea of an institutional approach for donor suitability; Koval CE, van Duin D, Mawhorter SD, Brizendine KD, Guedes de Morais A, Gonzalez BE, Cober ED, Miranda C, Shrestha RK, Teixeira L and Mossad S wrote material from which sections were synthesized and reviewed and approved the final draft; Kovacs Jr CS and Mossad SB coordinated and synthesized data, wrote, and compiled the final submitted draft.
Correspondence to: Sherif B Mossad, MD, Department of Infectious Diseases, Medicine Institute, Cleveland Clinic, 9500 Euclid Ave, Mailstop G-21, room 131, Cleveland, OH 44195, United States. mossads@ccf.org
Telephone: +1-216-4452572   Fax: +1-216-4459446
Received: December 20, 2013 Revised: March 21, 2014
Accepted: May 14, 2014
Published online: June 24, 2014

Abstract

Selection of the appropriate donor is essential to a successful allograft recipient outcome for solid organ transplantation. Multiple infectious diseases have been transmitted from the donor to the recipient *via* transplantation. Donor-transmitted infections cause increased morbidity and mortality to the recipient. In recent years, a series of high-profile transmissions of infections have occurred in organ recipients prompting increased attention on the process of improving the selection of an appropriate donor that balances the shortage of needed allografts with an approach that mitigates the risk of donor-transmitted infection to the recipient. Important advances focused on improving donor screening diagnostics, using previously excluded high-risk donors, and individualizing the selection of allografts to recipients based on their prior infection history are serving to increase the donor pool and improve outcomes after transplant. This article serves to review the relevant literature surrounding this topic and to provide a suggested approach to the selection of an appropriate solid organ transplant donor.

Core tip: The literature surrounding preventing donor-transmitted infections in solid organ transplant recipients has increased greatly in the last decade. Increased emphasis has been placed on improved diagnostics for screening of deceased donors. Importance has been placed on using donors who were previously thought to be high risk for transmitting infections to recipients and mitigating the risk to such recipients in an effort to increase the donor pool. Initiating the discussion around using human immunodeficiency virus (HIV) infected donors for HIV infected recipients has important implications for addressing the problem of allograft shortages.

Kovacs Jr CS, Koval CE, van Duin D, Guedes de Morais A, Gonzalez BE, Avery RK, Mawhorter SD, Brizendine KD, Cober ED, Miranda C, Shrestha RK, Teixeira L, Mossad SB. Selecting suitable solid organ transplant donors: Reducing the risk of donor-transmitted infections. *World J Transplant* 2014; 4(2): 43-56 Available from: URL: http://www.wjgnet.com/2220-3230/

full/v4/i2/43.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i2.43

INTRODUCTION

Selection of the appropriate donor is the cornerstone of achieving a positive outcome after solid organ transplantation (SOT). This selection requires screening potential donors for infectious diseases that can be transmitted to the allograft recipient[1]. Screening for transmissible infections allows timely disqualification of a donor if the risk of developing illness in the recipient is deemed prohibitive. Screening also allows risk reduction by identifying and actively treating infection in the donor prior to procurement or preemptively treating the recipient following transplantation. Selecting the suitable donor is of paramount importance to reducing the risk of infectious morbidity and mortality from donor-transmitted infections (DTI).

It has become necessary to consider donors who may have active infection, high-risk infectious serologic profile, or high-risk behavior for human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infection at the time of donation due to an inadequate supply of needed allografts[2]. As more patients rely on organ transplantation to manage end-stage disease processes, the available donor pool will only shrink further. Important, evidence-based, decisions regarding risk stratification and risk *vs* benefit analyses are needed in order to increase the donor pool. The risk of death while on the waiting list for many organs needs to be cautiously weighed with the risk for mortality after transplant when considering using expanded donor criteria in order to first do no harm to the recipient (Table 1)[3-8].

A number of incidents of DTI have brought this topic to the forefront of attention, as renewed evaluations of the donor screening process have been undertaken. Recent cases of rabies, lymphocytic choriomeningitis virus (LCM), West Nile virus (WNV), HIV, and HCV have all been confirmed as donor transmitted[9-13]. In 2005, the Disease Transmission Advisory Committee (DTAC) was created to aid the Organ Procurement and Transplantation Network/United Network for Organ Sharing in identifying and reviewing potential DTI. This committee has served an essential role in systematizing the collection and evaluation of nationwide information about suspected DTI. This includes: a thorough review of each case by an expert appointed by the committee, facilitation of communication between centers, and tabulating information to a growing database that provides critical information about donor derived risks[14-16]. Extensive deceased donor testing is often not feasible given the time constraints in which such screening must be carried out. Concerns exist regarding sensitivity of tests used for pathogens such as HIV and HCV, which may be negative prior to antibody production[17]. Infections that are reliant on microbiologic methods to diagnose, such as donor blood and urine cultures, may not be resulted until after

transplant has taken place. New technologies and donor screening strategies using nucleic acid amplification testing (NAAT) may help provide important information earlier, but developing approaches on how best to utilize these tests has been controversial[1,18,19].

Multiple pathogens have been shown to have the potential to be transmitted by SOT[20,21]. DTIs are estimated to occur in 0.2%-1.7% of all transplant procedures, with varying morbidity and mortality[22,23]. Bacterial, mycobacterial, viral, fungal, and parasitic pathogens all need to be contemplated by the transplant physician when called for opinion regarding donor suitability. This article serves to summarize the current literature about commonly encountered DTI and to offer an approach for decisions regarding donor suitability (Table 2).

BACTERIAL INFECTIONS

Transplantation of allografts taken from donors with underlying sepsis syndrome of unknown etiology is not recommended. Bacterial DTIs have been linked to increased morbidity and mortality as well as allograft loss[24-26]. As previously mentioned, however, underlying bacteremia in the donor may not be recognized until after transplantation has occurred. In one study, 60% of bacteremic donors were afebrile during the 24-h period prior to organ procurement[27]. The outcome of allograft donation from a bacteremic donor depends on the type of bacteria causing infection, previous antimicrobial therapy in the donor prior to organ procurement, and timely recognition of donor bacteremia so therapy can be instituted in the recipient[28,29].

An estimated 5% of organ donors have unrecognized bacteremia at the time of donation[27,30]. Some studies have shown that use of organs from bacteremic donors, especially when the organism is community acquired and not highly resistant to antimicrobials, is not associated with higher incidence of allograft dysfunction[27,30,31]. Thirty-day graft and patient survival for recipients of organs from bacteremic donors were not significantly different than those who received organs from non-bacteremic donors[30]. Recipients included in these series had been given broad-spectrum antibiotics during the perioperative period and were given tailored antibiotic therapy once donor bacteremia was identified. This suggests that allografts from bacteremic donors are suitable for transplantation if the donor is on appropriate antibiotic therapy for ≥ 24 h and if tailored antibiotic therapy can be initiated in the recipient in a timely manner. Recipients should be treated for a minimum of 7 d, depending on the posttransplant course and perhaps longer if the pathogen has the potential to disrupt an anastomosis or seed an endovascular source. In the event a donor is being treated for endocarditis, the recipient should receive organism-specific antimicrobial therapy for at least 2 wk, and if the organism is *Staphylococcus aureus*, 6 wk of therapy is appropriate[32]. If donor cultures are repeatedly positive for pathogenic bacteria or yeast, then additional consent from the recipient and/or family should

Table 1 Mortality figures by type of transplant for 2010 according to the Scientific Registry of Transplant Recipients 2011 Annual Report[1]		
Organ tansplanted[2]	Waiting list mortality incidence density[3] (deaths per 1000 patient-years)	1 yr posttransplant mortality incidence density (deaths per 1000 patient-years)
Kidney	56.5	34.9
Liver	115.6	123.7
Intestine	71.6	193.5
Heart	115.8	91.8
Lung	154.1	164.2

[1]The data and analyses reported in the 2011 Annual Data Report of the United States Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients have been supplied by UNOS and the Minneapolis Medical Research Foundation under contract with HHS/HRSA. The authors alone are responsible for reporting and interpreting these data; the views expressed herein are those of the authors and not necessarily those of the United States Government; [2]Data reported in table is for deceased donor only; [3]Incidence is reported as deaths per 1000 patient years at risk.

Table 2 Approach to selecting suitable donors for solid organ transplantation		
Infections	Diagnostic tools	Treatment considerations
Bacteremia	Blood cultures Antibiogram	Treat donor 24 h Tailored recipient therapy in posttransplant period
Resistant bacteria	Blood cultures Sterile site cultures Antibiogram	Tailored donor and recipient therapy
Meningoencephalitis	CSF analysis CSF culture and stain Cryptococcus antigen NAAT	Tailored therapy if meningitis only
Syphilis	Treponemal testing Nontreponemal testing	Treat recipients as late latent syphilis
Viral hepatitis	Serologic evaluation NAAT	Prophylaxis Tailored therapy HBIG Antivirals
Influenza	Influenza testing Respiratory virus PCR	Neuraminidase inhibitor
HTLV 1/2	Routine screening not recommended	No effective treatment, surveillance for recipients of positive donors
Candida infection	Blood cultures Sterile site cultures Antibiogram	Antifungal treatment of donor Treat colonization in certain settings
Cryptococcosis	CSF cryptococcal antigen Serum cryptococcal antigen	Antifungal treatment of donors prior to donation
Endemic fungi	Urine antigen testing Serologic evaluation Sterile site culture Histologic evaluation	Antifungal treatment of donors prior to donation
Schistosomiasis	Stool examination Serologic evaluation Rectal biopsy	Treat living donor successfully prior to donation
Strongyloidiasis	Serologic evaluation Stool examination	Treat recipients from positive donors
Chagas disease	Enzyme immunoassay Radioimmune precipitation assay	Treat recipient for positive surveillance testing

HTLV: Human T-lymphotropic virus; NAAT: Nucleic acid amplification testing; PCR: Polymerase chain reaction; HBIG: Hepatitis B immunoglobulin.

be obtained. Surveillance blood cultures of the recipient after transplant are prudent in this situation. Most studies evaluating donor bacteremia excluded donors with sepsis. This may have biased the data by selectively removing pathogens more likely to contribute significantly to post-transplant morbidity and mortality.

An emerging concern is the transmission of multi-drug resistant (MDR) bacteria. Management strategies for dealing with these donor-transmitted resistant infections, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococcus (VRE) species, and MDR gram-negative bacteria are not well established[33]. Resistant gram-positive bacteria are frequently encountered in the donor prior to organ procurement. Although less virulent gram-positive bacteria, such as coagulase-negative staphylococci are seemingly less likely to be transmitted from bacteremic donors and are less associated with poorer outcome after transplant, other more virulent gram-positive organisms such as VRE and MRSA do remain a source of concern regarding donor suitability[28]. MRSA colonization of an individual has been shown to increase their risk for infection[34]. Risk factors for MRSA infection and colonization include prolonged hospitalization, exposure to broad-spectrum antibiotics, intensive care unit (ICU) admission, and the presence of a central venous catheter, all of which are often present in deceased organ donors[35]. MRSA colonization in a donor should not prevent acceptance of the allograft; however, perioperative antibiotics should be adjusted to account for the potential increase in recipient infection risk. Mortality from deep-seated MRSA infection associated with bacteremia after transplant has been in excess of 80%[29]. Allografts from donors with deep-seated MRSA infections should only be accepted if the donor has been on appropriate antibiotic therapy for ≥ 48 h. If the potential allograft is the site of infection, the organ should be rejected. Vancomycin-intermediate *Staphylococcus aureus* and vancomycin-resistant *Staphylococ-*

cus aureus infections in the transplant population have not yet been reported[36]. Donor infection with these isolates should exclude them from donation. VRE is another common pathogen, specifically in the setting of transplantation of an intra-abdominal organ. Risk factors for VRE are similar to MRSA, and general guidelines for donor suitability pertaining to MRSA should be applied to reduce recipient risk for VRE infection[37].

Impact of infection with MDR gram-negative bacteria in transplant recipients is of special concern. Literature suggests that survival in transplant recipients with such infections is decreased[38]. These infections are problematic given limited antimicrobial options, need for potentially more toxic antimicrobials, more potential drug interactions, and fewer drugs in the developmental pipeline[33].

Transplant patients are especially vulnerable to infections with these organisms given end-stage disease processes, extensive healthcare contact before and after transplant, and the need for immunosuppression after transplant to maintain graft function. The most common MDR gram-negative infections encountered in the transplant population are carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and *Pseudomonas* species resistant to at least two different classes of antimicrobials (MDR). Donors with long-term stay in ICU, vasopressor requirement, and prolonged hospitalization are at increased risk for colonization and infection with MDR organisms that can be transmitted to the recipient, even in the absence of overt signs of infection in the donor[39-43]. Studies have shown that using an allograft from a donor with a deep-seated infection from MDR organisms can result in transmission to the recipient even when pathogen directed therapy is used in the recipient[39]. Horizontal transmission within a transplant unit can occur with devastating results. High rates of 30-d mortality have been reported when transplant recipients develop infection with carbapenem-resistant *Klebsiella pneumoniae,* with infection being a predictor of time-to-death[44,45]. The critical information involves whether the infection is sensitive to a carbapenem. If a donor is colonized with a MDR gram-negative organism that remains sensitive to a carbapenem, he may remain a candidate for donation. A donor with a deep-seated infection involving an organ not being transplanted can be considered only if treated with appropriate antibiotics for ≥ 48 h. Additional consent should be obtained from the recipient and/or family and a plan made to treat the recipient for ≥ 2 wk depending on the clinical course. As a general rule, donor bacteremia with CRE, CRAB, or MDR *Pseudomonas* infection should eliminate that donor from consideration. Infections stemming from MDR gram-negative organisms no longer susceptible to carbapenems should preclude donation. If a clear case of asymptomatic colonization with a MDR organism is identified in the donor, the allograft may be acceptable, unless noted in the urine or rectal swab of a planned kidney transplant or small bowel transplant, respectively. DTI with these organisms remains an area for study and optimal management strategies for MDR organisms are still to be defined.

Bacterial meningitis and syphilis may be present in a potential organ donor and, as such, may be transmitted to the allograft recipient. The disparity between available allografts and those awaiting transplantation has grown, such that, these two conditions are no longer deemed absolute contraindications for organ donation. Multiple cases of donor-transmitted syphilis have been reported[46-48]. The estimated prevalence of syphilis among potential organ donors based on the incidence in the general population is 0.15%[49]. Transmission of syphilis is a rare event, but if a donor tests positive for this organism additional consent from the recipient and/or family should be obtained. Most experts agree that if the organ is accepted the recipient should be treated with a regimen for late latent syphilis consisting of benzathine penicillin 2.4 million units intramuscularly every week for a total of 3 wk[1]. Syphilis IgG of the recipient should be assessed at time of transplant and at 1, 3, 6, and 12 mo. Patients with documented bacterial meningitis are also no longer considered to be excluded from organ donation, provided that pathogen-directed treatment has been initiated. Several instances of successful allograft procurement have been reported in the literature from donors with microbiologically proven bacterial meningitis[50-54]. Guidelines now recommend accepting an organ if the etiology of the meningitis is *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, or group B streptococcus. Meningitis must be confirmed as the sole site of infection in the donor and acceptance of donor allografts infected with highly virulent organisms such as *Listeria* species should be rejected. Ideally, the donor should be receiving appropriate therapy for 48 h prior to procurement with signs of clinical improvement. Additional consent from the recipient and/or family should be obtained and pathogen-directed therapy of the recipient should be continued for at least 2 wk[1].

Cultures of organ procurement fluid (OPF) have been studied as a potential source of DTI. OPF cultures are commonly positive for the growth of bacteria, with low-virulence bacteria such as coagulase-negative staphylococcus and *Corynebacterium*[55-60]. Studies are variable on whether positive OPF cultures portend an increased risk for posttransplant infection. Cultures of the OPF are rarely available to make donor suitability decisions, but should not prevent organ donation. The exception to this is OPF cultures growing *Candida*, which may be an important risk factor for graft-transmitted candidiasis[61-64]. The optimal strategy for managing recipients of allografts with positive OPF cultures is not known, but brief tailored treatment of the recipient for growth of virulent organisms is likely indicated[60].

TUBERCULOSIS

Almost 10000 cases of *Mycobacterium tuberculosis* (TB) infection were reported in the Unites States in 2012. The majority of these cases were in patients who were not born in the United States, but have emigrated from highly endemic areas, highlighting the need for close attention to donor demographics and travel history. It is estimated that rates of tuberculosis in patients from highly endemic areas are 20-74 times the general population with the prevalence of posttransplant tuberculosis approaching 12%[65,66]. Management of tuberculosis in transplant recipients is challenging on many fronts. Diagnosis can be difficult because disease presentation can be atypical, despite ongoing active disease, sputum smears can be negative with low mycobacterial burden, and tuberculin skin testing (TST) and interferon gamma release assays (IGRA) may be falsely negative in the setting of immunosuppression end-stage disease processes[65,67-69]. Treatment is also difficult with concerns for drug toxicity, interactions with immunosuppressive medications, and potential develop-

Table 3 Suggested approach to donor-transmitted *Mycobacterium tuberculosis*							
Deceased donors							
[1]TB Risk	[2]Suggestive radiology	[3]Donor testing	[4]Donor treated	Accept allograft	Additional consent	[5]Recipient treatment	Additional recipient testing
Low	No	Negative	N/A	Yes	None	None	None
Low	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Low	Yes	Pending	No/Yes	No	N/A	N/A	N/A
Elevated	No	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Elevated	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Elevated	Yes	Pending	No/Yes	No	N/A	N/A	N/A
Elevated	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Prior active TB	Yes	Negative	Yes	Yes	Yes	Chemoprophylaxis	None
Prior active TB	Yes	Pending	Yes	Yes	Yes	Chemoprophylaxis	None
Prior active TB	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Prior active TB	Yes	Positive	No	No	N/A	N/A	N/A
Active TB	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Living donors							
Low	No	Negative	N/A	Yes	No	None	None
LTBI	No	Positive	Yes	No	Yes	None	None
Active TB	No	Positive	No	No	N/A	N/A	N/A
Elevated	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None

[1]Based on history and physical examination; [2]Apical fibrosis and/or pleural thickening on chest radiograph or computerized tomography scan; [3]Sputum acid fast bacilli (AFB) smear and culture; molecular testing on smear-positive sputum; [4]Must be documented treatment with appropriate anti-TB therapy; [5]Refers to accepted regimen for treatment of latent tuberculosis infection (LTBI). TB: Tuberculosis; N/A: Not applicable.

ment of drug-resistant tuberculosis. *Mycobacterium tuberculosis* infection after transplant is associated with 20%-30% mortality rate[67,70].

Most cases of posttransplant tuberculosis are caused by reactivation of latent infection in the recipient following immunosuppressive therapy[71]. *Mycobacterium tuberculosis* can also be transmitted directly from the allograft to organ recipient[15,72,73]. This fact highlights the necessity of a thoughtful approach to the potential organ donor to limit the risk of a potentially catastrophic posttransplant infectious complication. Table 3 highlights one approach to evaluating the risk of donor-transmitted tuberculosis. There is no firm evidence from randomized clinical trials to make strong recommendations, and each center should factor in the incidence and prevalence of latent TB infection (LTBI) and active TB within their population. Assessment of the donor begins with identifying country of birth, a thorough historical evaluation with emphasis on epidemiological and associated disease-related TB risk factors, prior positive TST/IGRA, review of prior radiographic imaging, and in the case of prior active disease, documentation of completed appropriate anti-tuberculosis treatment. Risk factors for TB in the donor include substance abuse, malnutrition, HIV infection, and close household contact with TB smear-positive individuals[74-77]. Special attention should be paid to donors who have resided in homeless shelters, prisons, or highly endemic areas outside of the United States[78-81]. In donors with low TB risk accompanied by negative radiology, the allograft can be accepted without the need for chemoprophylaxis or additional informed consent on the part of the recipient. Donors, who have had active TB, particularly in the preceding 2 years, have higher relapse potential and increased risk of harboring drug-resistant TB isolates, which, may lead to increased risk of donor-

transmitted TB. This should be considered when monitoring and treating recipients of allografts from such donors[69,82].

VIRAL INFECTIONS

Viral infections are a common cause of morbidity and mortality after SOT. Infections that are potentially donor derived include HIV, HCV, HBV, human T-lymphotropic virus (HTLV- 1 and 2), etiologic agents of viral encephalitis, such WNV, LCM and rabies virus, and viral respiratory pathogens. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are commonly donor-transmitted but mainly affect outcomes after the initiation of posttransplant immunosuppression and thus are not addressed in this review. Criteria have been established by the CDC which, when present, may increase the risk of donor transmission of HIV, HBV, and HCV (Table 4)[28]. In the past, many centers have often rejected allografts from such high risk donors. However, availability of improved NAAT testing and closer surveillance monitoring of transplant recipients from CDC-defined high risk donors have allowed these transplants to be undertaken. Aiming to match the allograft to the most appropriate recipient to mitigate the overall risk by improved selection and monitoring has been an overall successful strategy. In such scenarios, additional consent and recipient screening at regular intervals during the first year after transplant should be performed[83].

Viral hepatitis is commonly encountered in both donors and recipients of SOT. HBV infects approximately 400 million people worldwide, with prevalence varying by geographic region[84,85]. As mentioned previously, the ever-enlarging pool of patients awaiting lifesaving transplants has necessitated relaxation of exclusion criteria used to

Table 4 Factors associated with increased risk for human immunodeficiency virus, hepatitis B virus, hepatitis C virus infection and potential donor transmission
People who have had sex with person known or suspected to have HIV, HBV, or HCV in the preceding 12 mo
MSM in the preceding 12 mo
Women who have had sex with a man with a history of MSM in the preceding 12 mo
People who have had sex in exchange for money or drugs in the preceding 12 mo
People who have had sex with a person who has had sex in exchange for money or drugs in the preceding 12 mo
People who have had sex with a person who has injected drugs for nonmedical reasons in the preceding 12 mo
A child who is ≤ 18 mo of age and born to a mother known to be infected with, or at risk for HIV, HBV or HCV infection
A child who has been breastfed within the preceding 12 mo and the mother is known to be infected with, or at risk for HIV, HBV or HCV infection
People who have injected drugs for nonmedical reasons in the preceding 12 mo
People who have been in lockup, jail, prison or a juvenile correctional facility for ≥ 72 consecutive hours in the preceding 12 h
People who have been newly diagnosed with, or have been treated for, syphilis, gonorrhea, *Chlamydia* or genital ulcers in the preceding 12 mo
People who have been on hemodialysis in the preceding 12 mo (HCV only)

HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MSM: Men who have sex with men.

select suitable organ donors. This has led to the usage of allografts taken from donors who have previously had HBV infection (anti-HB core antibody positive donors). The development of de novo hepatitis B infection in recipients of allografts from anti-HBc positive donors has been noted since 1992, but after initially excluding these donors, it has been found that allografts from these donors can be safely used[86-89]. Careful selection of the donor is essential when considering recipients coinfected with HBV and HDV as recurrence of disease is common in this setting and specific posttransplant treatments may need to be implemented to optimize outcomes[90]. HCV is a cause of chronic hepatitis in 3-4 million people in the United States and is the leading indication for liver transplantation[91]. As both HBV and HCV can be transmitted *via* organ donation, a thorough approach is needed for successful management of the recipient, and an emphasis on aggressive immunization and risk mitigation of transplant candidates prior to transplant should be pursued.

Decisions regarding donor suitability depend on whether living-related partial liver donation is planned and disease status of the donor and recipient at the time of allograft procurement. More stringent, evidence-based guidelines regarding the use of anti-HBc antibody donors are forthcoming, but currently it is felt that allografts from HBV infected donors should preferentially be given to recipients who are hepatitis B surface antigen positive, core antibody positive, or surface antibody positive[92]. In both hepatic and non-hepatic donors, an allograft from a donor with acute hepatitis B infection should not be accepted, regardless of the serologic status of the recipient. Hepatitis B surface antigen positive donors can donate to HB surface antigen positive recipients, but hepatitis B immunoglobulin (HBIG) and antiviral therapy should be given with advanced planning. Donors who are anti-HBc antibody positive and HBsAg negative are acceptable, but additional consent should be obtained from the recipient prior to transplant[65,93-95]. Antiviral treatment should be given at the time of transplant to recipients of liver allografts from donors with prior evidence of HBV infection. HBIG should be administered to liver allograft recipients who lack surface antibody to HBV[96-102]. Non-

immune non-hepatic allograft recipients should also receive antiviral prophylaxis if the donor is anti-HBc positive and HBV DNA is detected. HCV infected donors should be precluded from donating an allograft to a HCV naïve recipient[103]. HCV infected hepatic and non-hepatic allografts can be donated to HCV infected recipients with the caveat that donors with HCV genotype 1 infection should preferentially be used for recipients with HCV genotype 1 infection if that donor information is known prior to donation[92,104-108].

Influenza and other respiratory viruses are another potential cause of DTI. Influenza, respiratory syncytial virus (RSV), parainfluenza virus, human metapneumovirus (hMPV), adenovirus, and coronavirus are usually self-limited illnesses in healthy adults but have the potential for significant morbidity and mortality in the SOT population. These viruses cause a wide range of disease, and transplant recipients often have atypical presentations and more severe symptoms[109]. The burden of illness of these viruses follows a seasonal pattern, mainly occurring during the fall and winter months[110]. DTI with these respiratory viruses can increase the risk of secondary bacterial or fungal pneumonia in the recipient, lead to a prolonged period of viral shedding, and potentially contribute to increased risk of allograft rejection in lung transplant recipients[109,111-114]. DTI of respiratory viruses is further complicated by limited treatment options. Influenza and adenovirus have both been reported as DTI with devastating consequences[115-117]. As such, high index of suspicion is needed when evaluating a donor, especially during the peak seasons of respiratory viral infections within the community. During peak seasonal epidemic activity or in the setting of an ongoing pandemic, donors and recipients should be screened for clinical symptoms of an influenza-like illness. Lung and intestinal potential donors who have been diagnosed with influenza within the previous two weeks should be disqualified from donation. Other types of allografts can be accepted if additional consent is obtained, the donor has received anti-influenza treatment, and the recipient is given neuraminidase inhibitor chemoprophylaxis after transplant. Donors of any allograft with influenza diagnosed greater

than 2 wk prior to donation, who are adequately treated and no longer symptomatic can be utilized. Oseltamivir resistant influenza diagnosed in any donor should preclude his/her use as a donor[118]. Lung allografts from donors infected with other respiratory viruses should be rejected with the exception of resolved RSV infection with no residual symptoms. Non-lung allografts infected with respiratory viruses other than influenza can be accepted. If lower respiratory tract sampling shows viral respiratory infection other than influenza or radiograph show an infiltrate and that lung allograft is accepted for use in a dire situation, oral ribavirin can be considered as chemoprophylaxis for the recipient[109]. All allografts from donors with adenovirus infection should be rejected as adenovirus infections in the recipient tend to recur in the transplanted organ[117,119].

Additional viral infections that are potentially donor transmitted include HTLV-1/2 and the etiologic agents of viral encephalitis. Although no longer required as a screening test in deceased donors, concerns remain regarding donor-transmission of HTLV-1/2[120,121]. Rapid progression from infection to disease has been noted in transplant recipients, with the development of myelopathic spastic paraparesis and adult T-cell leukemia/lymphoma[122]. Donors who test positive for these viruses should be precluded from allograft donation unless required for an emergent life-threatening situation. If allograft is accepted, additional consent should be obtained, and the recipient should have virus-specific serology and polymerase chain reaction (PCR) testing at the time of transplant and 1, 3, and 12 mo[123]. Allografts from patients with suspected viral encephalitis should not be accepted given the risk of transmission of WNV, rabies, LCM and herpes simplex virus infections[124-126]. This recommendation may also extend to cerebrospinal fluid pleocytosis where bacterial meningitis has not been proven by either culture or antigen testing indicating a specific bacterial pathogen as the cause of infection.

FUNGAL INFECTIONS

Fungal infections often affect the critically ill potential organ donor and, as such, have the potential to be donor-transmitted. Recipient DTIs with *Candida* species, cryptococcosis, endemic fungal infections, aspergillosis, and non-*Aspergillus* mold infections have all been documented and, when they occur, are important causes of recipient morbidity and mortality[127].

Outcomes of fungal DTI depend on the type of fungal infection identified, the specific allograft donated, and antifungal susceptibilities of recovered isolates. Infections associated with *Candida* species may occur in the setting of positive preservation fluid cultures, possibly due to contamination at the time of organ procurement[61,63,128]. Bowel perforation in the donor is another common source of *Candida* contamination of the allograft[61]. In general, patients with untreated invasive fungal infections should not be used as organ donors. *Aspergillus* and other invasive mold infections result in significant morbidity and mortality from graft site abscesses and anastomotic

infections, despite treatment of both donor and recipient[127]. Renal allografts from donors with candiduria and lung allografts from donors with bronchial cultures positive for *Candida* species can be used with appropriate treatment. Recipients of lung allograft from a donor with documented *Candida* colonization of the airways have been shown to benefit from universal prophylaxis with an echinocandin for the prevention of early posttransplant infections; including empyema[127,129]. Treatment of renal allograft recipients from donors with candiduria should consist of a tailored antifungal agent for urinary tract involvement. Urinary levels of fluconazole exceed minimum inhibitory concentration values for most *Candida* species and can be used in most cases. Therapy should be continued for up to 6 wk depending on whether there is vascular involvement of the urinary tract[62,127,130]. After lung transplant, treatment should be continued until bronchoscopic evaluation confirms the integrity of the bronchial anastomosis[127].

Cryptococcosis can occur in up to 5% of SOT recipients[131]. Most infections after transplant represent reactivation of recipient latent infection, but DTIs do occur in a subset of patients[132,133]. The potential for cryptococcal DTI should be considered when a donor presents with undiagnosed neurological illness, unrecognized meningoencephalitis, or pulmonary nodules in the setting of risk factors for cryptococcosis, such as prior hematologic malignancy, steroid treatment, sarcoidosis, or other cell-mediated immune dysfunction[134]. Cerebrospinal fluid cryptococcal antigen and serum cryptococcal antigen should be obtained from donors who meet these clinical risk factors. Donors with active cryptococcal disease should be excluded from donation. Recovery of *Cryptococcus* in the recipient should not be treated as contamination or colonization, but should prompt initiation of therapeutic antifungal treatment[135].

Endemic fungal infection should be considered as a potential DTI when donors reside in endemic areas or travel frequently to areas with high incidence of histoplasmosis, blastomycosis, or coccidioidomycosis. These areas include the Ohio and Mississippi river valleys, the Great Lakes region, and Southwestern United States, respectively. Since histoplasmosis occurs in only 0.5% of SOT recipients residing in endemic regions, routine laboratory screening of all donors is not warranted[136]. Donors should be evaluated for a prior history or signs and symptoms compatible with active histoplasmosis. If current concerns or prior history exist, an assessment consisting of agar gel immunodiffusion, complement fixation antibody titers, and urine *Histoplasma* antigen should be undertaken. The presence of antigenuria, H precipitin bands, or complement fixation antibody titers ≥ 1:32 should lead to rejection of the donor allograft. Coccidioidomycosis is a dimorphic fungus that is endemic in the Southwestern United States, Mexico, Central and South America. Approximately 150000 infections occur annually in the US, with an estimated 1.4%-6.9% of transplant recipients becoming infected[137]. Reactivation of latent infection is the most common mode of

posttransplant infection, but multiple cases of DTI have been documented in patients from both endemic and non-endemic areas[138,139]. Patients with active coccidioidomycosis should not be permitted to donate an organ for transplantation. In donors with prior history of coccidioidomycosis, an evaluation should be undertaken to document clearance of infection; including history documenting the resolution of symptoms, resolution of radiographic abnormalities, and at least a 4-fold decrease in antibody titer[140]. Fluconazole or itraconazole can be used for the prevention of DTI in the event that a recipient receives an organ from a donor who in retrospect had evidence of remote infection[141]. Lifelong prophylaxis is indicated following treatment doses for at least one year. Fluconazole at an average daily dose of 200-400 mg can be used depending on whether prophylaxis is primary or secondary[137].

PARASITIC INFECTIONS

With increase in international travel and immigration, potential organ donors have greater risk for parasitic infections not endemic to the United States. Transmission of Chagas disease, schistosomiasis, and *Strongyloides* has been reported[142-144].

The optimal screening procedure for schistosomiasis in donors from endemic areas has not yet been established. Screening of living donors from endemic areas with fecal parasitological analysis paired with blood *Schistosoma* antibody detection assay is a reasonable starting point. This can be followed with a stepwise approach including rectal biopsy, liver biopsy, or both depending on the results of the initial screening tests. If stool analysis shows *Schistosoma* eggs, liver biopsy should be performed regardless of the result of *Schistosoma* serology. In the situation where *Schistosoma* eggs are not detected in the stools but the donor is noted to be seropositive for *Schistosoma*, further investigation with a rectal biopsy is indicated. If rectal biopsy demonstrates *Schistosoma* eggs, all allografts from this donor should be rejected. If eggs are found on initial screening, living donor treatment with praziquantel should be initiated followed by repeat testing of stools for *Schistosoma* eggs. Only if repeat stool testing is negative, should the patient be accepted to donate[145].

Screening of both donors and recipients for strongyloidiasis in the pretransplant period is recommended for those at epidemiologic risk and should include both serology and stool studies[146]. A donor with documented strongyloidiasis should not be precluded from donation, but additional consent from the recipient should be obtained. Recipients of organs from such donor should be prophylactically treated with ivermectin.

Chagas disease is an infection caused by the parasite *Trypanosoma cruzi*. It is endemic to Mexico, Central, and South America but has the potential to cause DTI in the setting of transplantation from a donor from an endemic region to a recipient in a non-endemic country[147]. Most posttransplant infections occurring in recipients from endemic regions occur due to reactivation of latent infec-

tion as a result of iatrogenic immunosuppression. Transmission rates from seropositive donors to seronegative recipients are approximately 20% for kidney transplant recipients and 30% for liver transplant recipients. Screening for Chagas disease should be performed on donors who were born or spent significant time living in an endemic country[148]. Donors who have a history of treated Chagas disease should also be screened using the Ortho enzyme immunoassay test (Ortho-Clinical Diagnostics, Inc.; Raritan, New Jersey) and the Abbot Prism Chagas test (Abbott Laboratories; Abbott Park, Illinois). If the initial screening of a living donor is positive, a second confirmatory test should be sent to the CDC; using the radioimmune precipitation assay. Deceased donor testing should also be performed but this information may not be available at the time of transplantation[149]. No allograft should be accepted from a donor who died from acute Chagas disease. When a donor has positive serology for Chagas disease or has a history of treated Chagas disease, organs other than the heart or intestine may be suitable for transplantation with additional consent and posttransplant screening of the recipient. Testing should include *T. cruzi* PCR and microscopy of blood peripheral smears at predetermined time intervals, or in the event of fever, and when rejection is present. Treatment is only indicated if surveillance testing of the recipient is consistent with *T. cruzi* infection. Heart or intestinal transplantation from a donor with a positive history or serology for *T. cruzi* is thought to represent too high of a potential risk for DTI to be acceptable[146,150-152].

CONCLUSION

The demand for allografts for the treatment of end-stage disease processes continues to grow. The need for a thoughtful and thorough approach to donor selection has never been more important in balancing unnecessarily discarding potentially lifesaving organs with reducing infectious complications for the recipient after transplant. Decisions regarding donor acceptability should be made in conjunction with a clinician who has special training and experience in dealing with infections related to transplantation. Donor history and physical examination should be meticulous with an emphasis on documenting current or latent infections that can be transmitted to the recipient. Screening using molecular and microbiological testing should be attempted, as time permits, prior to organ procurement in order to allow for rejection of an unacceptable allograft, or to allow for monitoring and treatment in the recipient. As the need for organs continues to rise, special attention will be focused on ways to expand the donor pool.

Multiple HIV-infected patients die each year awaiting organs that could be provided from living or deceased HIV-infected donors. Approximately 500 HIV positive deceased donors are not currently being utilized to donate organs to HIV-positive recipients[153]. Improvements in antiretroviral therapy and report of successful kidney transplantation from a donor with HIV infection in

South Africa make this an interesting, albeit complicated, area for future evaluation and research. A key advancement has recently occurred with the passage of the HIV Organ Policy Equity Act (HOPE Act) on 11/21/2013.

Improved development of NAAT in conjunction with defined and validated algorithms of application may allow for faster and more accurate testing of donor specimens enabling previously excluded donors to be accepted for donation. Focused efforts to reassess the risk of using high-risk donors should be undertaken, and methods for decreasing recipient risk of DTI are imperative. Finally, it is important to continue to build on the substantial contributions to quality and safety made by the DTAC in recent years. Providers should be strongly encouraged to report any possible donor-transmitted event in real time. Critical infrastructure is now in place to investigate potential DTI, to take appropriate action in the treatment of potential recipients at risk, and to analyze indispensable data in the pursuit of evidence-based decision making essential to improving outcomes in this unique patient population.

REFERENCES

1. **Fischer SA**, Lu K. Screening of donor and recipient in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 9-21 [PMID: 23464994 DOI: 10.1111/ajt.12094]
2. **Nadig SN**, Bratton CF, Karp SJ. Marginal donors in liver transplantation: expanding the donor pool. *J Surg Educ* 2007; **64**: 46-50 [PMID: 17320806 DOI: 10.1016/j.cursur.2006.08.001]
3. **Matas AJ**, Smith JM, Skeans MA, Lamb KE, Gustafson SK, Samana CJ, Stewart DE, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: kidney. *Am J Transplant* 2013; **13** Suppl 1: 11-46 [PMID: 23237695 DOI: 10.1111/ajt.12019]
4. **Kandaswamy R**, Stock PG, Skeans MA, Gustafson SK, Sleeman EF, Wainright JL, Carrico RJ, Ghimire V, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: pancreas. *Am J Transplant* 2013; **13** Suppl 1: 47-72 [PMID: 23237696 DOI: 10.1111/ajt.12020]
5. **Kim WR**, Stock PG, Smith JM, Heimbach JK, Skeans MA, Edwards EB, Harper AM, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: liver. *Am J Transplant* 2013; **13** Suppl 1: 73-102 [PMID: 23237697 DOI: 10.1111/ajt.12021]
6. **Smith JM**, Skeans MA, Thompson B, Horslen SP, Edwards EB, Harper AM, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: intestine. *Am J Transplant* 2013; **13** Suppl 1: 103-118 [PMID: 23237698 DOI: 10.1111/ajt.12022]
7. **Colvin-Adams M**, Smith JM, Heubner BM, Skeans MA, Edwards LB, Waller C, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: heart. *Am J Transplant* 2013; **13** Suppl 1: 119-148 [PMID: 23237699 DOI: 10.1111/ajt.12023]
8. **Valapour M**, Paulson K, Smith JM, Hertz MI, Skeans MA, Heubner BM, Edwards LB, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: lung. *Am J Transplant* 2013; **13** Suppl 1: 149-177 [PMID: 23237700 DOI: 10.1111/ajt.12024]
9. **Srinivasan A**, Burton EC, Kuehnert MJ, Rupprecht C, Sutker WL, Ksiazek TG, Paddock CD, Guarner J, Shieh WJ, Goldsmith C, Hanlon CA, Zoretic J, Fischbach B, Niezgoda M, El-Feky WH, Orciari L, Sanchez EQ, Likos A, Klintmalm GB, Cardo D, LeDuc J, Chamberland ME, Jernigan DB, Zaki SR. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med* 2005; **352**: 1103-1111 [PMID: 15784663 DOI: 10.1056/NEJMoa043018]
10. **Fischer SA**, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL, Shieh WJ, Erickson BR, Bandy U, DeMaria A, Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand RF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N Engl J Med* 2006; **354**: 2235-2249 [PMID: 16723615 DOI: 10.1056/NEJMoa053240]
11. **Iwamoto M**, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, Pham SM, Zaki S, Lanciotti RS, Lance-Parker SE, DiazGranados CA, Winquist AG, Perlino CA, Wiersma S, Hillyer KL, Goodman JL, Marfin AA, Chamberland ME, Petersen LR. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003; **348**: 2196-2203 [PMID: 12773646 DOI: 10.1056/NEJMoa022987]
12. **Ison MG**, Llata E, Conover CS, Friedewald JJ, Gerber SI, Grigoryan A, Heneine W, Millis JM, Simon DM, Teo CG, Kuehnert MJ. Transmission of human immunodeficiency virus and hepatitis C virus from an organ donor to four transplant recipients. *Am J Transplant* 2011; **11**: 1218-1225 [PMID: 21645254 DOI: 10.1111/j.1600-6143.2011.03597.x]
13. **Ahn J**, Cohen SM. Transmission of human immunodeficiency virus and hepatitis C virus through liver transplantation. *Liver Transpl* 2008; **14**: 1603-1608 [PMID: 18975294 DOI: 10.1002/lt.21534]
14. **Ison MG**, Nalesnik MA. An update on donor-derived disease transmission in organ transplantation. *Am J Transplant* 2011; **11**: 1123-1130 [PMID: 21443676 DOI: 10.1111/j.1600-6143.2011.03493.x]
15. **Ison MG**, Hager J, Blumberg E, Burdick J, Carney K, Cutler J, Dimaio JM, Hasz R, Kuehnert MJ, Ortiz-Rios E, Teperman L, Nalesnik M. Donor-derived disease transmission events in the United States: data reviewed by the OPTN/UNOS Disease Transmission Advisory Committee. *Am J Transplant* 2009; **9**: 1929-1935 [PMID: 19538493 DOI: 10.1111/j.1600-6143.2009.02700.x]
16. **Nalesnik MA**, Woodle ES, Dimaio JM, Vasudev B, Teperman LW, Covington S, Taranto S, Gockerman JP, Shapiro R, Sharma V, Swinnen LJ, Yoshida A, Ison MG. Donor-transmitted malignancies in organ transplantation: assessment of clinical risk. *Am J Transplant* 2011; **11**: 1140-1147 [PMID: 21645251 DOI: 10.1111/j.1600-6143.2011.03565.x]
17. **Fishman JA**, Strong DM, Kuehnert MJ. Organ and tissue safety workshop 2007: advances and challenges. *Cell Tissue Bank* 2009; **10**: 271-280 [PMID: 19016348 DOI: 10.1007/s10561-008-9114-z]
18. **Humar A**, Morris M, Blumberg E, Freeman R, Preiksaitis J, Kiberd B, Schweitzer E, Ganz S, Caliendo A, Orlowski JP, Wilson B, Kotton C, Michaels M, Kleinman S, Geier S, Murphy B, Green M, Levi M, Knoll G, Segev D, Brubaker S, Hasz R, Lebovitz DJ, Mulligan D, O'Connor K, Pruett T, Mozes M, Lee I, Delmonico F, Fischer S. Nucleic acid testing (NAT) of organ donors: is the 'best' test the right test? A consensus conference report. *Am J Transplant* 2010; **10**: 889-899 [PMID: 20121734 DOI: 10.1111/j.1600-6143.2009.02992.x]
19. **Shafer TJ**, Schkade D, Schkade L, Geier SS, Orlowski JP, Klintmalm G. Zero risk tolerance costs lives: loss of transplantable organs due to human immunodeficiency virus nucleic acid testing of potential donors. *Prog Transplant* 2011; **21**: 236-247; quiz 248 [PMID: 21977885]
20. **Avery RK**, Ljungman P. Prophylactic measures in the solid-organ recipient before transplantation. *Clin Infect Dis* 2001; **33** Suppl 1: S15-S21 [PMID: 11389517 DOI: 10.1086/320899]
21. **Fishman JA**, Issa NC. Infection in organ transplantation: risk factors and evolving patterns of infection. *Infect Dis Clin*

North Am 2010; **24**: 273-283 [PMID: 20466270 DOI: 10.1016/j.idc.2010.01.005]

22   **Grossi PA**, Costa AN, Fehily D, Blumberg EA, Kuehnert MJ, Fishman JA, Ison MG, Lattes R, Kotton CN, Lilleri D, Kabanova A, Lanzavecchia A, Gerna G, Razonable RR, Comoli P, Zecca M, Basso S, Ginevri F, Grossi A, Schena FP, Rimola A, Burra P, De Martin E, Rodriguez-Castro KI, Fagiuoli S, Pasulo L, Bruno R, Andreone P, Loggi E, Arena F, Maria Rossolini G, Sganga G, Cozza V. Infections and organ transplantation: new challenges for prevention and treatment--a colloquium. *Transplantation* 2012; **93**: S4-S39 [PMID: 22374265 DOI: 10.1097/TP.0b013e3182481347]

23   **Len O**, Gavaldà J, Blanes M, Montejo M, San Juan R, Moreno A, Carratalà J, de la Torre-Cisneros J, Bou G, Cordero E, Muñoz P, Cuervas-Mons V, Alvarez MT, Borrell N, Fortun J, Pahissa A. Donor infection and transmission to the recipient of a solid allograft. *Am J Transplant* 2008; **8**: 2420-2425 [PMID: 18925908 DOI: 10.1111/j.1600-6143.2008.02397.x]

24   **Weber TR**, Freier DT, Turcotte JG. Transplantation of infected kidneys: clinical and experimental results. *Transplantation* 1979; **27**: 63-65 [PMID: 375497 DOI: 10.1097/00007890-197901000-00016]

25   **Berggren H**, Berglin E, Kjellman U, Mantovani V, Nilsson B. Successful outcome after massive bleeding in a heart transplant recipient with mycotic aortitis. Case report. *Scand J Thorac Cardiovasc Surg* 1994; **28**: 45-47 [PMID: 7939507 DOI: 10.3109/14017439409098710]

26   **Nery JR**, Weppler D, Ketchum P, Olson L, Fragulidis GP, Khan MF, Webb MG, Miller J, Tzakis AG. Donor infection and primary nonfunction in liver transplantation. *Transplant Proc* 1997; **29**: 481-483 [DOI: 10.1016/S0041-1345(96)00214-X]

27   **Lumbreras C**, Sanz F, González A, Pérez G, Ramos MJ, Aguado JM, Lizasoain M, Andrés A, Moreno E, Gómez MA, Noriega AR. Clinical significance of donor-unrecognized bacteremia in the outcome of solid-organ transplant recipients. *Clin Infect Dis* 2001; **33**: 722-726 [PMID: 11477528 DOI: 10.1086/322599]

28   **Ison MG**, Grossi P. Donor-derived infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 22-30 [PMID: 23464995 DOI: 10.1111/ajt.12095]

29   **Singh N**. Impact of donor bacteremia on outcome in organ transplant recipients. *Liver Transpl* 2002; **8**: 975-976 [PMID: 12360445 DOI: 10.1053/jlts.2002.0080975]

30   **Freeman RB**, Giatras I, Falagas ME, Supran S, O'Connor K, Bradley J, Snydman DR, Delmonico FL. Outcome of transplantation of organs procured from bacteremic donors. *Transplantation* 1999; **68**: 1107-1111 [PMID: 10551637 DOI: 10.1097/00007890-199910270-00008]

31   **Kubak BM**, Gregson AL, Pegues DA, Leibowitz MR, Carlson M, Marelli D, Patel J, Laks H, Kobashigawa JA. Use of hearts transplanted from donors with severe sepsis and infectious deaths. *J Heart Lung Transplant* 2009; **28**: 260-265 [PMID: 19285618 DOI: 10.1016/j.healun.2008.11.911]

32   **Caballero F**, Lopez-Navidad A, Perea M, Cabrer C, Guirado L, Solà R. Successful liver and kidney transplantation from cadaveric donors with left-sided bacterial endocarditis. *Am J Transplant* 2005; **5**: 781-787 [PMID: 15760402 DOI: 10.1111/j.1600-6143.2005.00773.x]

33   **Sifri CD**, Ison MG. Highly resistant bacteria and donor-derived infections: treading in uncharted territory. *Transpl Infect Dis* 2012; **14**: 223-228 [PMID: 22676635 DOI: 10.1111/j.1399-3062.2012.00752.x]

34   **Wertheim HF**, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis* 2005; **5**: 751-762 [DOI: 10.1016/S1473-3099(05)70295-4]

35   **Asensio A**, Guerrero A, Quereda C, Lizán M, Martinez-Ferrer M. Colonization and infection with methicillin-resistant Staphylococcus aureus: associated factors and eradication. *Infect Control Hosp Epidemiol* 1996; **17**: 20-28 [PMID: 8789683

DOI: 10.1086/647184]

36   **Garzoni C**, Vergidis P. Methicillin-resistant, vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 50-58 [PMID: 23464998 DOI: 10.1111/ajt.12098]

37   **Patel G**, Snydman DR. Vancomycin-resistant Enterococcus infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 59-67 [PMID: 23464999 DOI: 10.1111/ajt.12099]

38   **van Duin D**, van Delden C. Multidrug-resistant gram-negative bacteria infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 31-41 [PMID: 23464996 DOI: 10.1111/ajt.12096]

39   **Ariza-Heredia EJ**, Patel R, Blumberg EA, Walker RC, Lewis R, Evans J, Sankar A, Willliams MD, Rogers J, Milano C, Razonable RR. Outcomes of transplantation using organs from a donor infected with Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae. *Transpl Infect Dis* 2012; **14**: 229-236 [PMID: 22624726 DOI: 10.1111/j.1399-3062.2012.00742.x]

40   **Watkins AC**, Vedula GV, Horan J, Dellicarpini K, Pak SW, Daly T, Samstein B, Kato T, Emond JC, Guarrera JV. The deceased organ donor with an "open abdomen": proceed with caution. *Transpl Infect Dis* 2012; **14**: 311-315 [PMID: 22283979 DOI: 10.1111/j.1399-3062.2011.00712.x]

41   **Goldberg E**, Bishara J, Lev S, Singer P, Cohen J. Organ transplantation from a donor colonized with a multidrug-resistant organism: a case report. *Transpl Infect Dis* 2012; **14**: 296-299 [PMID: 22176504 DOI: 10.1111/j.1399-3062.2011.00697.x]

42   **Simkins J**, Muggia V. Favorable outcome in a renal transplant recipient with donor-derived infection due to multidrug-resistant Pseudomonas aeruginosa. *Transpl Infect Dis* 2012; **14**: 292-295 [PMID: 22093290 DOI: 10.1111/j.1399-3062.2011.00674.x]

43   **Martins N**, Martins IS, de Freitas WV, de Matos JA, Magalhães AC, Girão VB, Dias RC, de Souza TC, Pellegrino FL, Costa LD, Boasquevisque CH, Nouér SA, Riley LW, Santoro-Lopes G, Moreira BM. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant Acinetobacter baumannii. *Transpl Infect Dis* 2012; **14**: 316-320 [PMID: 22168176 DOI: 10.1111/j.1399-3062.2011.00701.x]

44   **Bergamasco MD**, Barroso Barbosa M, de Oliveira Garcia D, Cipullo R, Moreira JC, Baia C, Barbosa V, Abboud CS. Infection with Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae in solid organ transplantation. *Transpl Infect Dis* 2012; **14**: 198-205 [PMID: 22093103 DOI: 10.1111/j.1399-3062.2011.00688.x]

45   **Kalpoe JS**, Sonnenberg E, Factor SH, del Rio Martin J, Schiano T, Patel G, Huprikar S. Mortality associated with carbapenem-resistant Klebsiella pneumoniae infections in liver transplant recipients. *Liver Transpl* 2012; **18**: 468-474 [PMID: 22467548 DOI: 10.1002/lt.23374]

46   **Cortes NJ**, Afzali B, MacLean D, Goldsmith DJ, O'Sullivan H, Bingham J, Lewis DA, MacMahon E, Tong CY, Koffman G. Transmission of syphilis by solid organ transplantation. *Am J Transplant* 2006; **6**: 2497-2499 [PMID: 16827785 DOI: 10.1111/j.1600-6143.2006.01461.x]

47   **Marek A**, Inkster T. A syphilis-positive organ donor -- management of the cardiac transplant recipient: a case report and review of the literature. *Sex Transm Dis* 2012; **39**: 485-486 [PMID: 22592837 DOI: 10.1097/OLQ.0b013e318249db35]

48   **Tariciotti L**, Das I, Dori L, Perera MT, Bramhall SR. Asymptomatic transmission of Treponema pallidum (syphilis) through deceased donor liver transplantation. *Transpl Infect Dis* 2012; **14**: 321-325 [PMID: 22624823 DOI: 10.1111/j.1399-3062.2012.00745.x]

49   **Gibel LJ**, Sterling W, Hoy W, Harford A. Is serological evidence of infection with syphilis a contraindication to kidney donation? Case report and review of the literature. *J Urol*

1987; **138**: 1226-1227 [PMID: 3312642]

50   **Caballero F**, Puig M, Santos JA, Deulofeu R, Ballarín J, Charco R, Leal J. Successful transplantation of organs from a donor with postneurosurgical meningitis caused by Escherichia coli. *Transplantation* 2012; **93**: e11-e13 [PMID: 22277959 DOI: 10.1097/TP.0b013e31823f9093]

51   **Cantarovich M**, Tchervenkov J, Loertscher R. Transplantation of kidneys from a donor with Neisseria meningitidis infection. *Am J Nephrol* 1993; **13**: 171-172 [PMID: 8342586 DOI: 10.1159/000168611]

52   **López-Navidad A**, Domingo P, Caballero F, González C, Santiago C. Successful transplantation of organs retrieved from donors with bacterial meningitis. *Transplantation* 1997; **64**: 365-368 [PMID: 9256203 DOI: 10.1097/00007890-199707270-00033]

53   **Issa NC**, Patel R. Potential for expansion of the donor pool using liver allografts from donors with bacterial meningitis. *Liver Transpl* 2002; **8**: 977-979 [PMID: 12360446 DOI: 10.1053/jlts.2002.0080977]

54   **Bahrami T**, Vohra HA, Shaikhrezai K, Tadjkarimi S, Banner N, Amrani M, Yacoub M, Khaghani A. Intrathoracic organ transplantation from donors with meningitis: a single-center 20-year experience. *Ann Thorac Surg* 2008; **86**: 1554-1556 [PMID: 19049748 DOI: 10.1016/j.athoracsur.2008.07.047]

55   **Yansouni CP**, Dendukuri N, Liu G, Fernandez M, Frenette C, Paraskevas S, Sheppard DC. Positive cultures of organ preservation fluid predict postoperative infections in solid organ transplantation recipients. *Infect Control Hosp Epidemiol* 2012; **33**: 672-680 [PMID: 22669228 DOI: 10.1086/666344]

56   **Grąt M**, Ligocka J, Lewandowski Z, Barski K, Hołówko W, Skalski M, Kornasiewicz O, Usarek P, Zieniewicz K, Młynarczyk G, Krawczyk M. Incidence, pattern and clinical relevance of microbial contamination of preservation fluid in liver transplantation. *Ann Transplant* 2012; **17**: 20-28 [PMID: 23018252 DOI: 10.12659/AOT.883454]

57   **Sauget M**, Verdy S, Slekovec C, Bertrand X, Talon D. Bacterial contamination of organ graft preservation solution and infection after transplantation. *Transpl Infect Dis* 2011; **13**: 331-334 [PMID: 21281417 DOI: 10.1111/j.1399-3062.2010.00597.x]

58   **Ruiz P**, Gastaca M, Gonzalez J, Hernandez MJ, Ventoso A, Valdivieso A, Montejo M, Ortiz de Urbina J. Incidence and clinical relevance of bacterial contamination in preservation solution for liver transplantation. *Transplant Proc* 2009; **41**: 2169-2171 [PMID: 19715863 DOI: 10.1016/j.transproceed.2009.06.036]

59   **Janny S**, Bert F, Dondero F, Durand F, Guerrini P, Merckx P, Nicolas-Chanoine MH, Belghiti J, Mantz J, Paugam-Burtz C. Microbiological findings of culture-positive preservation fluid in liver transplantation. *Transpl Infect Dis* 2011; **13**: 9-14 [PMID: 20738832 DOI: 10.1111/j.1399-3062.2010.00558.x]

60   **Mossad SB**, Avery RK, Goormastic M, Hobbs RE, Stewart RW. Significance of positive cultures from donor left atrium and postpreservation fluid in heart transplantation. *Transplantation* 1997; **64**: 1209-1210 [PMID: 9355844 DOI: 10.1097/00007890-199710270-00024]

61   **Albano L**, Bretagne S, Mamzer-Bruneel MF, Kacso I, Desnos-Ollivier M, Guerrini P, Le Luong T, Cassuto E, Dromer F, Lortholary O. Evidence that graft-site candidiasis after kidney transplantation is acquired during organ recovery: a multicenter study in France. *Clin Infect Dis* 2009; **48**: 194-202 [PMID: 19090753 DOI: 10.1086/595688]

62   **Mai H**, Champion L, Ouali N, Hertig A, Peraldi MN, Glotz D, Rondeau E, Costa MA, Snanoudj R, Benoit G, Charpentier B, Durrbach A. Candida albicans arteritis transmitted by conservative liquid after renal transplantation: a report of four cases and review of the literature. *Transplantation* 2006; **82**: 1163-1167 [PMID: 17102767 DOI: 10.1097/01.tp.0000239188.27153.23]

63   **Matignon M**, Botterel F, Audard V, Dunogue B, Dahan K, Lang P, Bretagne S, Grimbert P. Outcome of renal transplantation in eight patients with Candida sp. contamination of

preservation fluid. *Am J Transplant* 2008; **8**: 697-700 [PMID: 18294166 DOI: 10.1111/j.1600-6143.2007.02112.x]

64   **Veroux M**, Corona D, Scriffignano V, Caglià P, Gagliano M, Giuffrida G, Gona F, Sciacca A, Giaquinta A, Oliveri S, Sinagra N, Tallarita T, Zerbo D, Sorbello M, Parrinello L, Veroux P. Contamination of preservation fluid in kidney transplantation: single-center analysis. *Transplant Proc* 2010; **42**: 1043-1045 [PMID: 20534219 DOI: 10.1016/j.transproceed.2010.03.041]

65   **Muñoz SJ**. Use of hepatitis B core antibody-positive donors for liver transplantation. *Liver Transpl* 2002; **8**: S82-S87 [PMID: 12362304 DOI: 10.1053/jlts.2002.35783]

66   **Sakhuja V**, Jha V, Varma PP, Joshi K, Chugh KS. The high incidence of tuberculosis among renal transplant recipients in India. *Transplantation* 1996; **61**: 211-215 [PMID: 8600625 DOI: 10.1097/00007890-199601270-00008]

67   **Singh N**, Paterson DL. Mycobacterium tuberculosis infection in solid-organ transplant recipients: impact and implications for management. *Clin Infect Dis* 1998; **27**: 1266-1277 [PMID: 9827281 DOI: 10.1086/514993]

68   **Currie AC**, Knight SR, Morris PJ. Tuberculosis in renal transplant recipients: the evidence for prophylaxis. *Transplantation* 2010; **90**: 695-704 [PMID: 20647975 DOI: 10.1097/TP.0b013e3181ecea8d]

69   **Horne DJ**, Narita M, Spitters CL, Parimi S, Dodson S, Limaye AP. Challenging issues in tuberculosis in solid organ transplantation. *Clin Infect Dis* 2013; **57**: 1473-1482 [PMID: 23899676 DOI: 10.1093/cid/cit488]

70   **Holty JE**, Sista RR. Mycobacterium tuberculosis infection in transplant recipients: early diagnosis and treatment of resistant tuberculosis. *Curr Opin Organ Transplant* 2009; **14**: 613-618 [PMID: 19741533 DOI: 10.1097/MOT.0b013e3283324dfc]

71   **Subramanian AK**, Morris MI. Mycobacterium tuberculosis infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 68-76 [PMID: 23465000 DOI: 10.1111/ajt.12100]

72   **Winthrop KL**, Kubak BM, Pegues DA, Hufana C, Costamagna P, Desmond E, Sanders C, Shen P, Flores-Ibarra L, Osborne E, Bruckner D, Flood J. Transmission of mycobacterium tuberculosis via lung transplantation. *Am J Transplant* 2004; **4**: 1529-1533 [PMID: 15307842 DOI: 10.1111/j.1600-6143.2004.00536.x]

73   **Kumar D**, Budev M, Koval C, Hellinger WC, Gordon SM, Tomford JW. Donor-derived tuberculosis (TB) infection in lung transplant despite following recommended algorithm. *Am J Transplant* 2013; **13**: 2225-2226 [PMID: 23837505 DOI: 10.1111/ajt.12344]

74   **Oeltmann JE**, Kammerer JS, Pevzner ES, Moonan PK. Tuberculosis and substance abuse in the United States, 1997-2006. *Arch Intern Med* 2009; **169**: 189-197 [PMID: 19171816 DOI: 10.1001/archinternmed.2008.535]

75   **Cegielski JP**, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int J Tuberc Lung Dis* 2004; **8**: 286-298 [PMID: 15139466]

76   **Guelar A**, Gatell JM, Verdejo J, Podzamczer D, Lozano L, Aznar E, Miró JM, Mallolas J, Zamora L, González J. A prospective study of the risk of tuberculosis among HIV-infected patients. *AIDS* 1993; **7**: 1345-1349 [PMID: 8267907 DOI: 10.1097/00002030-199310000-00007]

77   **Marks SM**, Taylor Z, Qualls NL, Shrestha-Kuwahara RJ, Wilce MA, Nguyen CH. Outcomes of contact investigations of infectious tuberculosis patients. *Am J Respir Crit Care Med* 2000; **162**: 2033-2038 [PMID: 11112109 DOI: 10.1164/ajrccm.162.6.2004022]

78   **MacIntyre CR**, Kendig N, Kummer L, Birago S, Graham NM. Impact of tuberculosis control measures and crowding on the incidence of tuberculous infection in Maryland prisons. *Clin Infect Dis* 1997; **24**: 1060-1067 [PMID: 9195058 DOI: 10.1086/513632]

79   **Schieffelbein CW**, Snider DE. Tuberculosis control among

homeless populations. *Arch Intern Med* 1988; **148**: 1843-1846 [PMID: 3401108 DOI: 10.1001/archinte.1988.00380080109029]

80 **Cain KP**, Haley CA, Armstrong LR, Garman KN, Wells CD, Iademarco MF, Castro KG, Laserson KF. Tuberculosis among foreign-born persons in the United States: achieving tuberculosis elimination. *Am J Respir Crit Care Med* 2007; **175**: 75-79 [PMID: 17038659 DOI: 10.1164/rccm.200608-1178OC]

81 **Zuber PL**, McKenna MT, Binkin NJ, Onorato IM, Castro KG. Long-term risk of tuberculosis among foreign-born persons in the United States. *JAMA* 1997; **278**: 304-307 [PMID: 9228436 DOI: 10.1001/jama.1997.03550040060038]

82 **Morris MI**, Daly JS, Blumberg E, Kumar D, Sester M, Schluger N, Kim SH, Schwartz BS, Ison MG, Humar A, Singh N, Michaels M, Orlowski JP, Delmonico F, Pruett T, John GT, Kotton CN. Diagnosis and management of tuberculosis in transplant donors: a donor-derived infections consensus conference report. *Am J Transplant* 2012; **12**: 2288-2300 [PMID: 22883346 DOI: 10.1111/j.1600-6143.2012.04205.x]

83 **Seem DL**, Lee I, Umscheid CA, Kuehnert MJ. Excerpt from PHS guideline for reducing HIV, HBV and HCV transmission through organ transplantation. *Am J Transplant* 2013; **13**: 1953-1962 [PMID: 23890284 DOI: 10.1111/ajt.12386]

84 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745 [PMID: 9392700 DOI: 10.1056/NEJM199712113372406]

85 **Ueda Y**, Marusawa H, Egawa H, Okamoto S, Ogura Y, Oike F, Nishijima N, Takada Y, Uemoto S, Chiba T. De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation. *Antivir Ther* 2011; **16**: 479-487 [PMID: 21685535 DOI: 10.3851/IMP1771]

86 **Chazouilleres O**, Mamish D, Kim M, Carey K, Ferrell L, Roberts JP, Ascher NL, Wright TL. "Occult" hepatitis B virus as source of infection in liver transplant recipients. *Lancet* 1994; **343**: 142-146 [DOI: 10.1016/S0140-6736(94)90934-2]

87 **Uemoto S**, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, Kiuchi T, Miyake Y, Tanaka K, Chiba T. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. *Transplantation* 1998; **65**: 494-499 [PMID: 9500622 DOI: 10.1097/00007890-199802270-00007]

88 **Douglas DD**, Rakela J, Wright TL, Krom RA, Wiesner RH. The clinical course of transplantation-associated de novo hepatitis B infection in the liver transplant recipient. *Liver Transpl Surg* 1997; **3**: 105-111 [PMID: 9346723 DOI: 10.1002/lt.500030202]

89 **Fabia R**, Levy MF, Crippin J, Tillery W, Netto GJ, Aguanno J, Dysert P, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. De novo hepatitis B infection after liver transplantation: source of disease, incidence, and impact. *Liver Transpl Surg* 1998; **4**: 119-127 [PMID: 9516563 DOI: 10.1002/lt.500040210]

90 **Franchello A**, Ghisetti V, Marzano A, Romagnoli R, Salizzoni M. Transplantation of hepatitis B surface antigen-positive livers into hepatitis B virus-positive recipients and the role of hepatitis delta coinfection. *Liver Transpl* 2005; **11**: 922-928 [PMID: 16035057 DOI: 10.1002/lt.20471]

91 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948 DOI: 10.1056/NEJM200107053450107]

92 **Levitsky J**, Doucette K. Viral hepatitis in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 147-168 [PMID: 23465008 DOI: 10.1111/ajt.12108]

93 **Terrault N**, Roche B, Samuel D. Management of the hepatitis B virus in the liver transplantation setting: a European and an American perspective. *Liver Transpl* 2005; **11**: 716-732 [PMID: 15973718 DOI: 10.1002/lt.20492]

94 **Chung RT**, Feng S, Delmonico FL. Approach to the management of allograft recipients following the detection of hepatitis B virus in the prospective organ donor. *Am J Transplant* 2001; **1**: 185-191 [PMID: 12099368 DOI: 10.1034/j.1600-6143.2001.10214.x]

95 **Manzarbeitia C**, Reich DJ, Ortiz JA, Rothstein KD, Araya VR, Munoz SJ. Safe use of livers from donors with positive hepatitis B core antibody. *Liver Transpl* 2002; **8**: 556-561 [PMID: 12037788 DOI: 10.1053/jlts.2002.33451]

96 **Prakoso E**, Strasser SI, Koorey DJ, Verran D, McCaughan GW. Long-term lamivudine monotherapy prevents development of hepatitis B virus infection in hepatitis B surface-antigen negative liver transplant recipients from hepatitis B core-antibody-positive donors. *Clin Transplant* 2006; **20**: 369-373 [PMID: 16824156 DOI: 10.1111/j.1399-0012.2006.00495.x]

97 **Suehiro T**, Shimada M, Kishikawa K, Shimura T, Soejima Y, Yoshizumi T, Hashimoto K, Mochida Y, Maehara Y, Kuwano H. Prevention of hepatitis B virus infection from hepatitis B core antibody-positive donor graft using hepatitis B immune globulin and lamivudine in living donor liver transplantation. *Liver Int* 2005; **25**: 1169-1174 [PMID: 16343068 DOI: 10.1111/j.1478-3231.2005.01165.x]

98 **Saab S**, Waterman B, Chi AC, Tong MJ. Comparison of different immunoprophylaxis regimens after liver transplantation with hepatitis B core antibody-positive donors: a systematic review. *Liver Transpl* 2010; **16**: 300-307 [PMID: 20209589]

99 **Loss GE**, Mason AL, Nair S, Blazek J, Farr G, Guo L, Cohen AJ, Eason JD. Does lamivudine prophylaxis eradicate persistent HBV DNA from allografts derived from anti-HBc-positive donors? *Liver Transpl* 2003; **9**: 1258-1264 [PMID: 14625825 DOI: 10.1016/j.lts.2003.09.010]

100 **Loss GE**, Mason AL, Blazek J, Dick D, Lipscomb J, Guo L, Perrillo RP, Eason JD. Transplantation of livers from hbc Ab positive donors into HBc Ab negative recipients: a strategy and preliminary results. *Clin Transplant* 2001; **15** Suppl 6: 55-58 [PMID: 11903388 DOI: 10.1034/j.1399-0012.2001.00010.x]

101 **Jain A**, Orloff M, Abt P, Kashyap R, Mohanka R, Lansing K, Kelley M, Bozorgzadeh A. Use of hepatitis B core antibody-positive liver allograft in hepatitis C virus-positive and -negative recipients with use of short course of hepatitis B immunoglobulin and Lamivudine. *Transplant Proc* 2005; **37**: 3187-3189 [PMID: 16213345 DOI: 10.1016/j.transproceed.2005.07.049]

102 **Vizzini G**, Gruttadauria S, Volpes R, D'Antoni A, Pietrosi G, Filì D, Petridis I, Pagano D, Tuzzolino F, Santonocito MM, Gridelli B. Lamivudine monoprophylaxis for de novo HBV infection in HBsAg-negative recipients with HBcAb-positive liver grafts. *Clin Transplant* 2011; **25**: E77-E81 [PMID: 21039887 DOI: 10.1111/j.1399-0012.2010.01329.x]

103 **Pereira BJ**, Wright TL, Schmid CH, Levey AS. A controlled study of hepatitis C transmission by organ transplantation. The New England Organ Bank Hepatitis C Study Group. *Lancet* 1995; **345**: 484-487 [PMID: 7532254 DOI: 10.1016/S0140-6736(95)90583-9]

104 **Northup PG**, Argo CK, Nguyen DT, McBride MA, Kumer SC, Schmitt TM, Pruett TL. Liver allografts from hepatitis C positive donors can offer good outcomes in hepatitis C positive recipients: a US National Transplant Registry analysis. *Transpl Int* 2010; **23**: 1038-1044 [PMID: 20444239 DOI: 10.1111/j.1432-2277.2010.01092.x]

105 **Delladetsima I**, Psichogiou M, Sypsa V, Psimenou E, Kostakis A, Hatzakis A, Boletis JN. The course of hepatitis C virus infection in pretransplantation anti-hepatitis C virus-negative renal transplant recipients: a retrospective follow-up study. *Am J Kidney Dis* 2006; **47**: 309-316 [PMID: 16431260 DOI: 10.1053/j.ajkd.2005.11.008]

106 **Maluf DG**, Archer KJ, Mas VR. Kidney grafts from HCV-positive donors: advantages and disadvantages. *Transplant Proc* 2010; **42**: 2436-2446 [PMID: 20832522 DOI: 10.1016/j.transproceed.2010.04.056]

107 **Bucci JR**, Matsumoto CS, Swanson SJ, Agodoa LY, Holtzmuller KC, Peters TG, Abbott KC. Donor hepatitis C seropositivity: clinical correlates and effect on early graft and patient survival in adult cadaveric kidney transplantation.

J Am Soc Nephrol 2002; **13**: 2974-2982 [PMID: 12444217 DOI: 10.1097/01.ASN.0000034944.90425.75]

108 **Kucirka LM**, Singer AL, Ros RL, Montgomery RA, Dagher NN, Segev DL. Underutilization of hepatitis C-positive kidneys for hepatitis C-positive recipients. *Am J Transplant* 2010; **10**: 1238-1246 [PMID: 20353475 DOI: 10.1111/j.1600-6143.2010.03091.x]

109 **Manuel O**, Estabrook M. RNA respiratory viruses in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 212-219 [PMID: 23465014 DOI: 10.1111/ajt.12113]

110 Couch RB, Englund JA, Whimbey E. Respiratory viral infections in immunocompetent and immunocompromised persons. *Am J Med* 1997; **102**: 2-9; discussion 25-26 [DOI: 10.1016/S0002-9343(97)00003-X]

111 **Ison MG**, Gubareva LV, Atmar RL, Treanor J, Hayden FG. Recovery of drug-resistant influenza virus from immunocompromised patients: a case series. *J Infect Dis* 2006; **193**: 760-764 [PMID: 16479508 DOI: 10.1086/500465]

112 **Billings JL**, Hertz MI, Savik K, Wendt CH. Respiratory viruses and chronic rejection in lung transplant recipients. *J Heart Lung Transplant* 2002; **21**: 559-566 [DOI: 10.1016/S1053-2498(01)00405-3]

113 **Khalifah AP**, Hachem RR, Chakinala MM, Schechtman KB, Patterson GA, Schuster DP, Mohanakumar T, Trulock EP, Walter MJ. Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. *Am J Respir Crit Care Med* 2004; **170**: 181-187 [PMID: 15130908 DOI: 10.1164/rccm.200310-1359OC]

114 **Kumar D**, Erdman D, Keshavjee S, Peret T, Tellier R, Hadjiliadis D, Johnson G, Ayers M, Siegal D, Humar A. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. *Am J Transplant* 2005; **5**: 2031-2036 [PMID: 15996256 DOI: 10.1111/j.1600-6143.2005.00971.x]

115 **Meylan PR**, Aubert JD, Kaiser L. Influenza transmission to recipient through lung transplantation. *Transpl Infect Dis* 2007; **9**: 55-57 [PMID: 17313474 DOI: 10.1111/j.1399-3062.2006.00175.x]

116 **Le Page AK**, Kainer G, Glanville AR, Tu E, Bhonagiri D, Rawlinson WD. Influenza B virus transmission in recipients of kidney and lung transplants from an infected donor. *Transplantation* 2010; **90**: 99-102 [PMID: 20606569 DOI: 10.1097/TP.0b013e3181da1933]

117 **Kozlowski T**, Nickeleit V, Andreoni K. Donor-transmitted adenovirus infection causing kidney allograft nephritis and graft loss. *Transpl Infect Dis* 2011; **13**: 168-173 [PMID: 20854282 DOI: 10.1111/j.1399-3062.2010.00572.x]

118 **Kumar D**, Morris MI, Kotton CN, Fischer SA, Michaels MG, Allen U, Blumberg EA, Green M, Humar A, Ison MG. Guidance on novel influenza A/H1N1 in solid organ transplant recipients. *Am J Transplant* 2010; **10**: 18-25 [PMID: 19958321 DOI: 10.1111/j.1600-6143.2009.02960.x]

119 **Florescu DF**, Hoffman JA. Adenovirus in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 206-211 [PMID: 23465013 DOI: 10.1111/ajt.12112]

120 **Shames BD**, D'Alessandro AM, Sollinger HW. Human T-cell lymphotrophic virus infection in organ donors: a need to reassess policy? *Am J Transplant* 2002; **2**: 658-663 [PMID: 12201368 DOI: 10.1034/j.1600-6143.2002.20712.x]

121 **Armstrong MJ**, Corbett C, Rowe IA, Taylor GP, Neuberger JM. HTLV-1 in solid-organ transplantation: current challenges and future management strategies. *Transplantation* 2012; **94**: 1075-1084 [PMID: 23060278 DOI: 10.1097/TP.0b013e318263ad7a]

122 **Toro C**, Rodés B, Poveda E, Soriano V. Rapid development of subacute myelopathy in three organ transplant recipients after transmission of human T-cell lymphotropic virus type I from a single donor. *Transplantation* 2003; **75**: 102-104 [PMID: 12544880 DOI: 10.1097/00007890-200301150-00019]

123 **Kaul DR**, Taranto S, Alexander C, Covington S, Marvin M, Nowicki M, Orlowski J, Pancoska C, Pruett TL, Ison MG. Donor screening for human T-cell lymphotrophic virus 1/2: changing paradigms for changing testing capacity. *Am J Transplant* 2010; **10**: 207-213 [PMID: 19839982 DOI: 10.1111/j.1600-6143.2009.02867.x]

124 **Waggoner JJ**, Soda EA, Deresinski S. Rare and emerging viral infections in transplant recipients. *Clin Infect Dis* 2013; **57**: 1182-1188 [PMID: 23839998 DOI: 10.1093/cid/cit456]

125 **Rabe IB**, Schwartz BS, Farnon EC, Josephson SA, Webber AB, Roberts JP, de Mattos AM, Gallay BJ, van Slyck S, Messenger SL, Yen CJ, Bloch EM, Drew CP, Fischer M, Glaser CA. Fatal transplant-associated west nile virus encephalitis and public health investigation-california, 2010. *Transplantation* 2013; **96**: 463-468 [PMID: 23823653 DOI: 10.1097/TP.0b013e31829b4142]

126 **Singh N**, Levi ME. Arenavirus and West Nile virus in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 361-371 [PMID: 23465029 DOI: 10.1111/ajt.12128]

127 **Singh N**, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, infectious diseases community of practice. *Am J Transplant* 2012; **12**: 2414-2428 [PMID: 22694672 DOI: 10.1111/j.1600-6143.2012.04100.x]

128 **Canaud G**, Timsit MO, Zuber J, Bougnoux ME, Méjean A, Thervet E, Snanoudj R, Sberro R, Martinez F, Legendre C, Mamzer-Bruneel MF. Early conservative intervention for candida contamination of preservative fluid without allograft nephrectomy. *Nephrol Dial Transplant* 2009; **24**: 1325-1327 [PMID: 19004850 DOI: 10.1093/ndt/gfn622]

129 **Koo S**, Kubiak DW, Issa NC, Dietzek A, Boukedes S, Camp PC, Goldberg HJ, Baden LR, Fuhlbrigge AL, Marty FM. A targeted peritransplant antifungal strategy for the prevention of invasive fungal disease after lung transplantation: a sequential cohort analysis. *Transplantation* 2012; **94**: 281-286 [PMID: 22790447 DOI: 10.1097/TP.0b013e318255f864]

130 **Laouad I**, Buchler M, Noel C, Sadek T, Maazouz H, Westeel PF, Lebranchu Y. Renal artery aneurysm secondary to Candida albicans in four kidney allograft recipients. *Transplant Proc* 2005; **37**: 2834-2836 [PMID: 16182825 DOI: 10.1016/j.transproceed.2005.05.017]

131 **Sun HY**, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: evidence-based evolving trends. *Clin Infect Dis* 2009; **48**: 1566-1576 [PMID: 19402789 DOI: 10.1086/598936]

132 **Saha DC**, Goldman DL, Shao X, Casadevall A, Husain S, Limaye AP, Lyon M, Somani J, Pursell K, Pruett TL, Singh N. Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin Vaccine Immunol* 2007; **14**: 1550-1554 [PMID: 17959819 DOI: 10.1128/CVI.00242-07]

133 **Baddley JW**, Schain DC, Gupte AA, Lodhi SA, Kayler LK, Frade JP, Lockhart SR, Chiller T, Bynon JS, Bower WA. Transmission of Cryptococcus neoformans by Organ Transplantation. *Clin Infect Dis* 2011; **52**: e94-e98 [PMID: 21220771 DOI: 10.1093/cid/ciq216]

134 **Pappas PG**, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ, Henderson H, Kauffman CA, Haas DW, Saccente M, Hamill RJ, Holloway MS, Warren RM, Dismukes WE. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* 2001; **33**: 690-699 [PMID: 11477526 DOI: 10.1086/322597]

135 **Perfect JR**, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, Harrison TS, Larsen RA, Lortholary O, Nguyen MH, Pappas PG, Powderly WG, Singh N, Sobel JD, Sorrell TC. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis* 2010; **50**: 291-322 [PMID: 20047480 DOI: 10.1086/649858]

136 **Cuellar-Rodriguez J**, Avery RK, Lard M, Budev M, Gordon SM, Shrestha NK, van Duin D, Oethinger M, Mawhorter

SD. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009; **49**: 710-716 [PMID: 19635026 DOI: 10.1086/604712]

137 **Blair JE**, Logan JL. Coccidioidomycosis in solid organ transplantation. *Clin Infect Dis* 2001; **33**: 1536-1544 [PMID: 11588699 DOI: 10.1086/323463]

138 **Vucicevic D**, Carey EJ, Blair JE. Coccidioidomycosis in liver transplant recipients in an endemic area. *Am J Transplant* 2011; **11**: 111-119 [PMID: 21087416 DOI: 10.1111/j.1600-6143.2010.03328.x]

139 **Miller MB**, Hendren R, Gilligan PH. Posttransplantation disseminated coccidioidomycosis acquired from donor lungs. *J Clin Microbiol* 2004; **42**: 2347-2349 [PMID: 15131231 DOI: 10.1128/JCM.42.5.2347-2349.2004]

140 **Miller R**, Assi M. Endemic fungal infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 250-261 [PMID: 23465018 DOI: 10.1111/ajt.12117]

141 **Wright PW**, Pappagianis D, Wilson M, Louro A, Moser SA, Komatsu K, Pappas PG. Donor-related coccidioidomycosis in organ transplant recipients. *Clin Infect Dis* 2003; **37**: 1265-1269 [PMID: 14557974 DOI: 10.1086/378741]

142 **Bern C**, Montgomery SP, Herwaldt BL, Rassi A, Marin-Neto JA, Dantas RO, Maguire JH, Acquatella H, Morillo C, Kirchhoff LV, Gilman RH, Reyes PA, Salvatella R, Moore AC. Evaluation and treatment of chagas disease in the United States: a systematic review. *JAMA* 2007; **298**: 2171-2181 [PMID: 18000201 DOI: 10.1001/jama.298.18.2171]

143 **Vincenzi R**, Neto JS, Fonseca EA, Pugliese V, Leite KR, Benavides MR, Cândido HL, Porta G, Miura IK, Pugliese R, Danesi VB, Guimarães TC, Porta A, Kondo M, Carone E, Chapchap P. Schistosoma mansoni infection in the liver graft: The impact on donor and recipient outcomes after transplantation. *Liver Transpl* 2011; **17**: 1299-1303 [PMID: 21504049 DOI: 10.1002/lt.22316]

144 **Roseman DA**, Kabbani D, Kwah J, Bird D, Ingalls R, Gautam A, Nuhn M, Francis JM. Strongyloides stercoralis transmission by kidney transplantation in two recipients from a common donor. *Am J Transplant* 2013; **13**: 2483-2486 [PMID: 23919410 DOI: 10.1111/ajt.12390]

145 **Mossad SB**. Region-specific challenges for minimizing endemic donor-transmitted infections. *Liver Transpl* 2011; **17**: 1241-1243 [PMID: 21656654 DOI: 10.1002/lt.22353]

146 **Schwartz BS**, Mawhorter SD. Parasitic infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 280-303 [PMID: 23465021 DOI: 10.1111/ajt.12120]

147 **Coura JR**, Borges-Pereira J. Chagas disease. What is known and what should be improved: a systemic review. *Rev Soc Bras Med Trop* 2012; **45**: 286-296 [PMID: 22760123 DOI: 10.1590/S0037-86822012000300002]

148 **Chin-Hong PV**, Schwartz BS, Bern C, Montgomery SP, Kontak S, Kubak B, Morris MI, Nowicki M, Wright C, Ison MG. Screening and treatment of chagas disease in organ transplant recipients in the United States: recommendations from the chagas in transplant working group. *Am J Transplant* 2011; **11**: 672-680 [PMID: 21401868 DOI: 10.1111/j.1600-6143.2011.03444.x]

149 **Schwartz BS**, Paster M, Ison MG, Chin-Hong PV. Organ donor screening practices for Trypanosoma cruzi infection among US Organ Procurement Organizations. *Am J Transplant* 2011; **11**: 848-851 [PMID: 21426487 DOI: 10.1111/j.1600-6143.2011.03436.x]

150 **Machado CM**, Levi JE. Transplant-associated and blood transfusion-associated tropical and parasitic infections. *Infect Dis Clin North Am* 2012; **26**: 225-241 [PMID: 22632636 DOI: 10.1016/j.idc.2012.02.008]

151 **Rassi A**, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). *Infect Dis Clin North Am* 2012; **26**: 275-291 [PMID: 22632639 DOI: 10.1016/j.idc.2012.03.002]

152 **Huprikar S**, Bosserman E, Patel G, Moore A, Pinney S, Anyanwu A, Neofytos D, Ketterer D, Striker R, Silveira F, Qvarnstrom Y, Steurer F, Herwaldt B, Montgomery S. Donor-derived Trypanosoma cruzi infection in solid organ recipients in the United States, 2001-2011. *Am J Transplant* 2013; **13**: 2418-2425 [PMID: 23837488 DOI: 10.1111/ajt.12340]

153 **Boyarsky BJ**, Hall EC, Singer AL, Montgomery RA, Gebo KA, Segev DL. Estimating the potential pool of HIV-infected deceased organ donors in the United States. *Am J Transplant* 2011; **11**: 1209-1217 [PMID: 21443677 DOI: 10.1111/j.1600-6143.2011.03506.x]

REVIEW

Calcineurin inhibitor sparing strategies in renal transplantation, part one: Late sparing strategies

Andrew Scott Mathis, Gwen Egloff, Hoytin Lee Ghin

Andrew Scott Mathis, Hoytin Lee Ghin, Pharmacy Department, Monmouth Medical Center, Long Branch, NJ 07740, United States
Gwen Egloff, School of Pharmacy, University of Connecticut, Storrs, CT 06268, United States
Author contributions: All authors have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published.
Correspondence to: Andrew Scott Mathis, RPh, BS, PharmD, Pharmacy Department, Monmouth Medical Center, 300 Second Avenue, Long Branch, NJ 07740,
United States. smathis@barnabashealth.org
Telephone: +1-732-9236753 Fax: +1-732-9236757
Received: February 8, 2014 Revised: March 24, 2014
Accepted: May 14, 2014
Published online: June 24, 2014

Abstract

Kidney transplantation improves quality of life and reduces the risk of mortality. A majority of the success of kidney transplantation is attributable to the calcineurin inhibitors (CNIs), cyclosporine and tacrolimus, and their ability to reduce acute rejection rates. However, long-term graft survival rates have not improved over time, and although controversial, evidence does suggest a role of chronic CNI toxicity in this failure to improve outcomes. Consequently, there is interest in reducing or removing CNIs from immunosuppressive regimens in an attempt to improve outcomes. Several strategies exist to spare calcineurin inhibitors, including use of agents such as mycophenolate mofetil (MMF), mycophenolate sodium (MPS), sirolimus, everolimus or belatacept to facilitate late calcineurin inhibitor withdrawal, beyond 6 mo post-transplant; or using these agents to plan early withdrawal within 6 mo; or to avoid the CNIs all together using CNI-free regimens. Although numerous reviews have been written on this topic, practice varies significantly between centers. This review organizes the data based on patient characteristics (*i.e.*, the baseline immunosuppressive regimen) as a means to aid the practicing clinician in caring for their patients, by matching up their situation with the relevant literature. The current review, the first in a series of two, examines the potential of immunosuppressive agents to facilitate late CNI withdrawal beyond 6 mo post-transplant, and has demonstrated that the strongest evidence resides with MMF/MPS. MMF or MPS can be successfully introduced/maintained to facilitate late CNI withdrawal and improve renal function in the setting of graft deterioration, albeit with an increased risk of acute rejection and infection. Additional benefits may include improved blood pressure, lipid profile and serum glucose. Sirolimus has less data directly comparing CNI withdrawal to an active CNI-containing regimen, but modest improvement in short-term renal function is possible, with an increased risk of proteinuria, especially in the setting of baseline renal dysfunction and/or proteinuria. Renal outcomes may be improved when sirolimus is used in combination with MMF. Although data with everolimus is less robust, results appear similar to those observed with sirolimus.

Key words: Kidney transplantation; Calcineurin inhibitor; Withdrawal; Sparing; Cyclosporine; Tacrolimus; Renal function; Graft survival

Core tip: Mycophenolic acid derivatives have been used successfully to facilitate late calcineurin inhibitor withdrawal to improve short-term renal function in kidney transplantation. The benefit carries an increased risk of acute cellular rejection. Sirolimus and everolimus are also options, but have comparatively less evidence and carry and increased risk of proteinuria, which is dependent on baseline renal function.

Mathis AS, Egloff G, Lee Ghin H. Calcineurin inhibitor sparing

INTRODUCTION

Compared with hemodialysis, kidney transplantation improves quality of life and reduces of mortality risk[1-3]. The survival benefit of kidney transplantation over hemodialysis applies even to the use of marginal donor kidneys[4]. Much of this success has been attributed to calcineurin inhibitors, cyclosporine and tacrolimus, and their ability to reduce acute rejection rates. However, despite dramatic reductions in acute rejection rates over time, long-term graft survival rates have not improved to an appreciable extent[5,6]. A number of factors have been postulated that contribute to the lack of improvement in graft survival, including donor factors, recipient factors, human leukocyte antigen matching, death with a functioning allograft, delayed graft function, calcineurin inhibitor toxicity, chronic allograft nephropathy, and infectious nephropathy (BK virus)[6].

Calcineurin inhibitor nephrotoxicity was recognized early after the use of cyclosporine began, and it comes in many forms[7]. Calcineurin inhibitors cause acute and chronic nephrotoxicity. The acute forms include arteriolopathy, tubular vacuolization and thrombotic microangiopathy. Chronic forms of nephrotoxicity include interstitial fibrosis and tubular atrophy, medial arteriolar hyalinosis, glomerular capsular fibrosis, global glomerulosclerosis, focal segmental glomerulosclerosis, juxtaglomerular apparatus hyperplasia, and tubular microcalcifications, many of which can be caused by other factors and tend to be nonspecific findings on post-transplant biopsy[7]. Because of the known contribution of calcineurin inhibitors to nephrotoxicity, there has been much interest in finding the optimal agent and/or regimen[8-14]. While many studies demonstrated improved renal function with reduced dose calcineurin inhibitor use, or an early benefit on renal function with tacrolimus use when compared to cyclosporine, improvements in long-term graft function were not demonstrated[9-14]. Additionally, there are numerous differences in the adverse event profile of cyclosporine and tacrolimus. Many outside factors differentiate the calcineurin inhibitors and influence their contribution to nephrotoxicity, including therapeutic drug monitoring strategy, dosing strategy, drug-drug interaction, pharmacogenetics, and non-adherence[15-20]. These medication-related variables make nephrotoxicity and decline in allograft function very difficult to predict in practice. The lack of surveillance biopsies also makes differentiation of outcomes related to calcineurin inhibitor use and non-medication related factors difficult in practice[21-25].

A long-term biopsy study helped determine the true consequence of calcineurin inhibitors on chronic allograft nephropathy and graft failure[26]. In a well-designed study, Nankivell *et al*[26] demonstrated the natural history of chronic allograft nephropathy in 120 type 1 diabetics who underwent kidney-pancreas transplant followed by routine biopsies over a 10-year period. The initial phase (year 1) in the development of chronic allograft nephropathy was characterized by early tubulointerstitial damage from ischemic injury, prior severe rejection, and subclinical rejection, where these findings were present in 94.2% of patients. Beyond year 1, chronic allograft nephropathy was characterized by microvascular and glomerular injury and chronic rejection, defined as subclinical rejection for two or more years, and was uncommon (5.8%). Progressive high-grade arteriolar hyalinosis with luminal narrowing, increasing glomerulosclerosis, and tubulointerstitial damage were linked to the calcineurin inhibitors, and were irreversible. Despite dose reductions of both cyclosporine and tacrolimus, calcineurin inhibitor nephrotoxicity was nearly universal by 10 years, and was found to be the chief cause of late injury and renal function decline[26].

The data from Nankivell *et al*[26] suggested that chronic allograft nephropathy was primarily a function of calcineurin inhibitor nephrotoxicity. This has been interpreted with controversy, but the data surrounding the definition and pathophysiology of chronic allograft nephropathy have always been controversial, due to varied definitions utilized in both practice and research[27,28]. In addition, many believe that calcineurin inhibitor nephrotoxicity is a non-specific finding[7,22]. Still, the evidence from Nankivell *et al*[26] is the among the most robust long-term evidence available on calcineurin inhibitors. It should also be mentioned that objective assessment is superior to clinical assessment, to determine the presence of chronic allograft nephropathy and calcineurin inhibitor nephrotoxicity, because clinicians underestimate the chronic renal toxicity[29,30]. Despite underestimation, clinicians have many ways of dealing with perceived medication toxicity. Commonly, when adverse effects are noted, adjustments are made in the regimen of the individual patient[31]. This may result in unintended consequences, such as acute rejection and graft loss[32-34].

Collectively, protocols have been developed to assess the conversion between calcineurin inhibitors, or to select a preferable one, in order to avoid certain toxicities, or promote renal function improvements or short-term graft survival[9-14,35]. However, in a paired kidney analysis from a database with 5-year follow-up, no difference could be determined between cyclosporine and tacrolimus with respect to allograft survival, despite superior renal function in the tacrolimus group[36]. These results were similar in a prospective study with mean 2.8 years follow-up, and supported a 5-year histologic study that determined similar development of moderate to severe arteriolar hyalinosis with cyclosporine or tacrolimus[37,38]. When patients are switched between the two calcineurin inhibitors, or one is used in preference to the other, the basic tenet that calcineurin inhibitors are the primary contributors to graft decline is ignored[30]. In addition, the graft decline appears to occur primarily between 5 and 10

years post transplant[26]. It must also be emphasized that switching agents off-protocol in an uncontrolled way may have harmful effects, and is inconsistent with evidence-based practice[32].

In recent years, various schools of thought have emerged with the introduction of newer agents and experience gained through research. The main strategies are based on personalization, corticosteroid minimization, and calcineurin inhibitor sparing[39,40]. It is too soon for personalized medicine, although the foundation has been laid[17-19,39]. Steroid avoidance strategies have been generally disappointing. They focus on minimizing adverse effects, and usually require calcineurin inhibitor persistence for successful outcome[40-47]. Calcineurin inhibitor sparing strategies also aim to reduce adverse effects, but also seek to improve graft survival[43-66]. Understanding the different treatment options may lead to improvement in long-term care.

Although the calcineurin inhibitor sparing strategies have been extensively reviewed, we aim to provide a unique approach to the issue. Since many transplant centers have set protocols for their specific populations, and clinical trial results or experiences of other centers may not be generalizable, we aim to review the literature according to general age groups (adult and pediatric) and therapeutic approaches (de novo, early or late) based on the specific baseline regimens used. We will analyze calcineurin inhibitor withdrawal and avoidance, and only touch on minimization when directly compared since exposure appears to lead to chronic toxicity and follow-up was usually inadequate to determine the true consequence on chronic allograft nephropathy[26,54].

Due to the expanse of the issue, we will divide the topic into two manuscripts. The first, herein, will cover late calcineurin inhibitor withdrawal, beyond 6 mo post-transplant, and the second will cover early withdrawal and de novo avoidance. We will focus primarily on renal function and graft survival as the main outcomes of interest, and make recommendations based on the available evidence for each clinical subgroup since data on predicting or monitoring the outcome of changes in immunsuppression are still lacking[67-78]. The intent of the article is to aid the practicing clinician in identifying studies relevant to their practice to assist in clinical decision making. The clinician may have to refer the cited articles to find more specific information, such as the countries where the analysis was performed, ethnic breakdown of the population, transplant characteristics, *etc.*

STRATEGIES

Three basic strategies are available for calcineurin-sparing, "Avoidance", and "Early" and "Late" reduction or withdrawal. Late, defined as calcineurin inhibitor reduction withdrawal or elimination beyond 6 mo (> 6 mo) after the kidney transplant, is a strategy that has been frequently used when patients are faced with diminishing renal function, possibly related to established toxicity, and is the focus of this manuscript. Early, defined as calci-

neurin inhibitor withdrawal or reduction within the first 6 mo ($\leqslant$ 6 mo) after the kidney transplant, is generally done to prevent anticipated calcineurin inhibitor toxicity or in response to early evidence of diminished renal function. Calcineurin inhibitor avoidance or calcineurin inhibitor-free regimens are typically a proactive strategy in response to the concerns about the potential toxicity of the calcineurin inhibitors and their failure to promote long-term graft survival, despite dramatic reduction in the risk of acute cellular rejection. Early and de novo are the focus of a second manuscript in this series.

Our search strategy involved PubMed database for all years until August 2013 for articles involving kidney or renal transplantation with the search terms calcineurin inhibitor "reduction", "withdrawal", "elimination", "avoidance", "minimization", "sparing" and "free". References of identified articles were reviewed to identify additional articles of interest. Articles were separated according to the post-transplant time period when the intervention took place, according to the three categories (avoidance, early, and late), and then arranged according to population and baseline regimen. Based on the assumption that most long-term calcineurin inhibitor nephrotoxicity is irreversible and progressive, and minimization articles were only included if they directly compared with avoidance or withdrawal/elimination regimens. The remainder of the article will summarize the available evidence by patient type, intervention and baseline regimen.

ADULT PATIENTS AT VARIABLE TIME POST-TRANSPLANT

Regimens utilizing older agents to eliminate calcineurin inhibitors

Baseline calcineurin inhibitor and corticosteroid with or without azathioprine: A meta-analysis by Kasiske *et al*[79] evaluated early studies of calcineurin inhibitor withdrawal in patients on a baseline regimen of azathioprine, cyclosporine and corticosteroid, and compared calcineurin inhibitor withdrawal with continuation (part 1), and calcineurin inhibitor withdrawal with patients who never received calcineurin inhibition (part 2)[79]. In part 1 of the meta-analysis, 17 studies were included, with 9 of them including patients withdrawn during the first 6 mo after the transplant. The mean duration of follow-up of the studies was 26.6 ± 7.5 mo. It should be noted that the meta-analysis included mixed populations, containing patients withdrawn due to toxicity of cyclosporine (3 studies), patients with stable renal function and/or without recent rejection (10 studies), recipients of living donor kidneys (6 studies), and patients with first transplant (4 studies). In part 1, there was a higher rate of acute rejection episodes per patient in the cyclosporine withdrawal group (0.126; 95%CI: 0.085-0.167; $P < 0.001$), but no difference in graft loss per patient per year (-0.009; 95%CI: -0.022-0.004, $P = 0.19$) or deaths per patient per year (-0.005; 95%CI: -0.016-0.006, $P = 0.4$). The authors noted a trend toward higher serum creatinine in the con-

trol group who continued cyclosporine relative to those who discontinued the agent (1.84 ± 0.29 mg/dL *vs* 1.63 ± 0.28 mg/dL; *P* = 0.17). In part 2 of the meta-analysis, consisting of 6 studies, 3 included stable patients, none involved withdrawal due to toxicity, 3 studies included living donor kidneys, and 2 studies included only the first allograft, and 5 were performed in the first 6 mo after the transplant. The mean duration of follow-up was 28.8 ± 11.6 mo. When the six studies were analyzed together, the rate of graft loss per patient per years was similar (-0.02; 95%CI: -0.022-0.003, *P* = 0.08), but when only the 3 randomized trials were considered, graft survival was better among those withdrawn from cyclosporine (0.0382; 95%CI: 0.0002-0.0762, *P* = 0.049). The deaths per patient per year were similar (0.001; 95%CI: -0.006-0.008, *P* = 0.87) and the serum creatinine was non significantly higher in the group who never received calcineurin inhibitors (1.71 ± 0.36 *vs* 1.50 ± 0.18 mg/dL; *P* = 0.2). The authors noted that none of the outcomes were affected by the timing (before or after 6 mo) or method (slow or rapid taper) of cyclosporine withdrawal[79].

This meta-analysis demonstrated that cyclosporine withdrawal resulted in an early increase in the risk of acute cellular rejection, but similar graft function, graft survival and patient survival at about 2-year follow-up to patients retained on cyclosporine or who never received cyclosporine[79]. Despite promising results, azathioprine as an antiproliferative has been largely replaced in practice with newer agents that are considered more potent immunosuppressants. Another study evaluated withdrawal of cyclosporine using azathioprine versus mycophenolate mofetil in patients 1 year post-transplant. The primary endpoint was development of donor-specific antibodies (DSAs), measured by complement-dependent cytotoxicity assay, enzyme-linked immunosorbent assay (ELISA) and flow-cytometry crossmatch with donor spleen cells. DSAs, by three methods were not detected during cyclosporine treatment or during acute rejection treatment while on cyclosporine, but after conversion to azathioprine, 3 of 8 (37.5%) had DSAs in the presence of acute rejection, while none (0 of 6) of the mycophenolate mofetil patients had DSAs during rejection. These results highlight the potential benefits of mycophenolic acid over azathioprine, which have been described previously[80-82].

ADULT PATIENTS 6 OR MORE MONTHS POST-TRANSPLANT

Regimens utilizing mycophenolic acid to eliminate calcineurin inhibitors

Baseline calcineurin inhibitor and corticosteroid: At least two studies[83,84] evaluated patients withdrawn late from a calcineurin inhibitor with a baseline regimen of calcineurin inhibitor and corticosteroid (Table 1)[83-93]. One study was designed to prospectively evaluate arterial distensibility and endothelial function before and after removal of cyclosporine in a population with biopsy-

proven CAN and deteriorating renal function. MMF was introduced at 500 mg per day and increased to a target dose of 2000 mg per day over 4 wk. The mean daily dose of MMF was 1700 mg at the end of the trial. Half the patients were randomized to withdrawal (tapered to off over 4 wk) and half to cyclosporine continuation. At 6 mo, serum creatinine increased slightly in both groups, but to a numerically greater extent on the control group who remained on cyclosporine. Though blood pressure improved from baseline in the intervention group, but not in the control group, there was no significant effect on brachial artery endothelial-dependent vasodilation. Acute rejection was not reported[83]. Another study performed by the same investigators also evaluated patients with biopsy-proven CAN, serum creatinine less than 4 mg/dL, and deteriorating renal function. That study introduced MMF more aggressively, at 1 g/d, and titrated to 2 g/d over 3 wk, and then patients were randomized to withdrawal or continuation of the calcineurin inhibitor. In patients randomized to withdrawal, the calcineurin inhibitor was reduced by 33% every 2 wk. The primary endpoint of slope of reciprocal serum creatinine per month at week 35 was positive and higher (0.00585 ± 0.01122) in the dual therapy group than the triple therapy group (-0.00728 ± 0.01105). Additional findings were the degree of proteinuria (*P* = 0.01), diastolic blood pressure (*P* = 0.04) and mean arterial pressure (*P* = 0.04), which were lower in the dual therapy group at follow-up. No episodes of acute rejection were reported[84]. These results provide modest evidence that late withdrawal of calcineurin inhibitor with replacement by MMF may improve renal function, or at least reduce the rate of deterioration of renal function, and improve blood pressure relative to calcineurin inhibitor continuation.

Baseline calcineurin inhibitor, corticosteroid and azathioprine: A prospective, single-center randomized trial randomized patients on cyclosporine, azathioprine and corticosteroid, with biopsy-proven CAN and deteriorating renal function to MMF or tacrolimus. In patients randomized to cyclosporine, it was discontinued 24 h before tacrolimus was initiated. In patients randomized to MMF, MMF was introduced at 500 mg twice daily and then titrated up over 2-4 wk to 2 g/d. After 6 wk, cyclosporine was incrementally reduced to achieve withdrawal by 14 wk. Azathioprine was discontinued at conversion. At 6-mo, measured glomerular filtration rate (GFR) and serum creatinine were not improved in the tacrolimus group, but in the MMF group, GFR (*P* < 0.001) and serum creatinine (*P* < 0.001) were improved. In contrast, total cholesterol and triglycerides improved from baseline in the tacrolimus group, but not in the MMF group, and systolic and diastolic blood pressure improved in the MMF group, but not the tacrolimus. There were no reported rejection episodes[85]. Another study evaluated consecutive patients converted from cyclosporine, azathioprine and corticosteroid to MMF plus corticosteroid for CAN. Azathioprine was immediately stopped and MMF was introduced over 1 wk, with target dose of

Ref.	Design	Population (*n*)	Baseline Regimen	*n*	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Kosch *et al*[83]	Prospective, randomized, single-center	6-mo of deteriorating renal function, BP-CAN	CsA, Prednisolone	12	MMF added, target 2 g per day; CsA withdrawn over 4 wk	6 mo	SCr + 0.03 mg/dL *vs* baseline (*P* = NS)	NA	NA	NA
				12	MMF added, target 2 g; CsA continued		SCr + 0.07 mg/dL *vs* baseline (*P* = NS)	NA	NA	NA
Suwelack *et al*[84]	Prospective, randomized, single-center	> 1-yr post transplant, SCr < 4 mg/dL, BP-CAN, deteriorating renal function	CsA or TAC, Prednisolone	18	MMF added, target 2 g; CsA withdrawn over 6 wk	35 wk	Slope 1/SCr 0.00585 ± 0.01122; 67% responders; Proteinuria 0.5 ± 0.55 g/24 h	0%	100%	NA
				20	MMF added, target 2 g; CsA continued		Slope 1/SCr -0.00728 ± 0.01105 (*P* = 0.0018); 25% responders (*P* = 0.021); Proteinuria 1.5 ± 0.48 g/24 h (*P* = 0.01)	0%	85%	NA
McGrath *et al*[85]	Prospective, randomized, single-center	> 1-yr post transplant, BP-CAN, deteriorating renal function	CsA, azathioprine, prednisolone	15	MMF added, target 2 g; CsA withdrawn by 14 wk	6 mo	SCr - 58 μmol/L *vs* baseline (*P* < 0.001); isotope GFR + 8.5 mL/min *vs* baseline (*P* < 0.01)	0%	NA	NA
				15	CsA switch to TAC		SCr + 15 μmol/L *vs* baseline (*P* = NS); isotope GFR -2.1 mL/min *vs* baseline (*P* = NS)	0%	NA	NA
Hanvesakul *et al*[86]	Retrospective, consecutive patients, single-center	> 1-yr post transplant, CAN	CsA or TAC, azathioprine, prednisolone	30	MMF added, target 1.5-2 g; azathioprine stopped; CNI withdrawn over 4 wk	1 yr	eGFR + 2 mL/min *vs* baseline	3.30%	86.70%	96.70%
Dudley *et al*[87]	Randomized, open, multicenter	> 6-mo post transplant, deteriorating renal function, no recent ACR	CsA monotherapy, or CsA/corticosteroid, or CsA/azathioprine/corticosteroids	73	MMF added, target 2 g; azathioprine discontinued, if applicable; CsA withdrawn over 6 wk, if needed corticosteroid added	1 yr	Response rate (6 mo): 58% stabilized or reduced SCr; Response rate (1 yr): 48%; Least squares mean SCr -24.9 μmol/L; Least squares mean CrCL +5 mL/min	0%	93.20%	95.90%
			CsA monotherapy, or CsA/corticosteroid, or CsA/azathioprine/corticosteroids	70	Continued regimen		Response rate (6 mo): 32% stabilized or reduced SCr (*P* = 0.006); Response rate (1 yr): 35% (*P* = 0.1885); Least squares mean SCr +22.2 μmol/L (*P* < 0.01); Least squares mean CrCL -0.7 mL/min (*P* < 0.01)	0%	94.3%	100%

Table 1 renal transplant studies utilizing mycophenolic acid to withdraw calcineurin inhibitor beyond 6 mo post-transplant ("Late")[83-93]

Study	Design	Characteristics	Baseline	N	Intervention	Follow-up	Outcome			
Weir *et al*[88]	Prospective, non-randomized, single-center	Mean 853.3 d post transplant, BP-CAN, deteriorating renal function, no ACR	CsA or TAC, prednisone, azathioprine or MMF	18	Azathioprine stopped; MMF added, target 2 g; CNI withdrawn	Mean 651 d	Response rate: 91.7% improved or lack of deterioration in renal function using least squares method slope 1/SCr (*P* = 0.038)	NCR	100%	NA
			CsA, prednisone, azathioprine or MMF	67	CsA dose reduced approximately 50%; azathioprine withdrawn; MMF added, target 2 g		Response rate: 51.7% improved or lack of deterioration	NCR	100%	NA
			TAC, prednisone, azathioprine or MMF	33	TAC dose reduced approximately 50%; azathioprine withdrawn; MMF added, target 2 g		59.3% improved or lack of deterioration	NCR	100%	NA
Weir *et al*[89]	Continuation of above trial			13	CNI withdrawn	76 mo	2.7 ± 0.2 mg/dL	7.7%	92.3%	100%
				64	CsA dose reduced	54 mo	3 ± 0.1 mg/dL	4.7%	62.5%	92.2%
				28	TAC dose reduced	42 mo	3 ± 0.2 mg/dL	7.1%	67.8%	100%
Abramowicz *et al*[90]	Randomized, controlled, multicenter	No recent ACR, ≤ 1 ACR overall, 12 to 30 mo post-transplant, stable renal function	CsA, prednisone, ± azathioprine or MMF	85	MMF added over 3 mo, target 2 g; CsA withdrawn over 3 mo	12 mo	CrCL improved 10% in 46%; SCr -1 μmol/L; CrCL + 4.5 mL/min *vs* control group (*P* = 0.16), eGFR + 2.3 mL/min *vs* control group (*P* = 0.24)	10.6%	100%	NA
				85	MMF added over 3 mo, target 2 g; continued triple therapy		SCr + 4 μmol/L	2.4% (*P* = 0.03)	100%	
Abramowicz *et al*[91]	Continuation of above trial			74	CsA withdrawn	60 mo	CrCL 67.4 mL/min	10%	88%	93%
				77	Triple therapy		CrCL 61.7 mL/min (*P* = 0.05)	1% (*P* = 0.028)	92%	95%
Heeg *et al*[92]	Retrospective	BP-CNI toxicity, deteriorating renal function, mean 11.2 mo post-transplant	CsA or TAC, Prednisolone, ± MMF or MPS	17	MPS added; CNI withdrawn; MMF withdrawn	48 mo	All *vs* Baseline. SCr at 6 mo -0.5 mg/dL (*P* < 0.05); eGFR at 6 mo + 11 mL/min; SCr at 36 mo -0.5 mg/dL (*P* = 0.063); eGFR at 36 mo +11 mL/min *P* = 0.022); SCr at 48 mo + 0.6 mg/dL (*P* = 0.27); eGFR at 48 mo +1 mL/min (*P* = 0.91)	NA	NA	NA
Mourer *et al*[93]	Prospective, randomized, single-center	No recent ACR, ≤ 2 ACR overall, at least 12 mo post-transplant, stable renal function	CsA or TAC, Prednisone, MMF	79	CNI withdrawn, MMF concentration controlled	36 mo	eGFR 59.5 ± 2.1 mL/min	5.1%	98.7%	94.9%
				79	MMF withdrawn, CNI concentration controlled		eGFR 51.1 ± 2.1 mL/min (*P* = 0.006)	2.5%	98.7%	92.4%

ACR: Acute cellular rejection; BP-CAN: Biopsy-proven chronic allograft nephropathy; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; eGFR: Estimated glomerular filtration rate; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; MPS: Mycophenolate sodium; NA: not assessed/applicable; NCR: Not clearly reported by group; NS: Not significant; SCr: Serum creatinine; TAC: Tacrolimus.

1500 to 2000 mg per day. Calcineurin inhibitor was withdrawn over 4 wk by 25% reduction. Estimated GFR improved from the time of conversion to 1-year follow-up by 2 mL/min, but the authors cautioned that there was a dramatic increase in the risk of infection in the patients converted to MMF[86].

Baseline calcineurin inhibitor monotherapy, calcineurin inhibitor with corticosteroid, calcineurin inhibitor with azathioprine, or calcineurin inhibitor, corticosteroid and azathioprine: The "Creeping Creatinine" study[87] evaluated patients on various calcineurin inhibitor-based regimen who did not receive MMF at baseline. In the open, randomized, multicentered trial, patients had a negative slope of reciprocal serum creatinine, baseline serum creatinine of 100 to 400 μmol/L and a calculated creatinine clearance of at least 20 mL/min. A biopsy had to show absence of transplant glomerulopathy, recurrent renal disease, de novo renal disease, obstruction, renal artery stenosis, acute rejection, or acute rejection within 3 mo. Patients were randomized to MMF or maintenance of cyclosporine according to normal practice. Those randomized to MMF had the drug introduced incrementally over 4 wk to a target dose of 2 g/d, and corticosteroids were introduced if not previously used. Cyclosporine was reduced in three steps over 6 wk to off. Patients randomized to maintain cyclosporine were continued as per usual practice with a permitted reduction of cyclosporine to a trough not less than 80 ng/mL. Baseline biopsies documented CAN in 78% of the MMF group and 77% of the cyclosporine group. A responder, defined as an improvement in the slope of 1/SCr with no change in the randomized treatment and no graft loss occurred in 58% of the MMF group and 32% of the control group (P = 0.006) at 6 mo and 48% of the MMF group and 35% of the control group (P = 0.1185) at 1 year. At 12-mo the least squares mean (LSM) creatinine clearance was +5 mL/min in the MMF group and -0.7 mL/min in the cyclosporine group (P < 0.01). LSM serum creatinine and serum cholesterol were lower in the MMF group at follow-up, and platelet count was higher, but triglycerides, hemoglobin, white blood cell count, systolic blood pressure and diastolic blood pressure were not significantly different. There were no acute rejection episodes in either group. The incidence of diarrhea, abdominal pain and opportunistic infections were numerically higher in the MMF group[87].

Baseline calcineurin inhibitor and corticosteroid with or without azathioprine or MMF: A study evaluated patients on calcineurin inhibitor and corticosteroid, with or without azathioprine or MMF, in a prospective non-randomized, single-centered fashion where decision to reduce or withdrawal CNI was arbitrary[88]. Patients with deteriorating renal function and CAN on biopsy were started on MMF (target 2 g/d) if it was not previously given, and azathioprine was stopped. Patients were analyzed in three groups, those who had CNI withdrawn (n = 18), those with a 50% reduction in cyclosporine after MMF introduction (n = 67), and those with 50% reduction in tacrolimus after MMF was introduced (n = 33). At mean 651 d follow-up, 91.7% of the withdrawal group, 51.7% of the reduced dose cyclosporine group, and 59.3% of the reduced dose tacrolimus group had improved or lack of deterioration in the LS 1/SCr (P = 0.038). The withdrawal group also had lower serum

glucose (P < 0.05) and total cholesterol (P < 0.05), but not systolic or diastolic blood pressure. It should be noted that patients selected for CNI withdrawal had a lower incidence of acute rejection prior to the intervention, but the nadir serum creatinine was similar in all three groups[88]. A continuation of the trial, out to 76 mo demonstrated that about one third of the CNI reduction patients and only 7.7% of the withdrawal group lost their graft during follow-up (P = 0.05). The serum creatinine at follow-up was 2.7 mg/dL in the withdrawal group and 3 mg/dL in the CNI reduction groups[89]. A randomized, controlled, multicenter trial also evaluated patients on cyclosporine and corticosteroid, with or without azathioprine or MMF. Patients were selected if they had a first or second cadaveric or living transplant, were between 12-30 mo post-transplant and maintained on a cyclosporine-based regimen. Patients had to have had no more than one acute rejection episode, with none in the last 3 mo, and a SCr less than 300 μmol/L for at least 3 mo. All patients had MMF introduced to a target of 2 g/d over 3 mo. Patients randomized to cyclosporine withdrawal had it tapered over 3 mo (n = 85) and those randomized to remain on cyclosporine (n = 85), continued on triple-drug therapy. Creatinine clearance improved by 10% in 46% of the withdrawal group, and the creatinine clearance difference was 4.5 mL/min higher in the withdrawal group 9 mo after randomization (P = 0.16). Serum creatinine improved by decreasing 1 μmol/L in the withdrawal group, and increased 4 μmol/L in the continuation group, creating a net effect of 5 μmol/L in favor of the withdrawal group (P = 0.34). Withdrawal improved the total (P = 0.02) and low density lipoprotein (LDL) cholesterol (P = 0.015), but blood pressure did not differ significantly. Acute rejection (10.6% *vs* 2.4%; P = 0.03) and diarrhea were more common in the withdrawal group[90]. A five-year follow-up publication demonstrated a creatinine clearance of 67.4 mL/min in the withdrawal group and 61.7 mL/min (P = 0.05) in the continuation group, but graft loss due to chronic rejection occurred in 12% of the withdrawal group and 8% of the continuation group, due to a respective acute rejection rate of 10% and 1% (P = 0.028)[91].

Baseline calcineurin inhibitor with or without corticosteroid and with or without MMF or mycophenolate sodium: One retrospective study analyzed 17 patients approximately 11 years post-transplant for 4 years before and after conversion to mycophenolate sodium (MPS) for biopsy-proven CNI toxicity (n = 7) or clinical deterioration of GFR and exclusion for other reasons for graft dysfunction. Patients on CNI and corticosteroid were converted to MPS and prednisolone, patients on CNI monotherapy were converted to MPS alone, and patients on triple therapy were converted to MPS with prednisolone. At conversion, GFR was 43 ± 15 mL/min. After conversion, graft function, as determined by GFR, improved within one month, and peaked at 55.7 ± 21.7 mL/min at one year (P = 0.00362), but then declined to near-baseline (44 ± 27 mL/min; P = 0.91) by four years,

indicating a slowing of progression. However, the overall slope of the regression line for GFR did not change significantly ($P = 0.116$). Three patients discontinued MPS due to infection ($n = 2$) and lost to follow-up ($n = 1$)[92]. A randomized trial compared CNI withdrawal ($n = 79$) with MMF withdrawal ($n = 79$) in patients who were on CNI/MMF/corticosteroid triple therapy. This trial used concentration controlled area-under-the-curve (AUC) monitoring for the CNIs (3250 ng/mL per hour for cyclosporine, 120 ng/mL per hour for tacrolimus) and MMF (75 μg/mL per hour). Estimated GFR was significantly better in the CNI withdrawal group at 6 wk (63.1 ± 1.9 mL/min *vs* 55.2 ± 1.9 mL/min; $P = 0.004$), 1-year (61.1 ± 1.8 mL/min *vs* 52.9 ± 1.8 mL/min; $P = 0.002$), and 3-year (59.5 ± 2.1 mL/min *vs* 51.1 ± 2.1 mL/min; $P = 0.006$). By 6 mo, 1.3% of the MMF withdrawal group and 3.8% of the CNI withdrawal group had biopsy-proven acute rejection. None were high immunologic risk. Three year graft survival did not differ. Blood pressure, lipid values, proteinuria and infections did not differ between the groups. Anemia was more frequent in the CNI withdrawal group[93].

Summary of MMF and mycophenolic acid studies: These studies suggest that MMF or MPS can be introduced or maintained to facilitate late (beyond 6-mo post-transplant) CNI withdrawal after kidney transplantation in the setting of graft deterioration and BP-CAN. Withdrawal of CNI using MMF or MPS appears to improve serum creatinine and creatinine clearance/GFR in a majority of patients, without an increased risk of proteinuria. The studies also demonstrate a potential for this strategy to improve blood pressure, lipid profile and serum glucose[94]. Benefits of mycophenolic acid derivatives may be offset by in increased risk of acute rejection and infection, so patients should be carefully selected. It appears that concentration-controlling the administration may limit the occurrence of these adverse events and possibly explain differences in adverse effects, such as diarrhea[93,95-97]. Taken individually, these studies were too small and too limited in follow-up to determine an improvement in graft survival, but a meta-analysis did demonstrate a trend toward improvement in graft survival (OR = 0.72, 95%CI: 0.52-1.01, $P = 0.06$) with CNI withdrawal using MMF in a mixed population that was not limited to late withdrawal[98]. Generally speaking, our findings are in line with other recent reviews and meta-analyses, and support a potential role of late CNI elimination with mycophenolic acid derivatives[98-101].

Regimens utilizing sirolimus to eliminate calcineurin inhibitors

Baseline regimen not specified: The mammalian target of rapamycin inhibitor (mTOR), sirolimus, has also been used to eliminate CNIs. A study[102] evaluated patients more than one year post-transplant with chronic allograft dysfunction according to baseline proteinuria stratification in 3 groups, and either withdrew CNI with addition of sirolimus or reduced the dose of CNI with addition

of sirolimus as shown in Table 2[102-118]. As shown, the patients who had sirolimus added demonstrated a statistically significant increase in proteinuria when CNI was withdrawn, but not when CNI was reduced. The post-conversion increase in proteinuria was greater, when the baseline proteinuria value was higher. In addition, when analyzed overall (both withdrawal and continuation combined based on baseline proteinuria) the group with negative baseline proteinuria had a mean 10.4 mL/min ($P = 0.05$) improvement in CrCL over about 2 years, while the group with baseline proteinuria 0.3-0.8 g/d had a mean 7 mL/min ($P = NS$) improvement in CrCL, and the group with baseline proteinuria > 0.8 g/d had a 5.5mL/min ($P = 0.05$) decline in CrCL. Taken together these results suggested that use of sirolimus to facilitate CNI withdrawal beyond 1 year had the potential for an adverse impact on renal function that was dependent on the baseline level of proteinuria. Another retrospective study[103] examined 30 patients with unspecified baseline regimen and with about 2 years of follow-up based on indication for switching from CNI to sirolimus, as shown in Table 2. They concluded that sirolimus was associated with an improvement in CrCL and an increase in proteinuria, but that the benefits were achieved only when the conversion occurred within the first year after the transplant[103].

Baseline corticosteroid and either azathioprine or calcineurin inhibitor: A cohort study evaluated 19 patients who had sirolimus added and CNI withdrawn by 3 mo for progressive CAN. At 6-mo follow-up, 36% demonstrated improvement in renal function, 21% exhibited stabilization, and 43% resulted in continued worsening. Patients who demonstrated improvement in renal function had lower baseline SCr (2.6 ± 0.9 mg/dL *vs* 3.3 ± 0.7 mg/dL)[104].

Baseline calcineurin inhibitor and corticosteroid with or without mycophenolate mofetil: A retrospective study[105] of patients more than 1 year post-transplant with CAN examined 32 patients for 8.5 mo who had sirolimus added to their regimen and CNI reduced. Only 3 patients had improved SCr (9.4%) and 13 (40.6%) had stable SCr, suggesting that 50% of the population achieved a benefit from the strategy of CNI dose reduction with sirolimus introduction. The authors suggested that the benefit was greater when the baseline SCr was less than 3 mg/dL.

Baseline tacrolimus and mycophenolate mofetil with or without corticosteroids: A prospective, randomized study of 200 patients more than 1 year post-transplant, with about 3.5 years follow-up, examined sirolimus addition with trough target 5-8 ng/mL and tacrolimus withdrawal by week 2 ($n = 123$) or continuation of the current regimen with target tacrolimus trough of 6-8 ng/mL. As shown in Table 2, the GFR decreased, and proteinuria increased to a similar degree in both groups during follow-up, with similar acute cellular rejection (ACR) and graft survival, suggesting no tangible benefit to the late switch[106]. In contrast, a cohort study analyzed

Table 2 Renal transplant studies utilizing sirolimus to withdraw calcineurin inhibitor beyond 6 mo post-transplant

Ref.	Design	Population (*n*)	Baseline regimen	*n*	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Gutierrez *et al*[102]	Cohort	> 1-yr post transplant, chronic allograft dysfunction, no proteinuria	Not specified	8	SRL added, CNI dose reduced 50%	24.6 mo	Proteinuria = +0.56 g/d *vs* baseline (*P* = NS)	NA	90.50%	85.70%
				13	SRL added, CNI withdrawn		Proteinuria = + 0.67 g/d *vs* baseline (*P* = 0.02)			
		> 1-yr post transplant, chronic allograft dysfunction, proteinuria = 0.3-0.8 g/d		10	SRL added, CNI dose reduced 50%	23.2 mo	Proteinuria = +0.5 g/d *vs* baseline (*P* = NS)	NA	83.30%	94.40%
				8	SRL added, CNI withdrawn		Proteinuria = +1.1 g/d *vs* baseline (*P* = 0.05)			
		> 1-yr post transplant, chronic allograft dysfunction, proteinuria > 0.8 g/d		14	SRL added, CNI dose reduced 50%	25.9 mo	Proteinuria = -0.1 g/d *vs* baseline (NS)	NA	79.20%	87.50%
				10	SRL added, CNI withdrawn		Proteinuria = +2.3 g/d *vs* baseline (*P* = 0.01)			
Maharaj *et al*[103]	Retrospective cohort	> 1-yr post transplant, CsA-induced biochemical toxicity	Not specified	6	SRL added, CNI withdrawn	25 mo	Proteinuria = +0.06 g/d *vs* baseline eGFR = +12.2 mL/min *vs* baseline	NA	NA	NA
		> 1-yr post transplant, CAN		6			Proteinuria = +0.85 g/d *vs* baseline eGFR = -9.7 mL/min *vs* baseline	NA	NA	NA
		> 1-yr post transplant, Severe gum hypertrophy		9			Proteinuria = +0.99 g/d *vs* baseline eGFR = -1.0 mL/min *vs* baseline	NA	NA	NA
		4.5 mo post transplant, Posttransplant diabetes		4			Proteinuria = -0.22 g/d *vs* baseline eGFR = +13.3 mL/min *vs* baseline	NA	NA	NA
		5.5 mo post transplant, CNI induced histological nephrotoxicty		2			Proteinuria = +0.63 g/d *vs* baseline eGFR = -10.0 mL/min *vs* baseline	NA	NA	NA
		> 1-yr post transplant, CNI associated malignancy		3			Proteinuria = +0.09 g/d *vs* baseline eGFR = +7.0 mL/min *vs* baseline	NA	NA	NA
Citterlo *et al*[104]	Cohort	> 6-mo post transplant, deteriorating renal function, sCr 2-4.5 mg/dL, proteinuria > 500 mg/d, biopsy confirmed fibrosis, tubular atrophy and intimal hyperplasia	CsA or TAC or azathioprine with corticosteroid	19	SRL added to target trough 8-12 ng/mL, CNI withdrawn by 3 mo	6 mo	Response rate: 57% improved or lacked deterioration in renal function	0%	NA	100%

Wu *et al*[105]	Retrospective cohort	> 1-yr post transplant, CAN	CsA or TAC/corticosteroids orCsA or TAC/corticosteroids/MMF	32	SRL added with CNI dose reduced	8.5 mo	Response rate: 50% improved or lacked deterioration in renal function	3.10%	87.50%	NA
Chhabra *et al*[106]	Randomized, prospective, open-label, single-center	> 1-yr post transplant	TAC, MMF	123	SRL added to target trough 5-8 ng/mL, TAC withdrawn by week 2	41.1 mo	eGFR = -3.3 mL/min per 1.73 m² *vs* baseline proteinuria > 1 g/d = + 4.7% *vs* baseline	5.70% (ACR) 4.1% (AHR)	97.60%	97%
				64	Continue TAC to target trough 6-8 ng/mL	40.7 mo	eGFR = -8.7 mL/min per 1.73 m² *vs* baseline, proteinuria > 1 g/d = + 7.4% *vs* baseline	6.40% (ACR) 3.1% (AHR)	97%	100%
Wali *et al*[107]	Cohort	Renal dysfunction and biopsy confirmed CAN	TAC/MMF or TAC/MMF/corticosteroids	159	SRL added, target trough 8-10 ng/mL, TAC withdrawn after second dose of SRL	24 mo	sCr = -1.1 mg/dL *vs* baseline (*P* < 0.0001) eGFR = +21 mg/dL *vs* baseline (*P* < 0.0001)	9.60%	65%	90%
Diekmann *et al*[108]	Cohort	> 1-yr post transplant, biopsy confirmed CNI toxicity	CsA or TAC/corticosteroids, or CsA or TAC/corticosteroids/azathioprine, or CsA or TAC/corticosteroids/MMF, or CsA or TAC/MMF, or TAC/MMF/corticosteroids CsA or TAC/azathioprine	22	SRL added, target trough 8-12 ng/mL, CsA or TAC reduced by 50% immediately then further reduced 10%-20% weekly	6 mo	sCr = -0.7 mg/dL *vs* baseline (%= NS), Response rate: 59.1% improved or lacked deterioration in renal function	NA	86%	100%
Bumbea *et al*[109]	Prospective, single-center cohort	>6-mo post transplant, chronic allograft dysfunction or recurrent cutaneous cancer	CsA or TAC/corticosteroids, or CsA or TAC/corticosteroids/azathioprine or CsA or TAC/corticosteroids/MMF	43	SRL added, target trough = 8-12 ng/mL, CNI withdrawn abruptly or by week 3	27 mo	sCr = -17.8 μmol/L *vs* baseline (*P* = NS) CrCL = +2.3 mL/min *vs* baseline (*P* = NS) Proteinuria (> 1g/d): 20.6% at 2 yr (*P* = 0.01)	0%	93%	95.30%
Boratynska *et al*[110]	Cohort	> 1-yr post transplant, biopsy confirmed CAN	CsA, prednisone, azathioprine	5	SRL added, target trough 10-18 ng/mL, CsA withdrawn immediately. After 5 mo, SRL withdrawn and CsA reinitiated	3 mo	sCr = +1.6 mg/dL and proteinuria = +461 mg/dL after 3 mo SRL *vs* baseline sCr = +1.1 mg/dL and proteinuria = +6 mg/dL 6 mo after reconversion to CsA *vs* baseline sCr = -0.5 mg/dL and proteinuria = -455 mg/dL after reconversion to CsA *vs* SRL	0%	40%	100%
Martínez-Mier *et al*[111]	Retrospective cohort	> 6-mo post transplant, > 20% sCr increase in 6 mo or sCr 2-4.5 mg/dL	CsA, prednisone, MMF	15	SRL added, target trough 8-12 ng/mL, CsA withdrawn immediately	6 mo	sCr = -0.78 mg/dL *vs* baseline (*P* = 0.003) BUN = - 9.84 mg/dL *vs* baseline (*P* = NS)	0%	100%	100%

Kamar *et al*[112]	Prospective, multicenter, noncomparative, open-label cohort	> 1-yr post transplant, moderate renal insufficiency, sCr 160-265 µmol/L	CsA or TAC, corticosteroids, MMF	44	SRL added to target trough 6-10 ng/mL, CNI withdrawn	6 mo	GFR = +7.09 mL/ min *vs* baseline (*P* = 0.03) Proteinuria = +0.57 g/d	2.30%	100%	100%
Chen *et al*[113]	Cohort	> 6-mo post transplant, biopsy confirmed CAN	CsA or TAC, prednisone, MMF	16	SRL added, target trough 5-8 ng/mL, CNI withdrawn	12 mo	Response rate: 43.8% improved or lacked deterioration in renal function	0%	88%	100%
Stallone *et al*[114]	Prospective, open-label, single-center	> 1-yr post transplant, Scr 1-3 mg/dL	CsA or TAC, corticosteroids, MMF	50	40% CNI dose reduction	24 mo	sCr = -0.02 mg/ dL *vs* baseline (*P* = NS) CrCL -3.0 mL/ min *vs* baseline (*P* = NS) Proteinuria = +0.17 *vs* baseline (*P* = NS) Follow-up biopsy: worsened CAN score, increased α-SMA	0%	84%	100%
				34	SRL added, CNI immediately withdrawn		sCr = -0.14 mg/ dL *vs* baseline (*P* = NS) CrCL = +3.0 mg/ dL *vs* baseline (*P* = NS) Proteinuria = +0.37 g/d *vs* baseline (*P* = NS) Follow-up biopsy: stable CAN score, improved α-SMA	0%	97% (*P* = 0.04)	100%
Paoletti *et al*[115]	Cohort	> 6-mo post transplant, biopsy confirmed renal allograft dysfunction	CsA or TAC, corticosteroids, MMF	13	SRL added, target trough 4-8 ng/mL, CNI withdrawn	3 yr	sCr = -0.3 mg/dL *vs* baseline (*P* = 0.016) eGFR = +5.5 mg/ dL *vs* baseline (*P* = 0.011) Proteinuria = +0.21 g/d *vs* baseline (*P* = 0.83)	8%	100%	100%
		> 6-mo post transplant with stable graft function		26	Continued regimen		sCr= +0.3 mg/dL *vs* baseline (*P* = 0.016) eGFR = -6.4 mg/ dL *vs* baseline (*P* = 0.011) Proteinuria = +0.17 g/d *vs* baseline (*P* = 0.83)	4%	96%	96%
Alarrayed *et al*[116]	Retrospective, Observational, single-center	> 1-yr post transplant, sCr < 140 µmol/L	CsA or TAC, corticosteroids, azathioprine or MMF	45	SRL added to target trough 5-8 ng/mL, CNI withdrawn immediately	72.8 mo	sCr = -6 µmol/L *vs* baseline (*P* = 0.001) Proteinuria = +0.2 g/d *vs* baseline (*P* = NS)	0%	100%	NA
		> 1-yr post transplant, sCr ≥ 140 µmol/L		19			sCr = -13 µmol/L *vs* baseline (*P* = 0.01) Proteinuria = +0.6 g/d *vs* baseline (*P* = 0.001)	36.40%	72.70%	NA

Fischereder *et al*[117]	Prospective cohort	> 1-yr post transplant, deteriorating renal function, Scr 1.8-4 mg/dL	CsA or TAC, corticosteroids, azathioprine or MMF	12	SRL added, target trough = 10-20 ng/mL, CNI withheld by 4 wk	12 mo	sCr = -0.3 mg/dL *vs* baseline (*P* = 0.198) CrCL = +5.8 mL/min (*P* = 0.0368) Proteinuria = +735 mg/g creatinine *vs* baseline (*P* = 0.13)	0%	100%	100%
Schena *et al*[118]	Randomized, prospective, open-label, multicenter, blinded, comparative trial	> 6-mo post transplant, baseline GFR > 40 mL/min	CsA or TAC, corticosteroids, azathioprine or MMF	497	SRL added, target trough 8-20 ng/mL, CNI withdrawn in 1 d, MMF or azathioprine dose reduced or withdrawn	24 mo	GFR = + 1.3 mL/min in patients converted to SRL as compared with patients continued on CNI at 12 mo (*P* = NS) GFR = +1.3 mL/min *vs* baseline, UPr/Cr = -84 *vs* baseline	7.80%	92.40%	95.60%
		> 6-mo post transplant, baseline GFR 20-40 mL/min		58			GFR = + 3.8 mL/min in patients converted to SRL as compared with patients continued on CNI at 24 mo (*P* = NS)	8.60%	65.50%	82.80%
		> 6-mo post transplant, baseline GFR > 40 mL/min		246	Continue regimen		GFR = -1.8 mL/min *vs* baseline, UPr/Cr = -31 *vs* baseline	6.50%	93.90%	96.30%
		> 6-mo post transplant, baseline GFR 20-40 mL/min		29			GFR = + 2.6 mL/min in patients continued on CNI as compared with patients converted to SRL at 12 mo (*P* = NS)	10.30%	62.10%	89.70%

α-SMA: A-smooth muscle actin; AHR: Acute humoral rejection; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; NS: Not significant; SCr: Serum creatinine; TAC: Tacrolimus.

patients on tacrolimus/MMF or tacrolimus/MMF/corticosteroids with biopsy-proven CAN and progressive renal dysfunction when tacrolimus was converted to sirolimus (10 mg per day for 3 d, then 5 mg/d targeting trough levels 8-10 ng/mL[107]. Overall, SCr decreased and GFR improved, as shown in Table 2. About 1/3 of the patients were non-responders. Although first ACR was about 10%, it was less than the rate observed prior to the conversion (17%). Follow-up biopsies demonstrated significant improvement in interstitial fibrosis and tubular atrophy relative to baseline. It is important to note that this study only included patients who tolerated 90 d of sirolimus therapy[107].

Baseline CNI with corticosteroids, or CNI with azathioprine, or CNI with mycophenolate mofetil, or CNI with corticosteroids and azathioprine or mycophenolate mofetil: Two studies evaluated patients with wide variability in baseline regimens[108,109]. One study evaluated patients more than one year post-transplant with biopsy proven CNI toxicity (*n* = 22) and demonstrated a modest decrease in SCr and a 59.1% response rate of improved or lack of progression in renal func-

tion deterioration at 6 mo after CNI conversion to sirolimus[108]. The other study evaluated patients more than 6 mo post-transplant with chronic allograft dysfunction or recurrent cancer and demonstrated a modest, nonsignificant reduction in SCr and increase in CrCL at 27 mo follow-up. However, proteinuria greater than 1 g/d occurred in 20.6% of the population at 2 years[109]. Neither study reported any episodes of ACR[108,109].

Baseline calcineurin inhibitor, corticosteroid and azathioprine: A 5-patient cohort with BP-CAN explored conversion from cyclosporine to sirolimus. After 3 mo, SCr nearly doubled and proteinuria increased, at which time patients were converted back to CNI and proteinuria decreased, but SCr continued to rise, and 3 (60%) patients returned to dialysis[110].

Baseline calcineurin inhibitor, corticosteroid and azathioprine or mycophenolate mofetil: A retrospective study of patients more than 6 mo post-transplant, with a 20% increase in SCr in 6 mo or a current SCr 2-4.5 mg/dL were converted to sirolimus with CNI withdrawn immediately. At 6 mo, there was a significant reduction in

SCr versus baseline, and no evidence of ACR, as shown in Table 2[111]. A prospective, multicentered study of 44 patients more than one year post-transplant with moderate renal insufficiency demonstrated a 7 mL/min (*P* = 0.03) improvement in GFR with a 0.57 g/d increase in proteinuria (*P* = 0.002). Adverse effects observed included an increase in triglycerides, total cholesterol and LDL cholesterol, and a decrease in hemoglobin levels, and one episode of mild ACR[112]. In a cohort of 16 patients with sirolimus added and CNI withdrawn for biopsy proven -chronic allograft nephropathy (BP-CAN), 43.8% demonstrated improved, or lack of deterioration in SCr, without an increased risk of ACR. Patients with SCr at baseline < 2.48 mg/dL were more likely to achieve improvement in SCr after the conversion, and patients with higher SCr or C4d deposition in peritubular capillaries were less likely to achieve success[113].

A prospective open-label single-center study conducted by Stallone and colleagues[114] compared a 40% dose reduction in CNI (*n* = 50) with sirolimus addition and CNI elimination (*n* = 34) at greater than 1 year post-transplant[114]. Compared with baseline, CNI reduction resulted in no significant change in SCr, CrCL or proteinuria versus baseline at 2 years follow-up. To a similar degree, SCr, CrCL or proteinuria were similar to baseline in the CNI withdrawal group, although graft survival was improved (84% *vs* 97%, *P* = 0.04). On follow-up biopsies, CAN grade and α-smooth muscle actin (α-SMA) protein expression worsened in the CNI reduction group, and α-SMA decreased (*P* = 0.005) and CAN grade remained stable in the sirolimus group[114]. Another study compared sirolimus addition and CNI elimination (*n* = 13) in patients with BP-CAN versus CNI continuation (*n* = 26) in patients with stable renal function, at least 6 mo post-transplant followed patients for 3 years[115]. In that study, sirolimus resulted in an improvement in SCr and GFR, with a statistically significant increase in proteinuria relative to baseline, while CNI continuation resulted in worsening of SCr and GFR and a similar degree of proteinuria. There were more cardiovascular events (*P* = 0.024) in the CNI continuation group, although patient survival was similar. The 3-year change in GFR was the only significant predictor of event-free survival by Cox regression analysis (HR = 0.96, 95%CI: 0.93-0.99, *P* = 0.017), and sirolimus was the strongest predictor of GFR[115].

One retrospective study compared the effects of sirolimus addition and CNI elimination relative to baseline SCr (≥ 140 μmol/L *vs* < 140 μmol/L) and found that patients with more baseline renal dysfunction had a larger decline in SCr relative to baseline, but also developed more proteinuria and had a higher rate of ACR (36.4% *vs* 0%)[116]. Another prospective study targeted sirolimus trough 10-20 ng/mL and CNI elimination over 4 wk, in patients more than 1 year post-transplant, and demonstrated a 5.8 mL/min improvement in CrCL along with a non-significant increase in proteinuria at 12 mo[117].

A randomized, prospective, open-label multicentered comparative trial (CONVERT) evaluated sirolimus to facilitate CNI withdrawal in the setting of concurrent azathioprine or MMF reduction or withdrawal versus continuation of the CNI-based regimen, according to baseline GFR (20-40 mL/min *vs* > 40 mL/min) in patients more than 6 mo post-transplant[118]. As shown in Table 2, patients with GFR > 40 mL/min and converted to sirolimus had a non-significant improvement in GFR relative to baseline and relative to CNI continuation at 24 mo. Patients with baseline GFR 20-40 mL/min had a slightly higher, but still non-significant improvement in GFR at 24 mo relative to CNI continuation. Graft survival was poor in all patients with baseline GFR 20-40 mL/min regardless of regimen (62%-66%). A post-hoc analysis revealed that patients with GFR > 40 mL/min who had a baseline urinary protein-to-creatinine ratio (U$_{Pr/Cr}$) less than or equal to 0.11 had more favorable outcome with sirolimus conversion[118].

Summary of sirolimus studies: There appears to be less data comparing sirolimus-facilitated late CNI withdrawal to an active CNI-containing regimen than was found for mycophenolic acid (MPA)-facilitated CNI withdrawal. Sirolimus has the potential to support late CNI withdrawal, through a modest improvement in short-term renal function, which has been corroborated in a systematic review[119]. However, the benefit of sirolimus is somewhat limited by an increased risk of proteinuria, especially in the setting of baseline renal dysfunction and/or proteinuria and high rate of discontinuation for adverse effects which ranges from 17% in nonrandomized trials to 28% of randomized trials[119-121]. Adverse effects of sirolimus on renal function were confirmed in a trial which evaluated late sirolimus withdrawal using MMF and found improvement in the slope 1/SCr in 15 of 17 (88%) patients[122]. Renal function results associated with use of sirolimus appear to be improved to a relatively greater degree when sirolimus is used in combination with mycophenolate mofetil[123]. This combination may increase the risk of MMF adverse effects, in part due to a drug-drug interaction[123,124]. It should also be noted that use of reduced dose CNI in conjunction with sirolimus may also suffer from a pharmacokinetic interaction, which potentiates each's nephrotoxicity[125].

Regimens utilizing everolimus to eliminate calcineurin inhibitors

Baseline calcineurin inhibitor and unspecified adjunctive agents: A second mTOR inhibitor, everolimus has also generated evidence on late CNI withdrawal in renal transplantation. As shown in Table 3[126-135], a small case series evaluated 21 Hispanic first renal transplant patients (15 cadaveric), including 5 children, who were undergoing conversion from CNI to everolimus with MPA at a mean 8 mo post-transplant, due to CAN or CNI toxicity. Over 10-mo follow-up there was no mortality or graft loss and a slight mean decline of SCr of 0.2 mg/dL, but ACR rate was 17%[126]. Another case series of 78 patients converted CNI to everolimus at a mean 77 mo post-transplant, without manipulation or addition of MPA, and noted a statistically significant mean increase

Table 3 Renal transplant studies utilizing everolimus to withdraw calcineurin inhibitor beyond 6 mo post-transplant

Ref.	Design	Population (*n*)	Baseline regimen	*n*	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Giron *et al*[126]	Case series	Conversion due to unspecified reasons in Hispanic renal transplant patients (15 from cadaveric donors), mean conversion 8 mo post-transplant	CsA or TAC, and unspecified regimen	21	Everolimus added with MPS or MMF with complete suspension of CNI	10 mo (range, 2 to 22)	Mean SCr showed a trend to decline: preconversion 1.7 mg/dL; post-conversion 1.5 mg/dL	17%	100%	100%
Sánchez Fructuoso *et al*[127]	Case series, prospective, open	CAN or other reasons, stable renal function, mean 77 mo post-transplant	CNI and unspecified regimen	78	Switched to everolimus with complete and quick elimination of the CNI: An initial dose of 3 mg/d was adequate to obtain the recommended trough levels between 5 and 10 ng/mL	12 mo	Baseline CrCL = 51.9 ± 2.7 mL/min, and 3 mo = 55.7 ± 3.2 (*P* = 0.02). 12-mo CrCL not stated. Proteinuria = increased at 3 mo (*P* < 0.001), decreased between 3 to 6 mo (*P* = 0.001), but remained higher than basal levels (*P* = 0.002). Everolimus stopped in 13 patients (16.7%)	NA	NA	NA
Ruiz *et al*[128]	Case Series	CAN with deteriorating renal function	CsA or TAC, and unspecified regimen; tripe drug (41%), double-drug (52%), monotherapy (7%)	32	Everolimus added, to eliminate CNI	6 mo	Baseline SCr 1.93 ± 0.13 mg/dL *vs* 1.86 ± 0.14, *P* = 0.07. Proteinuria = 1.62 ± 0.62 g/d *vs* 2.11 ± 0.73 (*P* = 0.11)	NA	NA	NA
Fernández *et al*[129]	Case series	Cadaveric renal transplant patients with CAN, at a mean 123.8 ± 74.2 mo post-transplant	CsA or TAC, ± MMF or azathioprine, corticosteroid not specified	17	Converted to everolimus with complete suspension of CNI	24 mo	Baseline SCr of 1.8 ± 0.4; after a year, 1.62 ± 0.49; and after 2 yr, 1.56 ± 0.49 mg/dL (*P* < 0.05). Proteinuria was baseline 0.30 ± 0.13 mg/mg, 1 yr = 0.63 ± 0.68 (*P* < 0.05), and 2 yr = 0.48 ± 0.34. Protein/creatinine quotient was: baseline 0.30 ± 0.13; one year 0.63 ± 0.68; and 2 yr 0.48 ± 0.34. CrCL was baseline 37.1 ± 11.14 mL/min and 2 yr = 46.6 ± 14.6 (*P* < 0.05)	NA	NA	100%
		Cadaveric renal transplant patients treated with non-CAN diagnosis at a mean 123.8 ± 74.2 mo post-transplant	CsA or TAC, ± MMF or azathioprine, corticosteroid not specified	10	Converted to everolimus with complete suspension of CNI	24 mo	Baseline SCr of 1.1 ± 0.32 mg/dL; , 1 yr 0.97 ± 0.15, and 2 yr 0.97 ± 0.15. Proteinuria at baseline 0.12 ± 0.07 mg/mg, 1 yr = 0.46 ± 0.68 (*P* < 0.05), and 2 yr = 0.32 ± 0.17 (*P* < 0.05). Protein/creatinine quotient was: baseline 0.2 ± 0.07, 1 yr = 0.73 ± 0.7, and 2 yr = 0.32 ± 0.17. CrCL was baseline 68.81 ± 19 mL/min and 2 yr 74.56 ± 12.3	NA	NA	50%, due to tumors

Kamar *et al*[130]	Retrospective case-control	DSA-free kidney transplant patients with CNI toxicity, CAN or other diagnosis	CsA or TAC or belatacept, ± MPA or azathioprine, ± corticosteroids	61	Converted to everolimus-based regimen without CNIs	36 ± 25 mo	SCr (mmol/L) baseline 135 ± 37 to 141 ± 54 (*P* = NS). aMDRD GFR (mL/min) 54 ± 18 to 56 ± 22 (*P* = NS)	NA	NA	NA
			CsA or TAC, ± MPA or azathioprine, ± corticosteroids	61	Matched control patients on CNI		SCr (mmol/L) baseline 133 ± 51 to 131 ± 45 (*P* = NS). aMDRD GFR (mL/min) 65.7 ± 25 to 62 ± 24 (*P* = NS)			
Morales *et al*[131]	Case series	1st or 2nd transplant, converted due to CAN, nephrotoxicty or malignancy, mean 5 yr post-transplant	CsA or TAC, ± MMF or azathioprine, ± corticosteroid	8	Everolimus added to replace (*n* = 6) or decrease (30% reduction) CNI dose (*n* = 2) Antiproliferative dose reduced.	1-16 mo	Mean baseline SCr was 1.96 ± 0.69 mg/dL *vs* 1.59 ± 0.52. Mean CrCL = 51 ± 34.6 mL/min *vs* 56.5 ± 25.5. Mean Proteinuria:creatinine ratio = 1.34 ± 2.17 *vs* 1.28 ± 1.19 mg/g.	NA	NA	NA
Holdaas *et al*[132]	Prospective, randomized, open-label, multi-center. ASCERTAIN study	> 6-mopost transplant, renal impairment, no recent ACR < 3 mo	CsA or TAC, ± MPA or azathioprine, ± corticosteroids	127	Everolimus added, target 8-12 ng/mL; to eliminate CNI	24 mo	Mean measured GFR at month 24, 48 ± 22 mL/min per 1.73 m^2 Difference *vs* control was 1.12 mL/min per 1.73 m^2, 95%CI : -3.51-5.76 (*P* = 0.63). Urine protein:creatinine (mg/mmol) median increased from baseline 16.6 (3.5-413.7) to 32.6 (4.1-665.9; *P* = 0.007 *vs* control)	5.50%	94.50%	97.60%
				144	Everolimus added, target 3-8 ng/mL; to decrease CNI dose		Mean measured GFR at month 24, 46.6 ± 21.1 mL/min per 1.73 m^2. Difference *vs* control was 0.59 mL/min per 1.73 m^2, 95%CI: -3.88-5.07 (*P* = 0.79). Urine protein:creatinine (mg/mmol) median increased from baseline 13.5 (2.4-319.4) to 22.4 (5.1-513.5; *P* = 0.54 *vs* control)	5.60%	92.40%	97.90%
				123	Controls maintained current CNI-based regimen		Mean measure GFR at month 24 46 ± 20.4 mL/min. Urine protein:creatinine (mg/mmol) median remained stable from baseline 14.3 (3.3-431.9) to 19.3 (3.3-431.9)	2.40%	95.10%	100%
Inza *et al*[133]	Case series	Cadaveric kidney allograft, SCr > 2 mg/dL, proteinuria < 1 g/ 24 h	CsA or TAC, ± MPA or sirolimus, corticosteroids	22	Switched CNI to Everolimus, mean starting dose 1.4 mg/d.	24 mo	Baseline CrCL 29.31 ± 10.15 mL/min to 3-mo 37.99 ± 14.44 (*P* = 0.0076). No results specified for 24 mo, but authors stated CrCL trended to decline (*P* = 0.6). Proteinuria (mg/24 h) increased from baseline 384 ± 26.13 to one month, 958 ± 1019.38 (*P* = 0.05), to month 12, 1295 ± 1200.83 (*P* = 0.0106)	4.50%	90.50%	100%

Cataneo-Dávila *et al*[134]	Prospective, randomized, open pilot	> 6-mo post transplant, stable renal function, Banff grade I or II CAN within 6 mo, without ACR or grade III CAN in last 3 mo	CsA or TAC, MMF or azathioprine, corticosteroids	10	MMF or azathioprine were withdrawn and Everolimus added to decrease CNI dose by 80%.	12 mo	Baseline and end-of-study data were as follows: SCr, 1.27 ± 0.35 mg/dL *vs* 1.24 ± 0.4 mg/dL; estimated GFR = 72.4 ± 19.86 mL/min *vs* 76.26 ± 22.69 mL/min (*P* = NS); microalbuminuria 0 mg/g (range 0-50) *vs* 0 (range 0-609; *P* = NS)	10%	NA	NA
			CsA or TAC, MMF or azathioprine, corticosteroids	10	Everolimus added to eliminate CNI gradually. MMF or azathioprine withdrawn, then re-introduced at CNI elimination		Baseline and end-of-study data were as follows: SCr 1.27 ± 0.36 mg/dL *vs* 1.25 ± 0.3 mg/dL; estimated GFR 66.2 ± 12.95 mL/min *vs* 66.2 ± 13.73 mL/min (*P* = NS); microalbuminuria 0 mg/g (range 0-60) *vs* 0 (range 0-34; *P* = NS)	0%	NA	NA
Albano *et al*[135]	Prospective, randomized, open-label, multi-center. FOREVER trial	Completion of CALLISTO study of patients at risk for DGF, from transplantation to month 12, with proteinuria < 1 g/24 h at month 12	Low-exposure CsA, everolimus, corticosteroids	15	Switch CsA to mycophenolate sodium 720 mg/d, increase everolimus, target trough goal 6-10 ng/mL	12 mo	Median (range) mGFR was 54 (21-87) mL/min at baseline (*P* = 0.053 *vs* CNI at baseline) *vs* 56 (18-126) mL/min at month 12 (*P* = 0.007 *vs* CNI continuation; *P* = 0.3 *vs* baseline). Difference in mGFR (SE) was +10.3 mL/min (4.8) *vs* baseline. SCr (SE) = 24 μmol/mL (27). Proteinuria least squares mean change from baseline (SE) = 0.16 g/24 h (0.2)	0%	100%	100%
				15	Continue CsA and everolimus unchanged, trough goal 3-8 ng/mL		Median (range) mGFR was 37 (range 18-69) mL/min at baseline (*P* = 0.053) *vs* 32 (12-63) mL/min at month 12 (*P* = 0.007). Difference in mGFR (SE) was -4.1 mL/min (5) *vs* baseline. Proteinuria least squares mean change from baseline (SE) = 0.08 g/24 h (0.23)	6.67%	100%	93.3%

ACR: Acute cellular rejection; aMDRD: Abbreviated modified diet in renal disease; BP-CAN: Biopsy-proven chronic allograft nephropathy; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; DGF: Delayed graft function; DSA: Donor specific antibody; eGFR: Estimated glomerular filtration rate; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; MPA: Mycophenolic acid (includes MMF and MPS); MPS: Mycophenolate sodium; NA: Not assessed/applicable; NCR: Not clearly reported by group; NS: Not significant; SCr: Serum creatinine; TAC: Tacrolimus.

in CrCL of 3.8 mL/min at 3 mo post-conversion, but 12-mo CrCL was not stated. It should be noted that proteinuria increased from baseline at all time points studied, and 16.7% of patients stopped everolimus due to worsening renal function (*n* = 5), dermal eruptions (*n* = 3), or other reasons (*n* = 5)[127]. A case series of 32 patients took patients with deteriorating renal function in the face of CAN and added everolimus to eliminate CNI. At 6-mo, SCr decreased slightly, but not significantly (*P* = 0.07), and proteinuria trended toward an increase (*P* = 0.11)[128]. Of particular interest, a small study retrospectively com-pared 17 patients with CAN converted to everolimus with 10 patients being converted to everolimus for other reasons. In the CAN group, SCr was higher and CrCL lower at baseline relative to the non-CAN group. SCr in the CAN group decreased steadily out to 2 years follow-up (*P* < 0.05), and CrCL improved significantly, with 100% patient survival. In contrast, the non-CAN group did not demonstrate a significant improvement in SCr or CrCL, and had a 50% mortality rate due to malignancy present at the time of the switch. An increase in protein-uria was observed in both groups[129].

Baseline calcineurin inhibitor or belatacept with or without mycophenolic acid or azathioprine or corticosteroids: A retrospective case-control study evaluated patients on a CNI or belatacept with or without MPA or azathioprine or corticosteroids (*n* = 61) converted to everolimus, and another 61 matched patients maintained on CNI-based regimen to determine if DSAs developed after conversion. At mean 36 mo follow-up there was no changes from baseline or between the groups in SCr or CrCL. None of the patients had DSAs at baseline, but the everolimus group had a follow-up incidence of 9.8% (*P* = 0.03) and the CNI continuation group had an incidence of 5% (*P* = NS). The only factor independently associated with DSA development was higher age at transplantation, associated with less DSA formation. Overall, 33% of everolimus patients withdrew from everolimus treatment at a mean 32 mo, due to DSA formation (*n* = 5), lymphedema (*n* = 4), proteinuria (*n* = 3), and other reasons. None of the patients switched back to CNI developed DSAs[130]. Another case series examined 8 patients converted from CNI to everolimus at approximately 5 years post-transplant for CAN or malignancy. Everolimus replaced the CNI in 6 patients and was used to lower the CNI dose 30% in 2 patients. At 1-16 mo, SCr reduced slightly, CrCL improved slightly, and proteinuria:creatinine ratio decreased slightly. Three of the 8 patients developed serious infections[131]. A more robust study, the ASCERTAIN study[132], was a prospective, randomized, open-label, multicenter study with 24 mo follow-up. Patients enrolled were at least 6 mo post-transplant (mean 5.6 years), with renal impairment, and without ACR within 3 mo. The study compared addition of everolimus to eliminate CNI (*n* = 127), addition of everolimus to decreased CNI dose (*n* = 144) and controls maintained on CNI (*n* = 123). Overall, at 24-mo follow-up, ACR rates, graft survival and patient survival were similar. The primary endpoint of the study, CrCL at 24 mo, was not met, because CrCL was similar in all the groups at baseline and at follow-up. Proteinuria increased from baseline and relative to control in the CNI elimination group. Post-hoc analysis showed that patients with a baseline CrCL > 50 mL/min had a larger improvement in CrCL after CNI elimination[132].

Baseline calcineurin inhibitor with mycophenolic acid or azathioprine and corticosteroids: In cadaveric recipients on a CNI with MPA or azathioprine and corticosteroids and a SCr > 2 mg/dL with proteinuria less than 1 g/24 h, everolimus was used to withdraw CNI. CrCL improved from baseline to 3 mo, but no results for 24 mo were presented, although the authors noted a trend toward decline. Proteinuria increased by one month (*P* = 0.05) and more than 3-fold by month 12 (*P* = 0.0106). Two of the 22 patients lost their grafts due to nephrotic syndrome and increasing SCr, and one patient developed ACR[133]. Another study compared 10 patients managed with everolimus to facilitate an 80% CNI dose reduction versus 10 patients with gradual complete CNI elimination. MMF or azathioprine were withdrawn when

everolimus was introduced in both groups, but were reintroduced only when the CNI was eliminated. At 12 mo, both groups had similar follow-up SCr, GFR and microalbuminuria, as well as similar changes from baseline. ACR occurred in 10% of the CNI reduction group and none of the CNI elimination group. It is interesting to note that in this study, many of the patients received angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs), which could have impacted the degree of proteinuria. Triglycerides and total cholesterol increased due to everolimus[134].

Baseline calcineurin inhibitor with everolimus and corticosteroids: The FOREVER trial[135] examined patients previously enrolled in another trial of CsA with everolimus who either switched CsA to MPS and increased everolimus (*n* = 15) or continued CsA and everolimus. This study, although prospective and randomized, suffered from differences in baseline GFR between the groups that impacted the interpretation of the results. The median (range) baseline measured GFR was 54 (21-87) mL/min in the CsA withdrawal group, and 37 (18-69) mL/min in the CsA continuation group (*P* = 0.053). The difference at follow-up in GFR was -14.4 mL/min for the CsA continuation group, which did not meet statistical significance. Study drugs were discontinued in 7% of the CNI-free patients and 20% of the CNI-treated patients. Adverse event rates were similar, except aphthous stomatitis and pyrexia were more common in the CNI-free group, and hypertension, proteinuria, acute renal failure and urinary tract infection were more common in the CNI-treated patients[135].

Summary of everolimus studies: Although the majority of evidence was from small, low-quality studies, there was clear evidence of an increase in proteinuria with conversion to everolimus from a CNI-based regimen, similar to what has been observed with sirolimus[119-121,127-129]. It is interesting to speculate that this may be manageable with ACEI or ARBs[134]. As expected, everolimus also had an adverse effect profile similar to sirolimus[112,122,134,135]. Here was evidence of a modest short-term improvement in renal function after CNI elimination with use of everolimus, and like sirolimus, combination of the mTOR inhibitor and the CNI resulted in enhanced adverse effect profile[122,125,135]. Also, like sirolimus, there was little evidence comparing late CNI withdrawal to an active CNI-containing regimen.

Regimens utilizing other agents to eliminate calcineurin inhibitors

Calcineurin inhibitor and variable adjunctive agents: A randomized, open label phase Ⅱ trial[136] evaluated the T cell costimulation blocker, belatacept for comparison with continued CNI in patients 6-36 mo post-transplant. Patients were randomized to switch to belatacept (*n* = 84) intermittent therapy (5 mg/kg on days 1, 15, 29, 43 and 57, followed by every 28 d thereafter), or to continue the current regimen, which consisted of CNI and the current

regimen (80.7% corticosteroid, 3.4% azathioprine, and 94.3% MMF or MPA). Patients randomized to belatacept underwent a progressive taper to eliminate CNI by day 29. The primary endpoint was renal function over 12 mo as determined by calculated GFR, and the belatacept group improved 7 ± 11.99 mL/min and the CNI group improved 2.1 ± 10.34 mL/min from baseline (*P* = 0.0058 for comparison at follow-up). Patients in the belatacept group with a baseline CrCL 45-60 mL/min exhibited the greatest numeric improvement (10 ± 13.41 mL/min). Belatacept patients with baseline CrCL < 45 mL/min improved 3.7 ± 11.01 mL/min and patients with CrCL > 60 mL/min improved 5.7 ± 10.17 mL/min. In contrast, patients remaining on CNI exhibited similar CrCL change according to baseline CrCL stratification, ranging from 1.9-2.8 mL/min. Mild to moderate ACR occurred in 6 patients in the belatacept group, all within the first 6 mo. Four of these patients were on belatacept therapy and 2 had discontinued belatacept. SCr returned to baseline in 4 of the 6 patients. No ACR episodes were reported in the CNI continuation group. Proteinuria occurred in one patient in each group. No grafts were lost in either group in the first 12 mo. One patient in the CNI group died with a functioning graft on day 142. Serious adverse event occurred in 24% of the belatacept group and 19% of the CNI continuation group. The biggest discrepancy in the adverse effects, pyrexia occurred in 4% of the belatacept group and 0% of the CNI group[136]. A 2-year follow-up to this study demonstrated 1 additional graft loss in each group, no additional ACR, and a mean change in CrCL from baseline 8.8 mL/min in the belatacept group and 0.3 mL/min in the CNI continuation group. Serious adverse events occurred in 37% of the belatacept group and 33% of the CNI group[137].

PEDIATRIC PATIENTS 6 OR MORE MONTHS POST-TRANSPLANT

Pediatric renal transplant patients also commonly receive CNIs and are at risk for potential CNI nephrotoxicity. Based on a comparison with adult kidney transplant recipients, pediatric patients have similar graft survival at 10 years (*P* = 0.4325), with similar rates of delayed graft function and SCr levels. However, acute rejections were more common in pediatric patients, and 10-year patient survival tends to be lower in the pediatric transplant group (90.3% *vs* 76.8%; *P* < 0.02)[138]. Consequently, pediatric patients are at similar or greater risks as adult patients, depending on the endpoint studied, and thus may be considered for immunosuppression changes from CNIs over time[139].

Regimens using mycophenolic acid or sirolimus to eliminate CNIs
CNI and variable regimen: Weintraub and colleagues retrospectively evaluated 17 patients on a baseline regimen of CNI plus either sirolimus, MMF or azathioprine, with or without corticosteroids who were being switched

to sirolimus or MMF for CNI toxicity (*n* = 9), CAN (*n* = 6) or diabetes mellitus (*n* = 2) at a mean 5.9 years post-transplant. Mean CrCL actually decreased from baseline after the switch at 6 mo (*P* = 0.04) and 12 mo (*P* = 0.02), and 41% of patient developed ACR. Risk of ACR was predicted by prior AR history, which was present in 9 of 17 patients, lower sirolimus trough levels, and lower calcineurin inhibitor toxicity scores. Graft loss occurred in 24% of patients and was associated with worse CrCL, proteinuria, and histologic chronicity. Proteinuria increased in a manner unrelated to sirolimus use. Four patients returned to a CNI-base regimen based on adverse effects. The authors suggested that worsened graft function and graft loss after conversion could be minimized by selecting patients with high CNI toxicity scores and low chronicity scores on biopsy, and excluding patients with a history of ACR[140].

Regimens using mycophenolic acid to eliminate CNIs
Baseline CNI, corticosteroid and azathioprine : In another study of patients averaging 40 mo post-transplant, but at least 3 mo post-transplant, conversion from CNI, azathioprine and corticosteroid to MMF plus corticosteroid (*n* = 29) or addition of MMF and elimination of azathioprine, without CNI withdrawal (*n* = 9) resulted in overall patient survival of 100% and graft survival of 94% at approximately 5-year follow-up. There was no significant difference in ACR or proteinuria between the groups. Introduction of MMF resulted in improvement in GFR over 2 year regardless of which group was evaluated, but the patients with CNI withdrawn had a numerically increased GFR[141].

Regimens using sirolimus and MMF to eliminate CNI
Baseline calcineurin inhibitor, corticosteroid and azathioprine: A group retrospectively analyzed addition of sirolimus and MMF to eliminate CNI, and compared the strategy to CNI minimization (39% dose reduction), MMF and corticosteroid. One year after conversion, the sirolimus group had a 10.3 ± 3 mL/min improvement in CrCL (*P* < 0.05) versus baseline, while the CNI minimization group had a 17.7 ± 7.1 mL/min (*P* < 0.05) improvement in CrCL. No patient experienced ACR in either group. The authors concluded that sirolimus and MMF introduction had similar benefit to MMF introduction with CNI minimization[142].

Summary of pediatric studies: Data is currently very limited on late CNI withdrawal to improve renal function and further study is required. Patient characteristics may impact the success of selected regimens.

CONCLUSION

This manuscript presents available evidence on late conversion, beyond 6 mo, from CNIs to alternative regimens as a means to aid practicing clinicians in determining therapeutic options for patients exhibiting CNI toxicity

or CAN. Although recent evidence suggests that CNI toxicity and CAN are non-specific findings, and graft dysfunction may alternatively or additionally be a function of C4d and DSA, it has been shown that 5-year graft survival is not independently predicted by DSA and C4d, suggesting that clinicians will still modify regimens based on the presence of CAN and CNI toxicity on biopsy[143-146]. These studies provide moderate-level evidence of a short-term improvement in renal function, that is not without regimen-specific risks, such as increased infection rate with MPA or proteinuria with mTOR inhibitors. There appears to be a "point of no return" after which kidney damage is irreversible and the patient stands to benefit less from withdrawal of CNI[103-105,132]. Since the benefit of late withdrawal appears to be modest and dependent on baseline renal function, the second manuscript in this series will evaluate the data surrounding early conversion and de novo CNI avoidance.

REFERENCES

1   **Wolfe RA**, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; **341**: 1725-1730 [PMID: 10580071 DOI: 10.1056/NEJM199912023412303]

2   **Tonelli M**, Wiebe N, Knoll G, Bello A, Browne S, Jadhav D, Klarenbach S, Gill J. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant* 2011; **11**: 2093-2109 [PMID: 21883901 DOI: 10.1111/j.1600-6143.2011.03686]

3   **Fiebiger W**, Mitterbauer C, Oberbauer R. Health-related quality of life outcomes after kidney transplantation. *Health Qual Life Outcomes* 2004; **2**: 2 [PMID: 14713316 DOI: 10.1186/1477-7525-2-2]

4   **Ojo AO**, Hanson JA, Meier-Kriesche H, Okechukwu CN, Wolfe RA, Leichtman AB, Agodoa LY, Kaplan B, Port FK. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. *J Am Soc Nephrol* 2001; **12**: 589-597 [PMID: 11181808]

5   **Meier-Kriesche HU**, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant* 2004; **4**: 378-383 [PMID: 14961990 DOI: 10.1111/j.1600-6143.2004.00332.x]

6   **Tantravahi J**, Womer KL, Kaplan B. Why hasn't eliminating acute rejection improved graft survival? *Annu Rev Med* 2007; **58**: 369-385 [PMID: 17002551 DOI: 10.1146/annurev.med.58.061705.145143]

7   **Naesens M**, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009; **4**: 481-508 [PMID: 19218475 DOI: 10.2215/CJN.04800908]

8   **Tanabe K**. Calcineurin inhibitors in renal transplantation: what is the best option? *Drugs* 2003; **63**: 1535-1548 [PMID: 12887261]

9   **Hernández D**, Miquel R, Porrini E, Fernández A, González-Posada JM, Hortal L, Checa MD, Rodríguez A, García JJ, Rufino M, Torres A. Randomized controlled study comparing reduced calcineurin inhibitors exposure versus standard cyclosporine-based immunosuppression. *Transplantation* 2007; **84**: 706-714 [PMID: 17893603]

10 **Artz MA**, Boots JM, Ligtenberg G, Roodnat JI, Christiaans MH, Vos PF, Blom HJ, Sweep FC, Demacker PN, Hilbrands LB. Improved cardiovascular risk profile and renal func-

tion in renal transplant patients after randomized conversion from cyclosporine to tacrolimus. *J Am Soc Nephrol* 2003; **14**: 1880-1888 [PMID: 12819249 DOI: 10.1097/01.ASN.0000071515.27754.67]

11 **Artz MA**, Boots JM, Ligtenberg G, Roodnat JI, Christiaans MH, Vos PF, Moons P, Borm G, Hilbrands LB. Conversion from cyclosporine to tacrolimus improves quality-of-life indices, renal graft function and cardiovascular risk profile. *Am J Transplant* 2004; **4**: 937-945 [PMID: 15147428 DOI: 10.1111/j.1600-6143.2004.00427.x]

12 **Shihab FS**, Waid TH, Conti DJ, Yang H, Holman MJ, Mulloy LC, Henning AK, Holman J, First MR. Conversion from cyclosporine to tacrolimus in patients at risk for chronic renal allograft failure: 60-month results of the CRAF Study. *Transplantation* 2008; **85**: 1261-1269 [PMID: 18475181 DOI: 10.1097/TP.0b013e31816b4388]

13 **Meier M**, Nitschke M, Weidtmann B, Jabs WJ, Wong W, Suefke S, Steinhoff J, Fricke L. Slowing the progression of chronic allograft nephropathy by conversion from cyclosporine to tacrolimus: a randomized controlled trial. *Transplantation* 2006; **81**: 1035-1040 [PMID: 16612281]

14 **Vincenti F**, Friman S, Scheuermann E, Rostaing L, Jenssen T, Campistol JM, Uchida K, Pescovitz MD, Marchetti P, Tuncer M, Citterio F, Wiecek A, Chadban S, El-Shahawy M, Budde K, Goto N. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007; **7**: 1506-1514 [PMID: 17359512 DOI: 10.1111/j.1600-6143.2007.01749.x]

15 **Nashan B**, Cole E, Levy G, Thervet E. Clinical validation studies of Neoral C(2) monitoring: a review. *Transplantation* 2002; **73**: S3-S11 [PMID: 12023607]

16 **Jacobson PA**, Schladt D, Israni A, Oetting WS, Lin YC, Leduc R, Guan W, Lamba V, Matas AJ. Genetic and clinical determinants of early, acute calcineurin inhibitor-related nephrotoxicity: results from a kidney transplant consortium. *Transplantation* 2012; **93**: 624-631 [PMID: 22334041 DOI: 10.1097/TP.0b013e3182461288]

17 **Kuypers DR**, Naesens M, de Jonge H, Lerut E, Verbeke K, Vanrenterghem Y. Tacrolimus dose requirements and CYP3A5 genotype and the development of calcineurin inhibitor-associated nephrotoxicity in renal allograft recipients. *Ther Drug Monit* 2010; **32**: 394-404 [PMID: 20526235 DOI: 10.1097/FTD.0b013e3181e06818]

18 **Kuypers DR**, de Jonge H, Naesens M, Vanrenterghem Y. A prospective, open-label, observational clinical cohort study of the association between delayed renal allograft function, tacrolimus exposure, and CYP3A5 genotype in adult recipients. *Clin Ther* 2010; **32**: 2012-2023 [PMID: 21118736 DOI: 10.1016/j.clinthera.2010.11.010]

19 **de Jonge H**, de Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. In vivo CYP3A activity is significantly lower in cyclosporine-treated as compared with tacrolimus-treated renal allograft recipients. *Clin Pharmacol Ther* 2011; **90**: 414-422 [PMID: 21753749 DOI: 10.1038/clpt.2011.130]

20 **Mino Y**, Naito T, Otsuka A, Takayama T, Ozono S, Kagawa Y, Kawakami J. Cyclosporine alters correlation between free and total mycophenolic acid in kidney transplant recipients in the initial phase. *J Clin Pharm Ther* 2011; **36**: 217-224 [PMID: 21366651 DOI: 10.1111/j.1365-2710.2010.01168.x]

21 **Cosio FG**, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; **5**: 2464-2472 [PMID: 16162196 DOI: 10.1111/j.1600-6143.2500.01050.x]

22 **Snanoudj R**, Royal V, Elie C, Rabant M, Girardin C, Morelon E, Kreis H, Fournet JC, Noël LH, Legendre C. Specificity of histological markers of long-term CNI nephrotoxicity in kidney-transplant recipients under low-dose cyclosporine therapy. *Am J Transplant* 2011; **11**: 2635-2646 [PMID:

21883915 DOI: 10.1111/j.1600-6143.2011.03718.x]

23   **Matas AJ**, Leduc R, Rush D, Cecka JM, Connett J, Fieberg A, Halloran P, Hunsicker L, Cosio F, Grande J, Mannon R, Gourishankar S, Gaston R, Kasiske B. Histopathologic clusters differentiate subgroups within the nonspecific diagnoses of CAN or CR: preliminary data from the DeKAF study. *Am J Transplant* 2010; **10**: 315-323 [PMID: 20041864 DOI: 10.1111/j.1600-6143.2009.02943.x]

24   **Terasaki PI**, Ozawa M. Predictive value of HLA antibodies and serum creatinine in chronic rejection: results of a 2-year prospective trial. *Transplantation* 2005; **80**: 1194-1197 [PMID: 16314785 DOI: 10.1097/01.tp.0000174338.97313.5a]

25   **Sellarés J**, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, Hidalgo LG, Famulski K, Matas A, Halloran PF. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388-399 [PMID: 22081892 DOI: 10.1111/j.1600-6143.2011.03840.x]

26   **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326-2333 [PMID: 14668458 DOI: 10.1056/NEJMoa020009]

27   **Paul LC**. Chronic allograft nephropathy: An update. *Kidney Int* 1999; **56**: 783-793 [PMID: 10469349 DOI: 10.1046/j.1523-1755.1999.00611.x]

28   **Chapman JR**, O'Connell PJ, Nankivell BJ. Chronic renal allograft dysfunction. *J Am Soc Nephrol* 2005; **16**: 3015-3026 [PMID: 16120819 DOI: 10.1681/ASN.2005050463]

29   **Grinyo JM**, Saval N, Campistol JM. Clinical assessment and determinants of chronic allograft nephropathy in maintenance renal transplant patients. *Nephrol Dial Transplant* 2011; **26**: 3750-3755 [PMID: 21474575 DOI: 10.1093/ndt/gfr091]

30   **Chapman JR**. Chronic calcineurin inhibitor nephrotoxicity-lest we forget. *Am J Transplant* 2011; **11**: 693-697 [PMID: 21446974 DOI: 10.1111/J.1600-6143.2011.03504.x]

31   **Weir MR**, Wali RK. Minimizing the risk of chronic allograft nephropathy. *Transplantation* 2009; **87**: S14-S18 [PMID: 19384181 DOI: 10.1097/TP.0b013e3181a079c0]

32   **Meier-Kriesche HU**, Chu AH, David KM, Chi-Burris K, Steffen BJ. Switching immunosuppression medications after renal transplantation--a common practice. *Nephrol Dial Transplant* 2006; **21**: 2256-2262 [PMID: 16574677 DOI: 10.1093/ndt/gfl134]

33   **Opelz G**, Döhler B. Effect on kidney graft survival of reducing or discontinuing maintenance immunosuppression after the first year posttransplant. *Transplantation* 2008; **86**: 371-376 [PMID: 18698238]

34   **Knoll GA**, MacDonald I, Khan A, Van Walraven C. Mycophenolate mofetil dose reduction and the risk of acute rejection after renal transplantation. *J Am Soc Nephrol* 2003; **14**: 2381-2386 [PMID: 12937317 DOI: 10.1097/01.ASN.0000079616.71891.F5]

35   **Pascual J**, Marcén R, Ortuño J. Renal function: defining long-term success. *Nephrol Dial Transplant* 2004; **19** Suppl 6: vi3-vi7 [PMID: 15575023 DOI: 10.1093/ndt/gfh1062]

36   **Kaplan B**, Schold JD, Meier-Kriesche HU. Long-term graft survival with neoral and tacrolimus: a paired kidney analysis. *J Am Soc Nephrol* 2003; **14**: 2980-2984 [PMID: 14569110 DOI: 10.1097/01.ASN.0000095250.92361.D5]

37   **Cheung CY**, Wong KM, Chan HW, Liu YL, Chan YH, Wong HS, Chak WL, Choi KS, Chau KF, Li CS. Paired kidney analysis of tacrolimus and cyclosporine microemulsion-based therapy in Chinese cadaveric renal transplant recipients. *Transpl Int* 2006; **19**: 657-666 [PMID: 16827683 DOI: 10.1111/j.1432-2277.2006.00335.x]

38   **Stegall MD**, Park WD, Larson TS, Gloor JM, Cornell LD, Sethi S, Dean PG, Prieto M, Amer H, Textor S, Schwab T, Cosio FG. The histology of solitary renal allografts at 1 and 5 years after transplantation. *Am J Transplant* 2011; **11**: 698-707 [PMID: 21062418 DOI: 10.1111/j.1600-6143.2010.03312.x]

39   **Sprangers B**, Kuypers DR, Vanrenterghem Y. Immunosuppression: does one regimen fit all? *Transplantation* 2011; **92**: 251-261 [PMID: 21593703]

40   **Srinivas TR**, Meier-Kriesche HU. Minimizing immunosuppression, an alternative approach to reducing side effects: objectives and interim result. *Clin J Am Soc Nephrol* 2008; **3** Suppl 2: S101-S116 [PMID: 18308998 DOI: 10.2215/CJN.03510807]

41   **Bestard O**, Cruzado JM, Grinyó JM. Corticosteroid-sparing strategies in renal transplantation: are we still balancing rejection risk with improved tolerability? *Drugs* 2006; **66**: 403-414 [PMID: 16597159 DOI: 10.2165/00003495-200666040-00001]

42   **Pascual J**, Royuela A, Galeano C, Crespo M, Zamora J. Very early steroid withdrawal or complete avoidance for kidney transplant recipients: a systematic review. *Nephrol Dial Transplant* 2012; **27**: 825-832 [PMID: 21785040 DOI: 10.1093/ndt/gfr374]

43   **Yang H**. Maintenance immunosuppression regimens: conversion, minimization, withdrawal, and avoidance. *Am J Kidney Dis* 2006; **47**: S37-S51 [PMID: 16567240 DOI: 10.1053/j.ajkd.2005.12.045]

44   **Kirk AD**, Mannon RB, Swanson SJ, Hale DA. Strategies for minimizing immunosuppression in kidney transplantation. *Transplant Int* 2005; **18**: 2-14 [PMID: 15612977 DOI: 10.1111/j.1432-2277.2004.00019.x]

45   **Giessing M**, Fuller TF, Tuellmann M, Slowinski T, Budde K, Liefeldt L. Steroid- and calcineurin inhibitor free immunosuppression in kidney transplantation: state of the art and future developments. *World J Urol* 2007; **25**: 325-332 [PMID: 17333201 DOI: 10.1007/s00345-007-0157-8]

46   **Augustine JJ**, Hricik DE. Minimization of immunosuppression in kidney transplantation. *Curr Opin Nephrol Hypertens* 2007; **16**: 535-541 [PMID: 18089967]

47   **Helal I**, Chan L. Steroid and calcineurin inhibitor-sparing protocols in kidney transplantation. *Transplant Proc* 2011; **43**: 472-477 [PMID: 21440737 DOI: 10.1016/j.transproceed.2011.0 1.054]

48   **Olyaei AJ**, de Mattos AM, Bennett WM. Nephrotoxicity of immunosuppressive drugs: new insight and preventive strategies. *Curr Opin Crit Care* 2001; **7**: 384-389 [PMID: 11805539]

49   **Flechner SM**. Minimizing calcineurin inhibitor drugs in renal transplantation. *Transplant Proc* 2003; **35**: 118S-121S [PMID: 12742481 DOI: 10.1016/S0041-1345(03)00218-5]

50   **Lo A**. Strategies to prevent chronic allograft nephropathy in kidney transplantation: focus on calcineurin inhibitors. *Prog Transplant* 2004; **14**: 157-164 [PMID: 15264460]

51   **Bestard O**, Cruzado JM, Grinyó JM. Calcineurin-inhibitor-sparing immunosuppressive protocols. *Transplant Proc* 2005; **37**: 3729-3732 [PMID: 16386520 DOI: 10.1016/j.transproceed. 2005.09.129]

52   **Guerra G**, Srinivas TR, Meier-Kriesche HU. Calcineurin inhibitor-free immunosuppression in kidney transplantation. *Transpl Int* 2007; **20**: 813-827 [PMID: 17645419 DOI: 10.1111/j.1432-2277.2007.00528.x]

53   **Barbari AG**, Stephan AG, Masri MA. Calcineurin inhibitor-free protocols: risks and benefits. *Saudi J Kidney Dis Transpl* 2007; **18**: 1-23 [PMID: 17237886]

54   **Flechner SM**, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. *Clin Transplant* 2008; **22**: 1-15 [PMID: 18217899 DOI: 10.1111/j.1399-0012.2007.00739.x]

55   **Golshayan D**, Pascual M. Minimization of calcineurin inhibitors to improve long-term outcomes in kidney transplantation. *Transpl Immunol* 2008; **20**: 21-28 [PMID: 18775494 DOI: 10.1016/j.trim.2008.08.006]

56   **Ekberg H**. Calcineurin inhibitor sparing in renal transplantation. *Transplantation* 2008; **86**: 761-767 [PMID: 18813097 DOI:

57   **Grinyó JM**, Bestard O, Torras J, Cruzado JM. Optimal immunosuppression to prevent chronic allograft dysfunction. *Kidney Int Suppl* 2010; **(119)**: S66-S70 [PMID: 21116321 DOI: 10.1038/ki.2010.426]

58   **Sharif A**, Shabir S, Chand S, Cockwell P, Ball S, Borrows R. Meta-analysis of calcineurin-inhibitor-sparing regimens in kidney transplantation. *J Am Soc Nephrol* 2011; **22**: 2107-2118 [PMID: 21949096 DOI: 10.1681/ASN.2010111160]

59   **Vincenti F**. Are calcineurin inhibitors-free regimens ready for prime time? *Kidney Int* 2012; **82**: 1054-1060 [PMID: 22622502 DOI: 10.1038/ki.2012.194]

60   **Ruiz R**, Klintmalm GB. Renal-sparing regimens employing new agents. *Curr Opin Organ Transplant* 2012; **17**: 619-625 [PMID: 23111644 DOI: 10.1097/MOT.0b013e328359886a]

61   **Mathis AS**. Kidney transplantation: a pharmacist-focused discussion of common clinical issues related to comorbidities, renal function, and organ rejection. *Pharmacy Times* 2013; **79**: 87-99

62   **Salvadori M**, Bertoni E. Is it time to give up with calcineurin inhibitors in kidney transplantation? *World J Transplant* 2013; **3**: 7-25 [PMID: 24175203 DOI: 10.5500/wjt.v3.i2.7]

63   **Tönshoff B**, Höcker B. Treatment strategies in pediatric solid organ transplant recipients with calcineurin inhibitor-induced nephrotoxicity. *Pediatr Transplant* 2006; **10**: 721-729 [PMID: 16911497 DOI: 10.1111/j.1399-3046.2006.00577.x]

64   **Sarwal M**, Pascual J. Immunosuppression minimization in pediatric transplantation. *Am J Transplant* 2007; **7**: 2227-2235 [PMID: 17711553 DOI: 10.1111/j.1600-6143.2007.01936.x]

65   **Tredger JM**, Brown NW, Dhawan A. Calcineurin inhibitor sparing in paediatric solid organ transplantation : managing the efficacy/toxicity conundrum. *Drugs* 2008; **68**: 1385-1414 [PMID: 18578558]

66   **Höcker B**, Tönshoff B. Calcineurin inhibitor-free immunosuppression in pediatric renal transplantation: a viable option? *Paediatr Drugs* 2011; **13**: 49-69 [PMID: 21162600 DOI: 10.2165/11538530-000000000-00000]

67   **He J**, Li Y, Zhang H, Wei X, Zheng H, Xu C, Bao X, Yuan X, Hou J. Immune function assay (ImmuKnow) as a predictor of allograft rejection and infection in kidney transplantation. *Clin Transplant* 2013; **27**: E351-E358 [PMID: 23682828 DOI: 10.1111/ctr.12134]

68   **Ashton-Chess J**, Giral M, Soulillou JP, Brouard S. Can immune monitoring help to minimize immunosuppression in kidney transplantation? *Transpl Int* 2009; **22**: 110-119 [PMID: 18764832 DOI: 10.1111/j.1432-2277.2008.00748.x]

69   **Danger R**, Giral M, Soulillou JP, Brouard S. Rationale and criteria of eligibility for calcineurin inhibitor interruption following kidney transplantation. *Curr Opin Organ Transplant* 2008; **13**: 609-613 [PMID: 19060551 DOI: 10.1097/MOT.0b013e3283193bd8]

70   **Henderson LK**, Nankivell BJ, Chapman JR. Surveillance protocol kidney transplant biopsies: their evolving role in clinical practice. *Am J Transplant* 2011; **11**: 1570-1575 [PMID: 21797971 DOI: 10.1111/j.1600-6143.2011.03677.x]

71   **Roos-van Groningen MC**, Scholten EM, Lelieveld PM, Rowshani AT, Baelde HJ, Bajema IM, Florquin S, Bemelman FJ, de Heer E, de Fijter JW, Bruijn JA, Eikmans M. Molecular comparison of calcineurin inhibitor-induced fibrogenic responses in protocol renal transplant biopsies. *J Am Soc Nephrol* 2006; **17**: 881-888 [PMID: 16467444 DOI: 10.1681/ASN.2005080891]

72   **Rowshani AT**, Scholten EM, Bemelman F, Eikmans M, Idu M, Roos-van Groningen MC, Surachno JS, Mallat MJ, Paul LC, de Fijter JW, Bajema IM, ten Berge I, Florquin S. No difference in degree of interstitial Sirius red-stained area in serial biopsies from area under concentration-over-time curves-guided cyclosporine versus tacrolimus-treated renal transplant recipients at one year. *J Am Soc Nephrol* 2006; **17**: 305-312 [PMID: 16306168 DOI: 10.1681/ASN.2005030249]

73   **Sis B**, Dadras F, Khoshjou F, Cockfield S, Mihatsch MJ, Solez K. Reproducibility studies on arteriolar hyaline thickening scoring in calcineurin inhibitor-treated renal allograft recipients. *Am J Transplant* 2006; **6**: 1444-1450 [PMID: 16686769 DOI: 10.1111/j.1600-6143.2006.01302.x]

74   **Gotti E**, Perico N, Perna A, Gaspari F, Cattaneo D, Caruso R, Ferrari S, Stucchi N, Marchetti G, Abbate M, Remuzzi G. Renal transplantation: can we reduce calcineurin inhibitor/stop steroids? Evidence based on protocol biopsy findings. *J Am Soc Nephrol* 2003; **14**: 755-766 [PMID: 12595513 DOI: 10.1097/01.ASN.0000048717.97169.29]

75   **Hauser IA**, Schaeffeler E, Gauer S, Scheuermann EH, Wegner B, Gossmann J, Ackermann H, Seidl C, Hocher B, Zanger UM, Geiger H, Eichelbaum M, Schwab M. ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. *J Am Soc Nephrol* 2005; **16**: 1501-1511 [PMID: 15772250 DOI: 10.1681/ASN.2004100882]

76   **van de Wetering J**, Koumoutsakos P, Peeters A, van der Mast BJ, de Kuiper P, IJzermans JN, Weimar W, Baan CC. Discontinuation of calcineurin inhibitors treatment allows the development of FOXP3+ regulatory T-cells in patients after kidney transplantation. *Clin Transplant* 2011; **25**: 40-46 [PMID: 20636406 DOI: 10.1111/j.1399-0012.2010.01311.x]

77   **Toz H**, Sen S, Celik HA, Yilmaz M, Hur E, Hoscoskun C, Ozkahya M, Aydin HH. Calcineurin inhibitor-based and free regimens have distinct gene expression patterns in subclinical graft fibrosis. *Ann Transplant* 2011; **16**: 76-87 [PMID: 21716190]

78   **Jevnikar AM**, Mannon RB. Late kidney allograft loss: what we know about it, and what we can do about it. *Clin J Am Soc Nephrol* 2008; **3** Suppl 2: S56-S67 [PMID: 18309004 DOI: 10.2215/CJN.03040707]

79   **Kasiske BL**, Heim-Duthoy K, Ma JZ. Elective cyclosporine withdrawal after renal transplantation. A meta-analysis. *JAMA* 1993; **269**: 395-400 [PMID: 8418349 DOI: 10.1001/jama.1993.03500030093040]

80   **van der Mast BJ**, van Besouw NM, Witvliet MD, de Kuiper P, Smak Gregoor P, van Gelder T, Weimar W, Claas FH. Formation of donor-specific human leukocyte antigen antibodies after kidney transplantation: correlation with acute rejection and tapering of immunosuppression. *Transplantation* 2003; **75**: 871-877 [PMID: 12660517]

81   **Ojo AO**, Meier-Kriesche HU, Hanson JA, Leichtman AB, Cibrik D, Magee JC, Wolfe RA, Agodoa LY, Kaplan B. Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. *Transplantation* 2000; **69**: 2405-2409 [PMID: 10868649]

82   **Busque S**, Shoker A, Landsberg D, McAlister V, Halloran P, Shapiro J, Peets J, Schulz M. Canadian multicentre trial of tacrolimus/azathioprine/steroids versus tacrolimus/mycophenolate mofetil/steroids versus neoral/mycophenolate mofetil/steroids in renal transplantation. *Transplant Proc* 2001; **33**: 1266-1267 [PMID: 11267285]

83   **Kosch M**, Hausberg M, Suwelack B. Studies on effects of calcineurin inhibitor withdrawal on arterial distensibility and endothelial function in renal transplant recipients. *Transplantation* 2003; **76**: 1516-1519 [PMID: 14657697]

84   **Suwelack B**, Gerhardt U, Hohage H. Withdrawal of cyclosporine or tacrolimus after addition of mycophenolate mofetil in patients with chronic allograft nephropathy. *Am J Transplant* 2004; **4**: 655-662 [PMID: 15023160 DOI: 10.1111/j.1600-6143.2004.00404.x]

85   **McGrath JS**, Shehata M. Chronic allograft nephropathy: prospective randomised trial of cyclosporin withdrawal and mycophenolate mofetil or tacrolimus substitution. *Transplant Proc* 2001; **33**: 2193-2195 [PMID: 11377500]

86   **Hanvesakul R**, Kubal C, Jham S, Sarkar E, Eardley K, Adu D, Cockwell P. Increased incidence of infections following the late introduction of mycophenolate mofetil in renal trans-

plant recipients. *Nephrol Dial Transplant* 2008; **23**: 4049-4053 [PMID: 18622022 DOI: 10.1093/ndt/gfn387]

87 **Dudley C**, Pohanka E, Riad H, Dedochova J, Wijngaard P, Sutter C, Silva HT. Mycophenolate mofetil substitution for cyclosporine a in renal transplant recipients with chronic progressive allograft dysfunction: the "creeping creatinine" study. *Transplantation* 2005; **79**: 466-475 [PMID: 15729174 DOI: 10.1097/01.TP.0000151632.21551.00]

88 **Weir MR**, Ward MT, Blahut SA, Klassen DK, Cangro CB, Bartlett ST, Fink JC. Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy. *Kidney Int* 2001; **59**: 1567-1573 [PMID: 11260422 DOI: 10.1046/j.1523-1755.2001.0590041567.x]

89 **Weir MR**, Blahut S, Drachenburg C, Young C, Papademitriou J, Klassen DK, Cangro CB, Bartlett ST, Fink JC. Late calcineurin inhibitor withdrawal as a strategy to prevent graft loss in patients with suboptimal kidney transplant function. *Am J Nephrol* 2004; **24**: 379-386 [PMID: 15237243 DOI: 10.1159/000079390]

90 **Abramowicz D**, Manas D, Lao M, Vanrenterghem Y, Del Castillo D, Wijngaard P, Fung S. Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen in stable kidney transplant recipients: a randomized, controlled study. *Transplantation* 2002; **74**: 1725-1734 [PMID: 12499889 DOI: 10.1097/01.TP.0000038729.43731.F6]

91 **Abramowicz D**, Del Carmen Rial M, Vitko S, del Castillo D, Manas D, Lao M, Gafner N, Wijngaard P. Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J Am Soc Nephrol* 2005; **16**: 2234-2240 [PMID: 15917338 DOI: 10.1681/ASN.2004100844]

92 **Heeg MH**, Mueller GA, Bramlage C, Homayounfar K, Muehlhausen J, Leha A, Koziolek MJ. Improvement of renal graft function after conversion from a calcineurin inhibitor including immunosuppression to a mycophenolate sodium including regimen: a 4-year follow-up. *Transplant Proc* 2013; **45**: 142-147 [PMID: 23375288 DOI: 10.1016/j.transproceed.2012.10.028]

93 **Mourer JS**, Hartigh Jd, van Zwet EW, Mallat MJ, Dubbeld J, de Fijter JW. Randomized trial comparing late concentration-controlled calcineurin inhibitor or mycophenolate mofetil withdrawal. *Transplantation* 2012; **93**: 887-894 [PMID: 22538450 DOI: 10.1097/TP.0b013e31824ad60a]

94 **Mathis AS**, Davé N, Knipp GT, Friedman GS. Drug-related dyslipidemia after renal transplantation. *Am J Health Syst Pharm* 2004; **61**: 565-585; quiz 586-587 [PMID: 15061429]

95 **Kuypers DR**, Vanrenterghem Y, Squifflet JP, Mourad M, Abramowicz D, Oellerich M, Armstrong V, Shipkova M, Daems J. Twelve-month evaluation of the clinical pharmacokinetics of total and free mycophenolic acid and its glucuronide metabolites in renal allograft recipients on low dose tacrolimus in combination with mycophenolate mofetil. *Ther Drug Monit* 2003; **25**: 609-622 [PMID: 14508385]

96 **Kuypers DR**, de Jonge H, Naesens M, de Loor H, Halewijck E, Dekens M, Vanrenterghem Y. Current target ranges of mycophenolic acid exposure and drug-related adverse events: a 5-year, open-label, prospective, clinical follow-up study in renal allograft recipients. *Clin Ther* 2008; **30**: 673-683 [PMID: 18498916]

97 **Hohage H**, Zeh M, Heck M, Gerhardt UW, Welling U, Suwelack BM. Differential effects of cyclosporine and tacrolimus on mycophenolate pharmacokinetics in patients with impaired kidney function. *Transplant Proc* 2005; **37**: 1748-1750 [PMID: 15919453]

98 **Moore J**, Middleton L, Cockwell P, Adu D, Ball S, Little MA, Ready A, Wheatley K, Borrows R. Calcineurin inhibitor sparing with mycophenolate in kidney transplantation: a systematic review and meta-analysis. *Transplantation* 2009; **87**: 591-605 [PMID: 19307799 DOI: 10.1097/TP.0b013e318195a421]

99 **Land W**, Vincenti F. Toxicity-sparing protocols using my-

cophenolate mofetil in renal transplantation. *Transplantation* 2005; **80**: S221-S234 [PMID: 16251855]

100 **Dalal P**, Grafals M, Chhabra D, Gallon L. Mycophenolate mofetil: safety and efficacy in the prophylaxis of acute kidney transplantation rejection. *Ther Clin Risk Manag* 2009; **5**: 139-149 [PMID: 19436616]

101 **Grinyó JM**, Cruzado JM. Mycophenolate mofetil and calcineurin-inhibitor reduction: recent progress. *Am J Transplant* 2009; **9**: 2447-2452 [PMID: 19775321 DOI: 10.1111/j.1600-6143.2009.02812.x]

102 **Gutiérrez MJ**, González E, Andrés A, Morales JM. Clinical implications of proteinuria in renal transplant recipients switching to rapamycin for chronic allograft dysfunction. *Transplant Proc* 2009; **41**: 2348-2350 [PMID: 19715916 DOI: 10.1016/j.transproceed.2009.06.163]

103 **Maharaj S**, Assounga AG. Conversion of cyclosporine to sirolimus before 12 months is associated with marked improvement in renal function and low proteinuria in a South African renal transplant population. *Exp Clin Transplant* 2010; **8**: 14-18 [PMID: 20199366]

104 **Citterlo F**, Scatà MC, Violi P, Romagnoli J, Pozzetto U, Nanni G, Castagneto M. Rapid conversion to sirolimus for chronic progressive deterioration of the renal function in kidney allograft recipients. *Transplant Proc* 2003; **35**: 1292-1294 [PMID: 12826140 DOI: 10.1016/S0041-1345(03)00375-0]

105 **Wu MJ**, Shu KH, Cheng CH, Chen CH. Sirolimus in chronic allograft nephropathy. *Transplant Proc* 2004; **36**: 2053-2055 [PMID: 15518743 DOI: 10.1016/j.transproceed.2004.08.005]

106 **Chhabra D**, Alvarado A, Dalal P, Leventhal J, Wang C, Sustento-Reodica N, Najafian N, Skaro A, Levitsky J, Mas V, Gallon L. Impact of calcineurin-inhibitor conversion to mTOR inhibitor on renal allograft function in a prednisone-free regimen. *Am J Transplant* 2013; **13**: 2902-2911 [PMID: 24007570 DOI: 10.1111/ajt.12437]

107 **Wali RK**, Mohanlal V, Ramos E, Blahut S, Drachenberg C, Papadimitriou J, Dinits M, Joshi A, Philosophe B, Foster C, Cangro C, Nogueira J, Cooper M, Bartlett ST, Weir MR. Early withdrawal of calcineurin inhibitors and rescue immunosuppression with sirolimus-based therapy in renal transplant recipients with moderate to severe renal dysfunction. *Am J Transplant* 2007; **7**: 1572-1583 [PMID: 17511682 DOI: 10.1111/j.1600-6143.2007.01825.x]

108 **Diekmann F**, Waiser J, Fritsche L, Dragun D, Neumayer HH, Budde K. Conversion to rapamycin in renal allograft recipients with biopsy-proven calcineurin inhibitor-induced nephrotoxicity. *Transplant Proc* 2001; **33**: 3234-3235 [PMID: 11750386 DOI: 10.1016/S0041-1345(01)02375-2]

109 **Bumbea V**, Kamar N, Ribes D, Esposito L, Modesto A, Guitard J, Nasou G, Durand D, Rostaing L. Long-term results in renal transplant patients with allograft dysfunction after switching from calcineurin inhibitors to sirolimus. *Nephrol Dial Transplant* 2005; **20**: 2517-2523 [PMID: 15985508 DOI: 10.1093/ndt/gfh957]

110 **Boratyńska M**, Banasik M, Watorek E, Falkiewicz K, Patrzałek D, Szyber P, Klinger M. Conversion to sirolimus from cyclosporine may induce nephrotic proteinuria and progressive deterioration of renal function in chronic allograft nephropathy patients. *Transplant Proc* 2006; **38**: 101-104 [PMID: 16504675 DOI: 10.1016/j.transproceed.2005.12.023]

111 **Martínez-Mier G**, Méndez-López MT, Estrada-Oros J, Budar-Fernandez LF, Soto-González JI, Méndez-Machado GF, Viñas Dozal JC. Conversion from calcineurin inhibitor to sirolimus for renal function deterioration in kidney allograft recipients. *Arch Med Res* 2006; **37**: 635-638 [PMID: 16740435 DOI: 10.1016/j.arcmed.2005.12.003]

112 **Kamar N**, Frimat L, Blancho G, Wolff P, Delahousse M, Rostaing L. Evaluation of the efficacy and safety of a slow conversion from calcineurin inhibitor- to sirolimus-based therapies in maintenance renal-transplant patients presenting with

moderate renal insufficiency. *Transpl Int* 2007; **20**: 128-134 [PMID: 17239020 DOI: 10.1111/j.1432-2277.2006.00409.x]

113 **Chen J**, Li L, Wen J, Tang Z, Ji S, Sha G, Cheng Z, Sun Q, Cheng D, Liu Z. Observation of efficacy and safety of converting the calcineurin inhibitor to sirolimus in renal transplant recipients with chronic allograft nephropathy. *Transplant Proc* 2008; **40**: 1411-1415 [PMID: 18589119 DOI: 10.1016/j.transproceed.2008.03.096]

114 **Stallone G**, Infante B, Schena A, Battaglia M, Ditonno P, Loverre A, Gesualdo L, Schena FP, Grandaliano G. Rapamycin for treatment of chronic allograft nephropathy in renal transplant patients. *J Am Soc Nephrol* 2005; **16**: 3755-3762 [PMID: 16236802 DOI: 10.16_1/ASN.2005060635]

115 **Paoletti E**, Ratto E, Bellino D, Marsano L, Cassottana P, Cannella G. Effect of early conversion from CNI to sirolimus on outcomes in kidney transplant recipients with allograft dysfunction. *J Nephrol* 2012; **25**: 709-718 [PMID: 22038336 DOI: 10.5301/jn.5000044]

116 **Alarrayed SM**, El-Agroudy AE, Alarrayed AS, Al Ghareeb SM, Garadah TS, El-Sharqawi SY, Al-Aradi AH, Dandi BG, Abdulla S. Sirolimus-based calcineurin inhibitor withdrawal immunosuppressive regimen in kidney transplantation: a single center experience. *Clin Exp Nephrol* 2010; **14**: 248-255 [PMID: 20232105 DOI: 10.1007/s10157-010-0269-0]

117 **Fischereder M**, Graeb C, Krüger B, Kammerl MC, Zülke C, Jauch KW, Krämer BK. Conversion from calcineurin inhibitors to sirolimus in patients with chronic renal allograft dysfunction. *Transplant Proc* 2006; **38**: 1295-1297 [PMID: 16797286 DOI: 10.1016/j.transproceed.2006.03.026]

118 **Schena FP**, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC, Campistol JM, Racusen L, Polinsky MS, Goldberg-Alberts R, Li H, Scarola J, Neylan JF. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation* 2009; **87**: 233-242 [PMID: 19155978 DOI: 10.1097/TP.0b013e3181927a41]

119 **Mulay AV**, Cockfield S, Stryker R, Fergusson D, Knoll GA. Conversion from calcineurin inhibitors to sirolimus for chronic renal allograft dysfunction: a systematic review of the evidence. *Transplantation* 2006; **82**: 1153-1162 [PMID: 17102766 DOI: 10.1097/01.tp.0000237101.58974.43]

120 **van den Akker JM**, Wetzels JF, Hoitsma AJ. Proteinuria following conversion from azathioprine to sirolimus in renal transplant recipients. *Kidney Int* 2006; **70**: 1355-1357 [PMID: 16912706 DOI: 10.1038/sj.ki.5001792]

121 **Rangan GK**. Sirolimus-associated proteinuria and renal dysfunction. *Drug Saf* 2006; **29**: 1153-1161 [PMID: 17147461]

122 **Kaplan B**, Schold J, Srinivas T, Womer K, Foley DP, Patton P, Howard R, Meier-Kriesche HU. Effect of sirolimus withdrawal in patients with deteriorating renal function. *Am J Transplant* 2004; **4**: 1709-1712 [PMID: 15367229 DOI: 10.111/j.1600-6143.2004.00569.x]

123 **Grinyó JM**, Cruzado JM. Mycophenolate mofetil and sirolimus combination in renal transplantation. *Am J Transplant* 2006; **6**: 1991-1999 [PMID: 16930395 DOI: 10.1111/j.1600-1643.2006.01398.x]

124 **Pescovitz MD**, Vincenti F, Hart M, Melton L, Whelchel J, Mulgaonkar S, McKay D, Leung M, Calleja E, Bouw MR. Pharmacokinetics, safety, and efficacy of mycophenolate mofetil in combination with sirolimus or ciclosporin in renal transplant patients. *Br J Clin Pharmacol* 2007; **64**: 758-771 [PMID: 17555465 DOI: 10.1111/j.1365-2125.2007.02934.x]

125 **Meier-Kriesche HU**, Schold JD, Srinivas TR, Howard RJ, Fujita S, Kaplan B. Sirolimus in combination with tacrolimus is associated with worse renal allograft survival compared to mycophenolate mofetil combined with tacrolimus. *Am J Transplant* 2005; **5**: 2273-2280 [PMID: 16095509 DOI: 10.1111/j.1600-1643.2005.01019.x]

126 **Giron F**, Baez Y, Niño-Murcia A, Rodríguez J, Salcedo S. Conversion therapy to everolimus in renal transplant recipients: results after one year. *Transplant Proc* 2008; **40**: 711-713 [PMID: 18454994 DOI: 10.1016/j.transproceed.2008.03.012]

127 **Sánchez Fructuoso A**, Ruiz San Millán JC, Calvo N, Rodrigo E, Moreno MA, Cotorruelo J, Conesa J, Gómez-Alamillo C, Arias M, Barrientos A. Evaluation of the efficacy and safety of the conversion from a calcineurin inhibitor to an everolimus-based therapy in maintenance renal transplant patients. *Transplant Proc* 2007; **39**: 2148-2150 [PMID: 17889120 DOI: 10.1016/j.transproceed.2007.06.030]

128 **Ruiz JC**, Sanchez-Fructuoso A, Rodrigo E, Conesa J, Cotorruelo JG, Gómez-Alamillo C, Calvo N, Barrientos A, Arias M. Conversion to everolimus in kidney transplant recipients: a safe and simple procedure. *Transplant Proc* 2006; **38**: 2424-2426 [PMID: 17097956 DOI: 10.1016/j.transproceed.2006.08.190]

129 **Fernández A**, Marcén R, Galeano C, Caldés S, Amezquita Y, Villafruela J, Pascual J, Burgos J, Rodríguez-Mendiola N, Ortuño J. Complete switch to everolimus in long-term kidney transplants: evolution of the renal function. *Transplant Proc* 2009; **41**: 2345-2347 [PMID: 19715915 DOI: 10.1016/j.transproceed.2009.06.162]

130 **Kamar N**, Del Bello A, Congy-Jolivet N, Guilbeau-Frugier C, Cardeau-Desangles I, Fort M, Esposito L, Guitard J, Gamé X, Rostaing L. Incidence of donor-specific antibodies in kidney transplant patients following conversion to an everolimus-based calcineurin inhibitor-free regimen. *Clin Transplant* 2013; **27**: 455-462 [PMID: 23621682 DOI: 10.1111/ctr.12127]

131 **Morales J**, Fierro A, Benavente D, Zehnder C, Ferrario M, Contreras L, Herzog C, Buckel E. Conversion from a calcineurin inhibitor-based immunosuppressive regimen to everolimus in renal transplant recipients: effect on renal function and proteinuria. *Transplant Proc* 2007; **39**: 591-593 [PMID: 17445551 DOI: 10.1016/j.transproceed.2007.12.026]

132 **Holdaas H**, Rostaing L, Serón D, Cole E, Chapman J, Fellström B, Strom EH, Jardine A, Midtvedt K, Machein U, Ulbricht B, Karpov A, O'Connell PJ. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation* 2011; **92**: 410-418 [PMID: 21697773 DOI: 10.1097/TP.0b013e318224c12d]

133 **Inza A**, Balda S, Alvarez E, Zárraga S, Gaínza FJ, Lampreabe I. Conversion to everolimus in kidney transplant recipients with decreased renal function. *Transplant Proc* 2009; **41**: 2134-2136 [PMID: 19715854 DOI: 10.1016/j.transproceed.2009.05.013]

134 **Cataneo-Dávila A**, Zúñiga-Varga J, Correa-Rotter R, Alberú J. Renal function outcomes in kidney transplant recipients after conversion to everolimus-based immunosuppression regimen with CNI reduction or elimination. *Transplant Proc* 2009; **41**: 4138-4146 [PMID: 20005355 DOI: 10.1016/j.transproceed.2009.08.065]

135 **Albano L**, Alamartine E, Toupance O, Moulin B, Merville P, Rerolle JP, Tetaz R, Moal MC, Kamar N, Legendre C, Quéré S, Di Giambattista F, Terpereau A, Dantal J. Conversion from everolimus with low-exposure cyclosporine to everolimus with mycophenolate sodium maintenance therapy in kidney transplant recipients: a randomized, open-label multicenter study. *Ann Transplant* 2012; **17**: 58-67 [PMID: 22466910 DOI: 10.12659/AOT.882637]

136 **Rostaing L**, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, Steinberg S, Vincenti F, Shi R, Di Russo G, Thomas D, Grinyó J. Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized phase II study. *Clin J Am Soc Nephrol* 2011; **6**: 430-439 [PMID: 21051752 DOI: 10.2215/CJN.05840710]

137 **Grinyo J**, Alberu J, Contieri FL, Manfro RC, Mondragon G, Nainan G, Rial Mdel C, Steinberg S, Vincenti F, Dong Y, Thomas D, Kamar N. Improvement in renal function in

kidney transplant recipients switched from cyclosporine or tacrolimus to belatacept: 2-year results from the long-term extension of a phase II study. *Transpl Int* 2012; **25**: 1059-1064 [PMID: 22816557 DOI: 10.1111/j.1432-2277.2012.01535.x]

138   **Parada B**, Figueiredo A, Nunes P, Bastos C, Macário F, Roseiro A, Dias V, Rolo F, Mota A. Pediatric renal transplantation: comparative study with renal transplantation in the adult population. *Transplant Proc* 2005; **37**: 2771-2774 [PMID: 16182806 DOI: 10.1016/j.transproceed.2005.05.046]

139   **Kari JA**, Trompeter RS. What is the calcineurin inhibitor of choice for pediatric renal transplantation? *Pediatr Transplant* 2004; **8**: 437-444 [PMID: 15367278]

140   **Weintraub L**, Li L, Kambham N, Alexander S, Concepcion W, Miller K, Wong C, Salvatierra O, Sarwal M. Patient selection critical for calcineurin inhibitor withdrawal in pediatric kidney transplantation. *Pediatr Transplant* 2008; **12**: 541-549 [PMID: 18564305 DOI: 10.1111/j.1399-3046.2007.00847.x]

141   **Krischock L**, Gullett A, Bockenhauer D, Rees L, Trompeter RS, Marks SD. Calcineurin-inhibitor free immunosuppression with mycophenolate mofetil and corticosteroids in paediatric renal transplantation improves renal allograft function without increasing acute rejection. *Pediatr Transplant* 2009; **13**: 475-481 [PMID: 18992054]

142   **Höcker B**, Feneberg R, Köpf S, Weber LT, Waldherr R, Wühl E, Tönshoff B. SRL-based immunosuppression vs. CNI mini- mization in pediatric renal transplant recipients with chronic CNI nephrotoxicity. *Pediatr Transplant* 2006; **10**: 593-601 [PMID: 16856996 DOI: 10.1111/j.1399-3046.2006.00526.x]

143   **Gourishankar S**, Leduc R, Connett J, Cecka JM, Cosio F, Fieberg A, Gaston R, Halloran P, Hunsicker L, Kasiske B, Rush D, Grande J, Mannon R, Matas A. Pathological and clinical characterization of the 'troubled transplant': data from the DeKAF study. *Am J Transplant* 2010; **10**: 324-330 [PMID: 20055809 DOI: 10.1111/j.1600-6143.2009.02954.x]

144   **Gaston RS**, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, Gourishankar S, Grande J, Halloran P, Hunsicker L, Mannon R, Rush D, Matas AJ. Evidence for antibody-me- diated injury as a major determinant of late kidney allograft failure. *Transplantation* 2010; **90**: 68-74 [PMID: 20463643 DOI: 10.1097/TP.0b013e3181e065de]

145   **Matas AJ,** Leduc R, Rush D, Cecka JM, Connett J, Fieberg A, Halloran P, Hunsicker L, Cosio F, Grande J, Mannon R, Gourishankar S, Gaston R, Kasiske B. Histopathologic clus- ters differentiate subgroups within the nonspecific diagnoses of CAN or CR: preliminary data from the DeKAF study. *Am J Transplant* 2013; **10**: 315-323 [PMID: 20041864]

146   **Rush D**, Leduc R, Connett J, Cosio F, Gaston R, Mannon R, Hunsicker L, Gourishankar S, Halloran P, Cecka M, Grande J, Kasiske B. DeKAF pathology clusters: follow up at 5 years. *Am j Transplant* 2013; **19**: 417 [DOI: 10.1111/ajt.12266]

REVIEW

Pre-and-post transplant considerations in patients with nonalcoholic fatty liver disease

Vikas Khullar, Angela Dolganiuc, Roberto J Firpi

Vikas Khullar, Angela Dolganiuc, Roberto J Firpi, Department of Medicine, University of Florida College of Medicine, Gainesville, FL 32608, United States
Roberto J Firpi, Section of Hepatobiliary Diseases and Transplantation, University of Florida College of Medicine, Gainesville, FL 32608, United States
Author contributions: Khullar V, Dolganiuc A and Firpi RJ solely contributed to this work.
Correspondence to: Roberto J Firpi, MD, MS, AGAF, Department of Medicine, University of Florida College of Medicine, 1600 S.W. Archer Road, MSB-440, Gainesville, FL 32608, United States. roberto.firpi@medicine.ufl.edu
Telephone: +1-352-2739466   Fax: +1-352-3927393
Received: November 27, 2013   Revised: February 17, 2014
Accepted: March 11, 2014
Published online: June 24, 2014

Abstract

Non-alcoholic fatty liver disease (NAFLD) is currently the third most common indication for liver transplantation in the United States. With the growing incidence of obesity, NAFLD is expected to become the most common indication for liver transplantation over the next few decades. As the number of patients who have undergone transplantation for NAFLD increases, unique challenges have emerged in the management and long-term outcomes in patients. Risk factors such as obesity, hypertension, diabetes, and hyperlipidemia continue to play an important role in the pathogenesis of the disease and its recurrence. Patients who undergo liver transplantation for NAFLD have similar long-term survival as patients who undergo liver transplantation for other indications. Research shows that post-transplantation recurrence of NAFLD is commonplace with some patients progressing to recurrent non-alcoholic steatohepatitis and cirrhosis. While treatment of comorbidities is important, there is no consensus on the management of modifiable risk factors or the role of pharmacotherapy and immunosuppression in patients who develop recurrent or *de novo* NAFLD post-transplant.

This review provides an outline of NAFLD as indication for liver transplantation with a focus on the epidemiology, pathophysiology and risk factors associated with this disease. It also provides a brief review on the pre-transplant considerations and post-transplant factors including patient characteristics, role of obesity and metabolic syndrome, recurrence and *de novo* NAFLD, outcomes post-liver transplantation, choice of medications, and options for immunosuppression.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Liver transplantation; Non-alcoholic fatty liver disease; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Nonalcoholic steatohepatitis; Cirrhosis; Obesity

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease and one of the leading indication for liver transplantation (LT) nowadays. Although, it remains the third most common indication for LT in the United States, it is projected to become the most common indication by 2025. It presents a unique challenge for the transplant community in terms of management and long-term outcomes. Many risk factors for NAFLD pre-transplant such as obesity, hypertension, hyperlipidemia, diabetes continue to play an important role in the pathogenesis of post-transplant NAFLD. In addition to therapy focused on prevention and management of coexisting medical conditions, physicians must weight the benefits and harms of both medical and surgical options in patients undergoing LT.

Khullar V, Dolganiuc A, Firpi RJ. Pre-and-post transplant considerations in patients with nonalcoholic fatty liver disease. *World J Transplant* 2014; 4(2): 81-92 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/i2/81.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i2.81

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a major etiology leading to chronic liver disease since its first description by Ludwig *et al*[1] in 1980. NAFLD has become an umbrella term to describe the pathologic picture of alcohol induced liver injury that occurs in the absence of alcohol abuse[2]. Histologically, NAFLD ranges from simple or bland steatosis to nonalcoholic steatohepatitis (NASH) and can progress to end-stage liver disease including fibrosis and cirrhosis. The pathologic definition of NASH is based on findings of macro vesicular steatosis, nuclear glycogenation, lobular and portal inflammation, and Mallory hyaline[1]. Progression of NASH to advanced fibrosis and cirrhosis is thought to be secondary to chronic inflammation and fibrosis[3]. Obesity has been strongly associated with NAFLD and NASH with some authors suggesting that NAFLD is the hepatic manifestation of metabolic syndrome[4]. With the global epidemic of obesity on the rise, there has been a consistent increase in NAFLD and NASH cases leading to increasing frequency of liver transplantation (LT) for this indication. According to the Scientific Registry of Transplant Recipients database (SRTR), NASH now represents the third most common indication for LT in the United States, surpassed only by hepatitis C and alcohol induced liver disease[5,6]. Furthermore, LT secondary to NASH is the only indication that has increased in frequency from 1.2% to 9.7% in less than a decade (from 2001-2009)[6]. Based on this data, end-stage liver failure secondary to NAFLD is estimated to become the most common indication for LT within the next two decades[5,6].

In this manuscript, we provide an overview of NAFLD in the context of LT. First, we review the epidemiology, pathophysiology and risk factors for NAFLD and how obesity and metabolic syndrome play a role in the development of the disease. We then explore the pretransplant factors affecting this patient population such as patient characteristics and availability of livers available for transplantation. Finally, we discuss the post-transplant considerations such as recurrence and de-novo NAFLD, outcomes, pharmacotherapy and immunosuppression. The goal of this review is to educate and assist in the management of unique challenges for patients with NAFLD both pre- and post LT.

DEFINITION OF NAFLD AND NASH

An early diagnosis of NAFLD is often difficult as many patients remain asymptomatic until the disease has progressed to fibrosis and cirrhosis. Biochemically, there are no reliable serum biomarkers for NAFLD at the present time. Patients may have elevated serum transaminase levels; however, normal transaminases do not exclude the diagnosis. Per the United States Third National Health and Nutrition Examination Survey (NHANES Ⅲ), the prevalence of NAFLD with and without elevated transaminases was found to be 3.1% and 16.4% respectively[7].

Table 1 Non-alcoholic fatty liver disease Activity Score

Component	Score
Steatosis grade	
< 5%	0
5%-33%	1
33%-66%	2
> 66%	3
Lobular inflammation	
No foci	0
< 2 foci per 200 × field	1
2-4 foci per 200 × field	2
> 4 foci per 200 × field	3
Ballooning	
None	0
Few balloon cells	1
Prominent/many cells	2

Scoring system assigns a score for steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2) and sum of the scores is correlated with a score of greater than or equal to five as "definite NASH" and a score of less than or equal to three as "not NASH"[66]. Adapted from Tanaka *et al*[66].

When elevated, aspartate aminotransferase and alanine aminotransferase are seldom greater than four times the upper limit of normal[8]. Therefore, the diagnosis of NAFLD remains a diagnosis of exclusion requiring elimination of other causes of abnormal liver function tests in presence of imaging or biopsy suggestive of steatosis. Liver biopsy remains the gold standard for its diagnosis. On biopsy, NAFLD must have histologic findings of macro vesicular steatosis in greater than 5% of hepatocytes[9]. For the diagnosis of NASH, most experts require additional findings suggestive of active inflammatory process including hepatocyte swelling, ballooning and degeneration with lobular inflammation[10]. The Nonalcoholic Steatohepatitis Clinical Research Network has designed and validated a histologic scoring system for NAFLD, called the NAFLD Activity Score that allows for evaluation of steatosis, inflammation and ballooning scores[11]. This scoring system assigns a score for steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2) and sum of the scores if greater than or equal to five is defined as "definite NASH" and a score of less than or equal to three as "not NASH" (Table 1). In general, the diagnosis of both NAFLD and NASH requires the presence of hepatic steatosis, no significant alcohol consumption and no other etiology to explain liver disease[12,13]. Figure 1 illustrates the microscopic findings in biopsies of patients suspected of having NAFLD and depicts hepatocyte ballooning (Figure 1A), steatosis (Figure 1B) and lobular inflammation (Figure 1C).

EPIDEMIOLOGY AND RISK FACTORS IN NAFLD PATIENTS

Although the prevalence of NAFLD is unknown, its incidence is estimated to be on the rise with the concurrent obesity epidemic. According to the National Center for Health Statistics, the prevalence of obesity in the United States in 2009-2010 is estimated to be 35.5% of
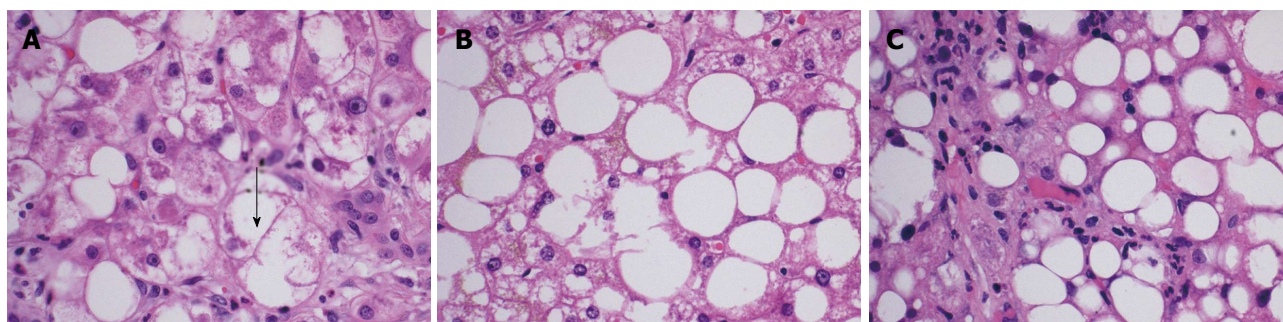
Figure 1 Microscopic findings in biopsies of patients' suspected of having non-alcoholic fatty liver disease/nonalcoholic steatohepatitis. A: H and E stained liver tissue at × 40 showing ballooning degeneration of a hepatocyte (marked with black arrow); B: H and E stained liver tissue at × 40 showing steatosis without steatohepatitis. C: H and E stained liver tissue at × 40 showing inflammation (neutrophilic inflammation surrounding fatty hepatocytes).

the male population and 35.8% of the female population[14]. A recent cross-sectional study in the setting of outpatient general internal medicine clinic in Texas shows the prevalence of NAFLD to be 46%, with findings of NASH in 12.2% of patients[15]. The projection from this study reports the anticipated prevalence of NASH in the US to be anywhere between three and eight million[15]. Despite these estimates, the frequency of progression from NAFLD to end-stage liver disease is unknown. In case series reports, transition from NASH to fibrosis are reported as high as a third of patients[16-18]. The rate of progression to decompensated cirrhosis and need for LT remains uncertain, however; this is the only indication for LT that has been steadily increasing[6]. Additionally, it is suggested that a high percentage of cases initially classified as cryptogenic cirrhosis may represent progression from NAFLD to cirrhosis[19]. As fibrosis distorts a fatty liver into a cirrhotic one, various histologic components such as steatosis and inflammatory changes become less evident and may even disappear[5]. Therefore, end-stage liver disease secondary to NAFLD is projected to become the most common indication for LT by 2025[6] given its increasing incidence and the steady decrease in frequency of hepatitis C infection and alcohol induced liver disease.

PATHOPHYSIOLOGY OF NAFLD AND NASH

NAFLD accounts for two types of fatty infiltration of the liver: simple steatosis and non-alcoholic steatohepatitis (NASH). Simple fatty liver infiltration, also called bland hepatic steatosis is a benign condition in which liver function tests are within normal limits or maybe slightly elevated. In this condition, liver biopsy shows liver tissue that is essentially normal except for fatty infiltration in hepatocytes. On the other hand, NASH is defined by the presence of inflammatory changes. The development of inflammation and subsequently NASH from hepatic steatosis is thought to be a complex mechanism involving insulin resistance, oxidative stress, and inflammatory cascade. Several models have been described in the literature to suggest the interplay between these

processes and how simple steatosis is transformed into steatohepatitis, including the "two-hit hypothesis". First described by Day *et al*[20], insulin resistance is the "first hit" that leads to steatosis in hepatocytes. During states of insulin resistance, both muscle and adipose tissues preferentially oxidize lipids, resulting in release of free-fatty acids. The liver incorporates these free fatty acids into triglycerides, and remaining free-fatty acids undergo oxidation in the mitochondria, peroxisomes or microsomes[21]. Then a "second hit" that occurs in the form of oxidative stress leads to inflammation and fibrosis[22]. Figure 2 summarizes the multiple factors that play a role in the development of NASH from steatosis. Others have also described a change in lipid metabolism through elevated peripheral fatty acids and *de novo* synthesis leading to an increase in fatty deposition in the liver. In patients with NAFLD, Donnelly *et al*[23] noted that the majority (60%) of the triacylglycerol in the liver arises from free fatty acids while 26% and 15% are attributable to *de novo* lipogenesis and diet, respectively[23,24]. Insulin resistance at the level of adipose tissue leads to an increased release of free fatty acids leading to an increased activation of macrophages and other immune cells. The entry of these free fatty acids in the liver also leads to the activation of intracellular inflammatory pathways causing hepatic inflammation and consequently fibrosis[25,26]. Furthermore, insulin resistance leads to hyperglycemia which in turn triggers stellate cell activation leading to fibrosis[27]. Genes also play an integral role in the development of NASH as evidenced by ethnic-specific allele frequencies and certain genotypes that purport a greater lipid content, more aggressive disease, and increase in serum aminotransferase levels[28].

Several studies have shown an increased prevalence of risk factors in the form of hypertension, diabetes, obesity and hyperlipidemia - all components of metabolic syndrome in patients' who have undergone LT[29]. In these patients, studies have also shown an increase in pro-steatotic cytokines such as leptin[30] and decrease in anti-steatotic cytokines such as adiponectin[31]. Additionally, the advanced age of the donors may exacerbate the effects of insulin resistance post-transplant due to accelerated fibrosis[32].
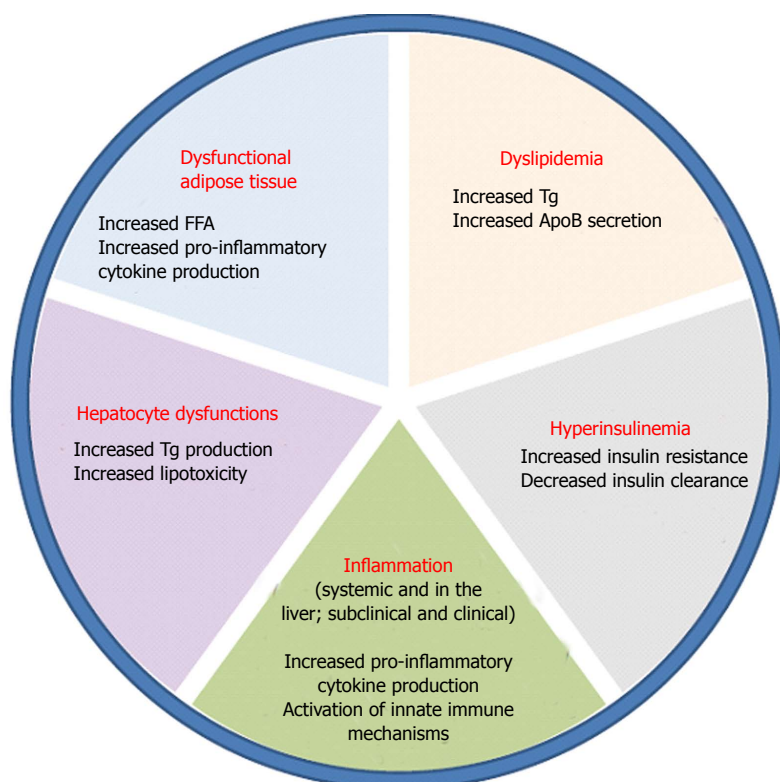
Figure 2 Multiple factors that play a role in the progression of steatosis to nonalcoholic steatohepatitis.

METABOLIC SYNDROME, OBESITY AND NAFLD

A large proportion of patients diagnosed with NAFLD have been identified to have the phenotype associated with metabolic syndrome. Although many organizations have defined the term "metabolic syndrome" differently, all definitions include risk factors for cardiovascular disease and type 2 diabetes such as hypertension, dyslipidemia (elevated triglycerides and lower high-density lipoprotein cholesterol), raised fasting glucose and central obesity[33]. Liver biopsies from patients who meet the strict definition of metabolic syndrome shows more advanced histologic changes and a high risk of severe fibrosis[34]. Additionally, obesity itself has been independently shown to be a predictor of advanced fibrosis in the liver. A study conducted by Dixon *et al*[35] showed that in 105 consecutive patients who underwent laparoscopic obesity surgery and had liver biopsies taken, there were findings of NASH in 25% with nearly half demonstrating findings of advanced fibrosis. Colicchio *et al*[36] also found severe steatosis to be uniformly present in non-diabetic patients with body mass index (BMI) greater than 39.9 kg/m^2 (grade III obesity) when evaluated using liver ultrasound. It is however, the central or visceral obesity that is associated with the development of NAFLD independent of overall obesity[37,38]. Dyslipidemia and diabetes have also been shown to have an independent association with NAFLD. One study by Assy *et al*[38] showed that in patients with hypertriglyceridemia, there is a significantly higher risk of fatty infiltration than in patients' with other forms of dyslipidemia, further supporting the association between metabolic syndrome and NAFLD.

PRE-TRANSPLANT CONSIDERATIONS

Patient characteristics

Obesity and insulin resistance have been implicated as the key pathogenic factors associated with NAFLD[39]. The risk factors associated with the histological severity of NASH in the non-transplant population include male sex, higher BMI, insulin resistance, hypertension, and presence of type II diabetes[18,40,41]. Analysis of the SRTR database by Charlton *et al*[6] showed that the people who underwent LT for NASH cirrhosis were older, had larger BMI, were more likely to be female, had a greater prevalence of diabetes and hypertension, and a lower incidence of hepatocellular carcinoma compared with other patients in the transplant cohort. Hence, prior to undergoing LT, optimization of modifiable factors in patients is essential for improved outcomes. In addition to medical optimization such as improved blood pressure and glycemic control, patients should strongly be encouraged to undergo supervised weight loss. A study by Nair *et al*[42] measured graft and patient survival in obese patients receiving LT in the United States. This study concluded that patients with morbid obesity (BMI > 40 kg/m^2) had significantly higher rates of primary graft non-function and significantly increased immediate, one and two year mortality. Five year mortality rates were also significantly higher in severely obese (BMI between 35.1 and 40 kg/m^2) and morbidly obese patients, secondary to increased cardiovascular mortality. Based on these findings, the American Association for the Study of Liver Disease (AASLD) considers morbid obesity a contraindication to LT[43], and recommends weight loss in all patients awaiting LT, especially if the patient's BMI is greater than 35

kg/m². Additionally, weight loss has been shown to help with improvement in the severity of steatosis and NASH prior to transplant. Meta-analysis by Mummadi et al[44] in the non-LT population who underwent bariatric surgery shows that a 19%-41% reduction in BMI was associated with improvement of steatosis in 91.6%, steatohepatitis in 81.3%, fibrosis in 65.5% and complete resolution of NASH in 69.5% of patient's post-bariatric surgery.

Concurrent bariatric surgery and LT has also been evaluated in obese patients. A recent study analyzed thirty-seven patients referred for LT with BMI > 35 who had achieved weight loss prior to transplant and underwent LT alone and compared them with seven patients who underwent LT with sleeve gastrectomy[45]. This study reported that in patients with LT alone, there was a higher frequency of weight gain, steatosis, post-transplant diabetes, graft loss and death when compared with the sleeve gastrectomy group. This small study suggests that although bariatric surgery may play a promising role in patients undergoing transplant, more studies are needed to evaluate long-term survival in these patients and it may be appropriate for some patients who have persistent obesity and fail non-invasive management.

Availability of livers for transplant in the NAFLD population

The increasing prevalence of obesity has led to further increases in hepatic steatosis in potential donors, which has reduced the number of transplantable livers available for any indication. The use of steatotic livers for transplant depends on the level of fatty infiltration. Donor livers with greater than 60% steatosis are deemed non-transplantable whereas those with less than 30% are deemed useable with good function. Even though livers with 30%-60% steatosis are potentially used for patients, they have been associated with poor results due to decreased function, graft survival and decreased patient survival[46]. The biggest concern remains primary non-function of the graft which has been reported as high as 13% in donor livers with greater than 30% steatosis compared with < 3% in those with no steatosis on biopsy prior to transplant[47,48]. More recent studies show the rate of primary non-function of the graft to be less than 5% in those undergoing LT with steatosis of less than 30%[49-51]. Increased hepatic graft steatosis has also been associated with intrahepatic cholestasis and transient hyperbilirubinemia during regeneration after living donor transplant but the mechanism remains elusive[52].

The use of living donors for LT also has its challenges. Although the maximum percentage of steatosis in living donors is unknown for LT, most centers are reluctant to transplant grafts with greater than 30% steatosis given the increased risk of primary non-function of the graft[53]. With the growing incidence of obesity, finding grafts with less than 10% steatosis (preferred by most centers) is difficult[54]. Studies report that one third to one half of potential living donors have steatosis on liver biopsies and in these studies more than one-third of biopsies showed steatosis greater than 10%[55,56]. The need for liver biopsy in living transplant donors is also not without risk, given that the sensitivity of imaging modalities is low for small amounts of steatosis and improves with increasing steatosis[55].

POST-TRANSPLANT CONSIDERATIONS

Recurrence of NAFLD and NASH

The development of steatosis post-LT in patients is common with some observational studies reporting prevalence as high as 100%[57]. One study of post-liver transplant patients by Maor-Kendler et al[58], showed the incidence of grade 2 steatosis or higher in 38% of recipients with pre-transplant diagnosis of NASH/cryptogenic cirrhosis when compared to 6% in cholestasic disease, 16% in alcoholic disease and 9% in patients with HCV cirrhosis. Table 2 summarizes several studies that evaluated the incidence of NAFLD, NASH and cirrhosis post LT[57,59-66]. A recent study by Dureja et al[59] analyzed post-transplant data in eighty-eight patients who underwent transplant for NAFLD and report prevalence of recurrent NAFLD to be 39%, recurrent NASH to be 28.4% and fibrosis (stage 3 and 4) to be 3.4% respectively. Moreover, according to Contos et al[57] when comparing the cases of cryptogenic cirrhosis with those transplanted for alcoholic liver disease, primary biliary cirrhosis and primary sclerosing cholangitis, the rates of steatosis and subsequent NASH were significantly higher in the cryptogenic cirrhosis group. Similarly, Bhagat et al[61] reported the recurrence of NASH in 33% of the patients who were transplanted for cryptogenic cirrhosis with NASH phenotype compared with those transplanted for alcohol related cirrhosis at six months post-LT. Tanaka et al[66] recently reported recurrence of NASH in one patient who underwent living donor LT for NAFLD; however, this study is limited by small sample size and had only seven patients who were transplanted for this indication. Based on the studies (summarized in Table 2), the recurrence of steatosis, NASH and cirrhosis in patients transplanted for NAFLD is clearly possible and further studies are needed to determine the risk of recurrence in patients' post-LT.

De novo NAFLD/NASH

Little is known about the prevalence of de novo NAFLD and NASH in patients who undergo liver transplantation for non-NASH cirrhosis and have been transplanted a donor graft free of steatosis. Report by Seo et al[63] who evaluated sixty-eight liver transplant patients with various causes of liver cirrhosis using pre-transplant and post-transplant biopsies, noted the prevalence of de novo steatosis in twelve patients (18%) with prevalence of de novo NASH in six patients (9%). In another study that evaluated thirty patients with mostly infectious cirrhosis from HBV and HCV, incidence of steatosis and NASH were 40% and 13% respectively, although it is unclear how much of this was de novo[62]. In another case series in which patients underwent transplantation for HCV and alcohol cirrhosis, four patients developed de novo NAFLD post-transplant in the absence of graft steatosis[67]. Thus,

Ref.	Year of publication	Indication of transplant	Number of patients	Findings of NAFLD post-transplant	Findings of NASH post-transplant	Findings of cirrhosis post-transplant	Mean follow-up duration
Tanaka *et al*[66]	2013	Living donor transplant for NAFLD	7	0 (0)	1 (14)	None	5.3 yr
Dureja *et al*[59]	2011	NAFLD	88	34 (39)	25 (28.4)	3 (3.4) (reported as fibrosis grade 3/4)	82 mo
Dumortier *et al*[60]	2010	Several indication	599	131 (31.1)	5 (3.8)	3 (2.25)	40 mo
Bhagat *et al*[61]	2009	Cryptogenic/NASH Cirrhosis *vs* alcoholic cirrhosis	71	N/A	31 (33)	None	1517 d
Lim *et al*[62]	2007	Non-NAFLD indication (18 HBV, 7 HCV, 5 others)	30	12 (40)	4 (13)	None	44 mo
Seo *et al*[63]	2007	68 various causes, 84% HCV	68	12[1] (18)	6[1] (9)	None	28 mo
Ong *et al*[64]	2001	Cryptogenic cirrhosis	51	13 (25.4)	8 (15.7)	None	26 mo
Contos *et al*[57]	2001	Cryptogenic/NASH cirrhosis	30	30 (100)	3 (10)	None	3.5 yr
Charlton *et al*[65]	2001	NASH cirrhosis	16	9 (60)	5 (33)	2 (12.5)	28.1 mo

Table 2 Various studies examining the incidence/recurrence of non-alcoholic fatty liver disease (*de novo* or recurrent), non-alcoholic steatohepatitis and Cirrhosis in the post-liver transplant population *n* (%)

[1]*De novo*. HCV: Hepatitis C virus; HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

the incidence, prevalence and the mechanism of *de novo* NAFLD or NASH remains unclear and there is an emerging need for studies in this area.

Influence of NAFLD/NASH on outcomes after liver transplantation

Data suggests that the outcome of LT in patients who undergo transplant for most common causes of cirrhosis in the United States, including cholestatic liver disease (primary biliary cirrhosis, primary sclerosing cholangitis), alcoholic liver disease, and HCV are excellent, with one year survival rates of 85%-90% and five year survival rates of 70%-80% respectively[6,68]. Review of literature for patients undergoing LT for NASH cirrhosis shows mortality after transplant to be similar at five years when compared with patients undergoing transplant for other indications, however the one and three year mortality in NASH cirrhosis patients were significantly higher[68]. Malik *et al*[68] reported a higher one year mortality in NASH patients with age $\geq$ 60 years and BMI $\geq$ 30 kg/m^2 with diabetes and hypertension. A more recent review of transplant patients by Charlton *et al*[6] however reports survival at one year and three years after LT for NASH to be 84% and 78%, respectively and similar for other indications. They also report that patient and graft survival was similar to values for other indications when adjusted for age, sex, BMI and serum creatinine. There is, however, a higher incidence of cardiac events following LT in a subset of patients with higher BMI, elevated serum creatinine, diabetes, systolic blood pressure elevation, hypercholesterolemia, and these may represent to some extent the cause of poor outcomes in LT patients with NASH cirrhosis[69]. Malik *et al*[68] reported statistically significant differences in infection as the cause of death is NASH cirrhosis patients post-LT when compared with other indications and explain the likely cause to be elevated hyperglycemia and diabetes which may predispose these

patients' to increased risk of infection. With the growing number of NAFLD and NASH patients' post-LT, it is expected that more studies would emerge in the upcoming years that would be high-powered to provide further details on these issues.

Management of NAFLD patients after liver transplant

Little data exists for the treatment of NAFLD patients' post-LT. All recommendations for management of NAFLD post-transplant are a reflection of studies done on the non-LT population and can be divided into three broad categories: Lifestyle modifications, Pharmacotherapy and Bariatric Surgery.

Lifestyle modifications: The mainstay of medical management includes weight reduction through physical activity and diet modification and pharmacological management of medical co-morbidities such as hypertension, hypercholesterolemia and diabetes[4]. A low-carbohydrate (< 60 g of carbs/d) low caloric diet when compared with high carbohydrate (> 180 g of carbs/d) low caloric diet has been shown to lead to a more pronounced reduction in intrahepatic triglyceride content and improves insulin sensitivity[70]. Weight loss has also been shown to improve hepatic steatosis and inflammation with weight loss of 3%-5% showing improvement in steatosis and 7%-10% weight loss showing improvement in the level of steatohepatitis[13]. Physical activity has an important effect on the level of NAFLD and should be encouraged in patients. Moderate and vigorous activity was compared with controls that were generally inactive. This study showed that vigorous activity was beneficial in preventing progression to fibrosis in NAFLD patients over moderate activity[71] and thus should be encouraged. The role of caffeine in coffee has also been evaluated in patients with NAFLD. Molloy *et al*[72] showed that when comparing 4 different groups (controls, bland steatosis/not-NASH,

NASH stage 0-1, and NASH stage 2-4), there was a significant reduction in the risk of fibrosis among patients with higher coffee consumption per day.

Pharmacotherapy: The use of insulin sensitizing medications including metformin and thiazolidinedione has been evaluated in patients with NAFLD and NASH. Although metformin use had been associated with normalization of aminotransferases and improvement in liver echographic findings in prior studies[73,74], pooled results from meta-analysis have found no significant improvement on steatosis, inflammation or fibrosis in metformin treated patients with NASH[75]. The study concluded that in patients without diabetes, targeted lifestyle interventions might be at least as beneficial as metformin and there is little evidence to suggest benefit of metformin in patients with NAFLD without pre-existing glucose intolerance regardless of the dose. Thiazolidinediones (TZDs), including rosiglitazone and pioglitazone, have been evaluated in multiple studies on its benefit in NASH patients. Rosiglitazone has however been shown to be associated with increased rate of myocardial infarction[76] and has been removed from European markets and highly restricted in the United States. Given the risk factors for NASH also mirror risk factors for coronary artery disease, rosiglitazone is likely not an optimal treatment option in patients. Pioglitazone was evaluated in a large multicenter study[77] for 96 wk at doses of 30 mg/d and compared with Vitamin E 800 IU/d or placebo in patients without diabetes with NASH. This study concluded that both treatment groups (Vitamin E and Pioglitazone) demonstrated improvement in hepatic steatosis, ballooning and inflammation, although only Vitamin E was associated with statistically significant improvements. Neither treatment had an effect on fibrosis but both Vitamin E and pioglitazone led to improvement in aminotransferase levels. Although Vitamin E may have a role in the treatment of NAFLD patients without diabetes, it is important to note that Vitamin E use has been associated with increased all-cause mortality and prostate cancer, especially at doses of 400 IU/d or higher[78,79]. Other small randomized control trials have also shown similar benefit of pioglitazone at 30-45 mg/d in NASH patients with or without diabetes demonstrating improvements in aminotransferase levels, hepatic steatosis, improved insulin sensitivity and inflammation[80,81] however no improvement in fibrosis were noted. Additionally, unlike rosiglitazone that has been associated with increased cardiovascular mortality[76], pioglitazone has only been associated with having a slightly positive or neutral effect on the cardiovascular system[82]. Based on this data, pioglitazone at doses of 30 mg/d and titrated up for glycemic control if necessary, may be recommended for patients with NAFLD, however should be used with caution in patients with history of heart failure and bladder cancer[82].

The use of statins has been investigated in small pilot studies for the treatment of NAFLD, although there have been mixed results. Rosuvastatin at dose of 10 mg/d given to NAFLD patients without diabetes, showed normalization of aminotransferase and cholesterol levels after follow-up for eight months[83] whereas another trial in NASH patients receiving simvastatin 40 mg/d demonstrated no significant differences in hepatocellular structure and aminotransferase levels when compared with placebo over a duration of one year[84]. Based on conflicting reports, AASLD has recommended against the use of statins in the treatment of NASH until more randomized clinical control trials can demonstrate its efficacy[13].

Ursodiol or ursodeoxycholic acid, approved for the treatment of primary biliary cirrhosis, has also been evaluated for NASH patients and trials thus far have not demonstrated significant differences in overall histology[85,86].

Pentoxifylline, a drug that inhibits the synthesis of TNF-α which is thought to be associated with possible progression to fibrosis[87] in NAFLD patients has also been studied for the treatment of NASH. A recent randomized control trial evaluated pentoxifylline 1200 mg/d compared to placebo in biopsy-confirmed NASH patients over a course of one year and found improvements in aminotransferase levels and histologic features from baseline but these were not significant when compared to placebo[88].

Use of pharmacological intervention to augment weight loss in NASH and NAFLD patients with orlistat has also shown improvement in steatosis and aminotransferase levels[89], however it is most likely the observed changes were associated with weight loss rather than the drug itself.

Role of bariatric surgery: As in the non-transplant population, weight loss has its own challenges in the post-LT population. In addition to obesity pre-transplant, many recipients experience rapid weight gain post-transplant that leads to recurrence and *de novo* steatosis in the graft liver[60]. Weight gain can partially be attributed to immunosuppressive medication such as steroids and calcineurin inhibitors taken to suppress the immune system post-LT. Few studies exist on the benefit of bariatric surgery post-OLT, mostly in the form of case reports and case series[90-93]. Duchini et al[92] reported Roux-en-Y bypass as a successful procedure in two NAFLD patients post-LT with morbid obesity demonstrating significant weight reduction, normalization of liver function and metabolic parameters, including lipid profile and hyperglycemia. A recent study from the University of Minnesota identified seven patients who underwent Roux-en-Y gastric bypass post-LT between 2001 and 2009[93], and reported therapeutic weight loss, improved glycemic control, and improved high-density lipoprotein in the presence of continued dyslipidemia. More studies however, are needed for consideration of bariatric surgery in post-LT patients before definite recommendations could be made.

Choice of Immunosuppression in NAFLD patients
Many immunosuppressive regimens used in the treatment

of post-LT patients are associated with diabetes, hypertension, hyperlipidemia, obesity and increased risk of infection[94]. Patients who undergo LT for NASH often have metabolic syndrome and are at increased risk for the development of major vascular events[68]. Some studies have shown an increased risk of recurrence of hepatocellular carcinoma[95] in addition to other known adverse effects from steroids including diabetes, osteoporosis and obesity. Given that steroids have been linked to much adverse effects, they should be withdrawn from maintenance therapy within three months post-LT. Moving away from a steroid based immunosuppressive regimen in LT patients was evaluated by Segev *et al*[94] in their meta-analysis of thirty publications, including nineteen randomized control trials which showed there was no difference in death, graft loss and infection rates in patients who were on steroid-free regimens when compared with steroid-based immunosuppression. Additionally, the analysis showed a trend towards reduced hypertension and statistically significant decrease in CMV infection and cholesterol levels in steroid-free regimens. The authors also reported that if the steroids were replaced by another immunosuppression medication, there is a reduced risk of diabetes, rejection and severe rejection. This would advocate for the role of avoidance of steroids post-LT for immunosuppression, especially in patients with NASH cirrhosis.

Calcineurin inhibitors include tacrolimus (FK506) and cyclosporine and act by inhibiting T-cell activation. Although these drugs are commonly used, studies have shown acute and chronic nephrotoxicity as a major adverse effect of both tacrolimus and cyclosporine, occurring in up to 20% of patients depending on the organ transplanted[96]. Due to these outcomes, studies have advocated for conversion to sirolimus therapy in patients who develop renal insufficiency due to calcineurin inhibitors[97], however their complete avoidance has been associated with higher rejection rates[98]. Additionally, tacrolimus has been associated with neurotoxicity and development of de-novo diabetes, while cyclosporine has been associated with hypertension and hyperlipidemia[99,100].

Mycophenolic acid and Azathioprine are two other medications commonly used post-LT however require close monitoring due to the risk of bone marrow suppression[101] and their experience in NASH-related LT is limited. The decision on the type of immunosuppression regimen to be used should be based on maintaining a balance between drug toxicity and efficacy and dictated by patient factors such as age, ethnicity and etiology of their liver disease.

CONCLUSION

NAFLD is increasingly recognized as a major etiology leading to chronic liver disease and remains the only indication for LT that has steadily and steeply increased in frequency over the past decades. As the third most common indication for LT in the United States after HCV and alcoholic liver disease, NAFLD is projected to become the most common indication by 2025. The increasing prevalence of NAFLD both pre- and post-transplant presents unique challenges for the transplant community in terms of management and long-term outcomes. Many risk factors for NAFLD pre-transplant such as obesity, hypertension, hyperlipidemia, diabetes continue to play an important role in the pathogenesis of post-transplant NAFLD. In addition to prevention and management of coexisting medical conditions, physicians must weigh the benefits and harms of both medical and surgical therapies in patients undergoing LT. New research in pharmacotherapy such as insulin sensitizing drugs, statins, metformin and others continues to emerge, yet more research is needed to help identify methods to reduce and possibly reverse progression to fibrosis in these patients. The recommendation on avoidance of steroids and minimization of calcineurin inhibitors in this patient population would likely be beneficial in decreasing the risk factors associated with post-transplant NAFLD and should be considered. Further research is still needed to better understand the issues that affect this unique patient population.

REFERENCES

1   **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

2   **Schaffner F**, Thaler H. Nonalcoholic fatty liver disease. *Prog Liver Dis* 1986; **8**: 283-298 [PMID: 3086934]

3   **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJM-ra011775]

4   **Saadeh S**. Nonalcoholic Fatty liver disease and obesity. *Nutr Clin Pract* 2007; **22**: 1-10 [PMID: 17242448 DOI: 10.1177/0115 42650702200101]

5   **Charlton M**. Nonalcoholic fatty liver disease: a review of current understanding and future impact. *Clin Gastroenterol Hepatol* 2004; **2**: 1048-1058 [PMID: 15625647 DOI: 10.1016/S1542-3565(04)00440-9]

6   **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

7   **Lazo M**, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, Clark JM. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. *BMJ* 2011; **343**: d6891 [PMID: 22102439 DOI: 10.1136/bmj.d6891]

8   **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271 [PMID: 10781624]

9   **McClain CJ**, Barve S, Deaciuc I. Good fat/bad fat. *Hepatology* 2007; **45**: 1343-1346 [PMID: 17538963 DOI: 10.1002/hep.21788]

10   **Brunt EM**. Nonalcoholic steatohepatitis: definition and pa-

thology. *Semin Liver Dis* 2001; **21**: 3-16 [PMID: 11296695 DOI: 10.1055/s-2001-12925]

11　**Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

12　**Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

13　**Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

14　**Flegal KM**, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 2012; **307**: 491-497 [PMID: 22253363 DOI: 10.1001/jama.2012.39]

15　**Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

16　**Evans CD**, Oien KA, MacSween RN, Mills PR. Non-alcoholic steatohepatitis: a common cause of progressive chronic liver injury? *J Clin Pathol* 2002; **55**: 689-692 [PMID: 12195000 DOI: 10.1136/jcp.55.9.689]

17　**Fassio E**, Alvarez E, Domínguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology* 2004; **40**: 820-826 [PMID: 15382171 DOI: 10.1002/hep.20410]

18　**Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

19　**Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: 10051466 DOI: 10.1002/hep.510290347]

20　**Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

21　**Edmison J**, McCullough AJ. Pathogenesis of non-alcoholic steatohepatitis: human data. *Clin Liver Dis* 2007; **11**: 75-104; ix [PMID: 17544973]

22　**Pessayre D**, Fromenty B, Mansouri A. Mitochondrial injury in steatohepatitis. *Eur J Gastroenterol Hepatol* 2004; **16**: 1095-1105 [PMID: 15489566]

23　**Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: 15864352 DOI: 10.1172/JCI23621]

24　**Tamura S**, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1139-1142 [PMID: 15864343 DOI: 10.1172/JCI24930]

25　**Cusi K**. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology* 2012; **142**: 711-725.e6 [PMID: 22326434 DOI: 10.1053/j.gastro.2012.02.003]

26　**Li ZZ**, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. *J Biol Chem* 2009; **284**: 5637-5644 [PMID: 19119140 DOI: 10.1074/jbc.M807616200]

27　**Paradis V**, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 2001; **34**: 738-744 [PMID: 11584370 DOI: 10.1053/jhep.2001.28055]

28　**Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

29　**Laryea M**, Watt KD, Molinari M, Walsh MJ, McAlister VC, Marotta PJ, Nashan B, Peltekian KM. Metabolic syndrome in liver transplant recipients: prevalence and association with major vascular events. *Liver Transpl* 2007; **13**: 1109-1114 [PMID: 17663411 DOI: 10.1002/lt.21126]

30　**Modan-Moses D**, Paret G. Leptin and transplantation: pieces are still missing in the puzzle. *Isr Med Assoc J* 2002; **4**: 207-208 [PMID: 11908266]

31　**Man K**, Zhao Y, Xu A, Lo CM, Lam KS, Ng KT, Ho JW, Sun CK, Lee TK, Li XL, Fan ST. Fat-derived hormone adiponectin combined with FTY720 significantly improves small-for-size fatty liver graft survival. *Am J Transplant* 2006; **6**: 467-476 [PMID: 16468955 DOI: 10.1111/j.1600-6143.2005.01201.x]

32　**Foxton MR**, Quaglia A, Muiesan P, Heneghan MA, Portmann B, Norris S, Heaton ND, O'Grady JG. The impact of diabetes mellitus on fibrosis progression in patients transplanted for hepatitis C. *Am J Transplant* 2006; **6**: 1922-1929 [PMID: 16780550 DOI: 10.1111/j.1600-6143.2006.01408.x]

33　**Alberti KG**, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]

34　**Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987]

35　**Dixon JB**, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**: 91-100 [PMID: 11438497]

36　**Colicchio P**, Tarantino G, del Genio F, Sorrentino P, Saldalamacchia G, Finelli C, Conca P, Contaldo F, Pasanisi F. Non-alcoholic fatty liver disease in young adult severely obese non-diabetic patients in South Italy. *Ann Nutr Metab* 2005; **49**: 289-295 [PMID: 16088092]

37　**Angulo P**. NAFLD, obesity, and bariatric surgery. *Gastroenterology* 2006; **130**: 1848-1852 [PMID: 16697746]

38　**Assy N**, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* 2000; **45**: 1929-1934 [PMID: 11117562]

39　**Milić S**, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. *Dig Dis* 2012; **30**: 158-162 [PMID: 22722431 DOI: 10.1159/000336669]

40　**Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/

j.jhep.2004.09.012]

41 **Machado M**, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. *J Hepatol* 2006; **45**: 600-606 [PMID: 16899321 DOI: 10.1016/j.jhep.2006.06.013]

42 **Nair S**, Verma S, Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002; **35**: 105-109 [PMID: 11786965 DOI: 10.1053/jhep.2002.30318]

43 **Murray KF**, Carithers RL. AASLD practice guidelines: Evaluation of the patient for liver transplantation. *Hepatology* 2005; **41**: 1407-1432 [PMID: 15880505 DOI: 10.1002/hep.20704]

44 **Mummadi RR**, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 1396-1402 [PMID: 18986848 DOI: 10.1016/j.cgh.2008.08.012]

45 **Heimbach JK**, Watt KD, Poterucha JJ, Ziller NF, Cecco SD, Charlton MR, Hay JE, Wiesner RH, Sanchez W, Rosen CB, Swain JM. Combined liver transplantation and gastric sleeve resection for patients with medically complicated obesity and end-stage liver disease. *Am J Transplant* 2013; **13**: 363-368 [PMID: 23137119 DOI: 10.1111/j.1600-6143.2012.04318.x]

46 **Perkins JD**. Saying "Yes" to obese living liver donors: short-term intensive treatment for donors with hepatic steatosis in living-donor liver transplantation. *Liver Transpl* 2006; **12**: 1012-1013 [PMID: 16721781 DOI: 10.1002/lt.20807]

47 **D'Alessandro AM**, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen D, Belzer FO. The predictive value of donor liver biopsies on the development of primary nonfunction after orthotopic liver transplantation. *Transplant Proc* 1991; **23**: 1536-1537 [PMID: 1989280]

48 **Ploeg RJ**, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, Sollinger HW, Belzer FO, Kalayoglu M. Risk factors for primary dysfunction after liver transplantation--a multivariate analysis. *Transplantation* 1993; **55**: 807-813 [PMID: 8475556 DOI: 10.1097/00007890-199304000-00024]

49 **Tevar AD**, Clarke C, Wang J, Rudich SM, Woodle ES, Lentsch AB, Edwards ML. Clinical review of nonalcoholic steatohepatitis in liver surgery and transplantation. *J Am Coll Surg* 2010; **210**: 515-526 [PMID: 20347746 DOI: 10.1016/j.jamcollsurg.2010.01.020]

50 **McCormack L**, Petrowsky H, Jochum W, Mullhaupt B, Weber M, Clavien PA. Use of severely steatotic grafts in liver transplantation: a matched case-control study. *Ann Surg* 2007; **246**: 940-946; discussion 946-948 [PMID: 18043095 DOI: 10.1097/SLA.0b013e31815c2a3f]

51 **Angele MK**, Rentsch M, Hartl WH, Wittmann B, Graeb C, Jauch KW, Loehe F. Effect of graft steatosis on liver function and organ survival after liver transplantation. *Am J Surg* 2008; **195**: 214-220 [PMID: 18154767 DOI: 10.1016/j.amjsurg.2007.02.023]

52 **Cho JY**, Suh KS, Lee HW, Cho EH, Yang SH, Cho YB, Yi NJ, Kim MA, Jang JJ, Lee KU. Hepatic steatosis is associated with intrahepatic cholestasis and transient hyperbilirubinemia during regeneration after living donor liver transplantation. *Transpl Int* 2006; **19**: 807-813 [PMID: 16961772 DOI: 10.1111/j.1432-2277.2006.00355.x]

53 **Brandhagen D**, Fidler J, Rosen C. Evaluation of the donor liver for living donor liver transplantation. *Liver Transpl* 2003; **9**: S16-S28 [PMID: 14528424 DOI: 10.1053/jlts.2003.50222]

54 **Trotter JF**. Thin chance for fat people (to become living donors). *Liver Transpl* 2001; **7**: 415-417 [PMID: 11349261 DOI: 10.1053/jlts.2001.24933]

55 **Ryan CK**, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl* 2002; **8**: 1114-1122 [PMID: 12474149 DOI: 10.1053/jlts.2002.36740]

56 AASLD abstracts (pp. 586a–686a). *Hepatology* 2003; **38**: 586A-686A. Available from: URL: http://www.readcube.com/articles/10.1002/hep.1840380508?locale=en

57 **Contos MJ**, Cales W, Sterling RK, Luketic VA, Shiffman ML, Mills AS, Fisher RA, Ham J, Sanyal AJ. Development of non-alcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. *Liver Transpl* 2001; **7**: 363-373 [PMID: 11303298 DOI: 10.1053/jlts.2001.23011]

58 **Maor-Kendler Y**, Batts KP, Burgart LJ, Wiesner RH, Krom RA, Rosen CB, Charlton MR. Comparative allograft histology after liver transplantation for cryptogenic cirrhosis, alcohol, hepatitis C, and cholestatic liver diseases. *Transplantation* 2000; **70**: 292-297 [PMID: 10933151 DOI: 10.1097/00007890-200007270-00009]

59 **Dureja P**, Mellinger J, Agni R, Chang F, Avey G, Lucey M, Said A. NAFLD recurrence in liver transplant recipients. *Transplantation* 2011; **91**: 684-689 [PMID: 21248661 DOI: 10.1097/TP.0b013e31820b6b84]

60 **Dumortier J**, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, Boillot O, Rubbia-Brandt L, Scoazec JY, Hadengue A. Non-alcoholic fatty liver disease in liver transplant recipients: another story of "seed and soil". *Am J Gastroenterol* 2010; **105**: 613-620 [PMID: 20040915 DOI: 10.1038/ajg.2009.717]

61 **Bhagat V**, Mindikoglu AL, Nudo CG, Schiff ER, Tzakis A, Regev A. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis versus patients with cirrhosis due to alcoholic liver disease. *Liver Transpl* 2009; **15**: 1814-1820 [PMID: 19938128 DOI: 10.1002/lt.21927]

62 **Lim LG**, Cheng CL, Wee A, Lim SG, Lee YM, Sutedja DS, Da Costa M, Prabhakaran K, Wai CT. Prevalence and clinical associations of posttransplant fatty liver disease. *Liver Int* 2007; **27**: 76-80 [PMID: 17241384 DOI: 10.1111/j.1478-3231.2006.01396.x]

63 **Seo S**, Maganti K, Khehra M, Ramsamooj R, Tsodikov A, Bowlus C, McVicar J, Zern M, Torok N. De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844-847 [PMID: 17029282 DOI: 10.1002/lt.20932]

64 **Ong J**, Younossi ZM, Reddy V, Price LL, Gramlich T, Mayes J, Boparai N. Cryptogenic cirrhosis and posttransplantation nonalcoholic fatty liver disease. *Liver Transpl* 2001; **7**: 797-801 [PMID: 11552214 DOI: 10.1053/jlts.2001.24644]

65 **Charlton M**, Kasparova P, Weston S, Lindor K, Maor-Kendler Y, Wiesner RH, Rosen CB, Batts KP. Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. *Liver Transpl* 2001; **7**: 608-614 [PMID: 11460228 DOI: 10.1053/jlts.2001.25453]

66 **Tanaka T**, Sugawara Y, Tamura S, Kaneko J, Takazawa Y, Aoki T, Hasegawa K, Sakamoto Y, Yamashiki N, Kokudo N. Living donor liver transplantation for non-alcoholic steatohepatitis: A single center experience. *Hepatol Res* 2013 Jul 9; Epub ahead of print [PMID: 23834427 DOI: 10.1111/hepr.12200]

67 **Poordad F**, Gish R, Wakil A, Garcia-Kennedy R, Martin P, Yao FY. De novo non-alcoholic fatty liver disease following orthotopic liver transplantation. *Am J Transplant* 2003; **3**: 1413-1417 [PMID: 14525603 DOI: 10.1046/j.1600-6143.2003.00203.x]

68 **Malik SM**, deVera ME, Fontes P, Shaikh O, Ahmad J. Outcome after liver transplantation for NASH cirrhosis. *Am J Transplant* 2009; **9**: 782-793 [PMID: 19344467 DOI: 10.1111/j.1600-6143.2009.02590.x]

69 **Guckelberger O**, Mutzke F, Glanemann M, Neumann UP, Jonas S, Neuhaus R, Neuhaus P, Langrehr JM. Validation of cardiovascular risk scores in a liver transplant population. *Liver Transpl* 2006; **12**: 394-401 [PMID: 16498651 DOI: 10.1002/lt.20722]

70 **Kirk E**, Reeds DN, Finck BN, Mayurranjan SM, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology* 2009; **136**: 1552-1560 [PMID: 19208352 DOI: 10.1053/

j.gastro.2009.01.048]

71   **Kistler KD**, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* 2011; **106**: 460-468; quiz 469 [PMID: 21206486 DOI: 10.1038/ajg.2010.488]

72   **Molloy JW**, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. *Hepatology* 2012; **55**: 429-436 [PMID: 21987293 DOI: 10.1002/hep.24731]

73   **Angelico F**, Burattin M, Alessandri C, Del Ben M, Lirussi F. Drugs improving insulin resistance for non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis. *Cochrane Database Syst Rev* 2007; **(1)**: CD005166 [PMID: 17253544 DOI: 10.1097/00005344-199100177-00078]

74   **Bugianesi E**, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; **100**: 1082-1090 [PMID: 15842582 DOI: 10.1111/j.1572-0241.2005.41583.x]

75   **Musso G**, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 79-104 [PMID: 20578268 DOI: 10.1002/hep.23623]

76   **Nissen SE**, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**: 2457-2471 [PMID: 17517853 DOI: 10.1056/NEJMoa072761]

77   **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

78   **Klein EA**, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Lieber MM, Walther PJ, Klotz L, Parsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL, Baker LH. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2011; **306**: 1549-1556 [PMID: 21990298 DOI: 10.1001/jama.2011.1437]

79   **Miller ER**, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005; **142**: 37-46 [PMID: 15537682 DOI: 10.7326/0003-4819-142-1-200501040-00110]

80   **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

81   **Aithal GP**, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J. Randomized, placebo-controlled trial of pioglitazone in non-diabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1176-1184 [PMID: 18718471 DOI: 10.1053/j.gastro.2008.06.047]

82   **Dormandy JA**, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspec-tive pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005; **366**: 1279-1289 [PMID: 16214598 DOI: 10.1016/s0140-6736(05)67528-9]

83   **Antonopoulos S**, Mikros S, Mylonopoulou M, Kokkoris S, Giannoulis G. Rosuvastatin as a novel treatment of non-alcoholic fatty liver disease in hyperlipidemic patients. *Atherosclerosis* 2006; **184**: 233-234 [PMID: 16168995 DOI: 10.1016/j.atherosclerosis.2005.08.021]

84   **Nelson A**, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *J Clin Gastroenterol* 2009; **43**: 990-994 [PMID: 19448566 DOI: 10.1097/MCG.0b013e31819c392e]

85   **Leuschner UF**, Lindenthal B, Herrmann G, Arnold JC, Rössle M, Cordes HJ, Zeuzem S, Hein J, Berg T. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology* 2010; **52**: 472-479 [PMID: 20683947 DOI: 10.1002/hep.23727]

86   **Lindor KD**, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, Burgart L, Colin P. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 2004; **39**: 770-778 [PMID: 14999696 DOI: 10.1002/hep.20092]

87   **Buechler C**, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; **17**: 2801-2811 [PMID: 21734787 DOI: 10.3748/wjg.v17.i23.2801]

88   **Van Wagner LB**, Koppe SW, Brunt EM, Gottstein J, Gardikiotes K, Green RM, Rinella ME. Pentoxifylline for the treatment of non-alcoholic steatohepatitis: a randomized controlled trial. *Ann Hepatol* 2011; **10**: 277-286 [PMID: 21677329]

89   **Harrison SA**, Fecht W, Brunt EM, Neuschwander-Tetri BA. Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. *Hepatology* 2009; **49**: 80-86 [PMID: 19053049 DOI: 10.1002/hep.22575]

90   **Butte JM**, Devaud N, Jarufe NP, Boza C, Pérez G, Torres J, Pérez-Ayuso RM, Arrese M, Martínez J. Sleeve gastrectomy as treatment for severe obesity after orthotopic liver transplantation. *Obes Surg* 2007; **17**: 1517-1519 [PMID: 18219781]

91   **Tichansky DS**, Madan AK. Laparoscopic Roux-en-Y gastric bypass is safe and feasible after orthotopic liver transplantation. *Obes Surg* 2005; **15**: 1481-1486 [PMID: 16354531 DOI: 10.1381/096089205774859164]

92   **Duchini A**, Brunson ME. Roux-en-Y gastric bypass for recurrent nonalcoholic steatohepatitis in liver transplant recipients with morbid obesity. *Transplantation* 2001; **72**: 156-159 [PMID: 11468551]

93   **Al-Nowaylati AR**, Al-Haddad BJ, Dorman RB, Alsaied OA, Lake JR, Chinnakotla S, Slusarek BM, Sampson BK, Ikramuddin S, Buchwald H, Leslie DB. Gastric bypass after liver transplantation. *Liver Transpl* 2013; **19**: 1324-1329 [PMID: 24039124 DOI: 10.1002/lt.23734]

94   **Segev DL**, Sozio SM, Shin EJ, Nazarian SM, Nathan H, Thuluvath PJ, Montgomery RA, Cameron AM, Maley WR. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. *Liver Transpl* 2008; **14**: 512-525 [PMID: 18383081 DOI: 10.1002/lt.21396]

95   **Mazzaferro V**, Rondinara GF, Rossi G, Regalia E, De Carlis L, Caccamo L, Doci R, Sansalone CV, Belli LS, Armiraglio E. Milan multicenter experience in liver transplantation for hepatocellular carcinoma. *Transplant Proc* 1994; **26**: 3557-3560 [PMID: 7998274]

96   **Ojo AO**, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931-940 [PMID: 12954741 DOI: 10.1056/NEJMoa021744]

97   **Fairbanks KD**, Eustace JA, Fine D, Thuluvath PJ. Renal function improves in liver transplant recipients when switched

from a calcineurin inhibitor to sirolimus. *Liver Transpl* 2003; **9**: 1079-1085 [PMID: 14526403 DOI: 10.1053/jlts.2003.50183]

98   **Farkas SA**, Schnitzbauer AA, Kirchner G, Obed A, Banas B, Schlitt HJ. Calcineurin inhibitor minimization protocols in liver transplantation. *Transpl Int* 2009; **22**: 49-60 [PMID: 19121146 DOI: 10.1111/j.1432-2277.2008.00796.x]

99   **Tueche SG**. Diabetes mellitus after liver transplant new etiologic clues and cornerstones for understanding. *Transplant Proc* 2003; **35**: 1466-1468 [PMID: 12826194 DOI: 10.1016/

S0041-1345(03)00528-1]

100   **Haddad EM**, McAlister VC, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. *Cochrane Database Syst Rev* 2006; **(4)**: CD005161 [PMID: 17054241 DOI: 10.1002/14651858]

101   **Taylor AL**, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. *Crit Rev Oncol Hematol* 2005; **56**: 23-46 [PMID: 16039869 DOI: 10.1016/j.critrevonc.2005.03.012]

REVIEW

Coronary microvasculopathy in heart transplantation: Consequences and therapeutic implications

Alessandra Vecchiati, Sara Tellatin, Annalisa Angelini, Sabino Iliceto, Francesco Tona

Alessandra Vecchiati, Sara Tellatin, Annalisa Angelini, Sabino Iliceto, Francesco Tona, Department of Cardiac, Thoracic and Vascular Sciences, Padova University Hospital, 35128 Padova, Italy

Author contributions: Vecchiati A wrote the manuscript; Tona F supervised and discussed this manuscript; all authors contributed to this work.

Correspondence to: Francesco Tona, MD, PhD, Department of Cardiac, Thoracic and Vascular Sciences, Padova University Hospital, via Giustiniani 2, 35128 Padova, Italy. francesco.tona@unipd.it

Telephone: +39-49-8211844   Fax: +39-49-8211802

Received: November 26,2013   Revised: January 11, 2014
Accepted: March 11, 2014
Published online: June 24, 2014

Abstract

Despite the progress made in the prevention and treatment of rejection of the transplanted heart, cardiac allograft vasculopathy (CAV) remains the main cause of death in late survival transplanted patients. CAV consists of a progressive diffuse intimal hyperplasia and the proliferation of vascular smooth muscle cells, ending in wall thickening of epicardial vessels, intramyocardial arteries (50-20 μm), arterioles (20-10 μm), and capillaries (< 10 μm). The etiology of CAV remains unclear; both immunologic and non-immunologic mechanisms contribute to endothelial damage with a sustained inflammatory response. The immunological factors involved are Human Leukocyte Antigen compatibility between donor and recipient, alloreactive T cells and the humoral immune system. The non-immunological factors are older donor age, ischemia-reperfusion time, hyperlipidemia and CMV infections. Diagnostic techniques that are able to assess microvascular function are lacking. Intravascular ultrasound and fractional flow reserve, when performed during coronary angiography, are able to detect epicardial coronary artery disease but are not sensitive enough to assess microvascular changes. Some authors have proposed an index of microcircula-
tory resistance during maximal hyperemia, which is calculated by dividing pressure by flow (distal pressure multiplied by the hyperemic mean transit time). Non-invasive methods to assess coronary physiology are stress echocardiography, coronary flow reserve by transthoracic Doppler echocardiography, single photon emission computed tomography, and perfusion cardiac magnetic resonance. In this review, we intend to analyze the mechanisms, consequences and therapeutic implications of microvascular dysfunction, including an extended citation of relevant literature data.

Core tip: In this review, we intend to analyze the mechanisms, consequences and therapeutic implications of microvascular dysfunction in heart transplantation recipients, including an extended citation of relevant data from the literature. We think that this manuscript could be of interest for many research workers and physicians working in the field of cardiovascular surgery, cardiology and transplant medicine.

INTRODUCTION

Heart transplantation (HT) is the most effective treatment for patients with end-stage heart failure. Recently, early survival after HT has been improved through the

use of immunosuppressive therapy and updated surgical procedures. Unfortunately, late survival is still limited by the onset of malignancies and cardiac allograft vasculopathy (CAV). CAV is a specific form of coronary artery disease that affects heart transplanted patients and is characterized by an early, diffuse intimal proliferation of both the epicardial and microvascular vessels, resulting in epicardial coronary artery stenosis and small vessel occlusion[1]. The 29th Official Adult Heart Transplant Report, edited by the Registry of Heart and Lung Transplantation, noted a relatively small decrease in the cumulative incidence of CAV: at 7 years after transplant, 37% of the patients transplanted between 2003 and June 2010 had CAV, compared with 42% of those transplanted between April 1994 and 2002. In fact, CAV affects 8% by year 1, 30% by year 5 and 50% by year 10 after transplant[2]. This decrease seems to be related to newer approaches to CAV treatment, such as targeting lower low-density lipoproteins (LDL)-cholesterol levels or the use of mammalian target of rapamycin (mTOR) inhibitors or drug-eluting coronary stents[3]. The 1-year survival rate after HT is 81%, and the 5-year survival rate is 69%, with a median survival of 11 years for all HT patients and 13 years for those surviving the first year. CAV causes approximately 10%-15% of the deaths between years 1 and 3 after HT and contributes to potentially more deaths resulting from graft dysfunction[4]. Epicardial coronary artery disease is detectable by intravascular ultrasound (IVUS) during coronary angiography. Coronary microvascular function can be assessed by transthoracic Doppler echocardiography (TDE) measuring coronary flow reserve (CFR)[5]. Understanding the physiopathology of endothelial and microvascular dysfunction in CAV plays a crucial role in the development of new therapies.

THE ROLE OF ENDOTHELIAL FUNCTION

Coronary endothelial vasodilator dysfunction is a common finding in HT recipients and is an early marker for the development of intimal thickening and graft atherosclerosis. Since 1988, a paradoxical coronary vasoconstriction to acetylcholine in allograft recipients with and without angiographic evidence of CAV has been observed[6]. Subsequently, other investigators have observed abnormal responses (vasoconstriction and/or impairment in coronary blood flow response) to serotonin, substance P, cold-pressor testing, and exercise[7-10]. The impairment of endothelial function is time-dependent. Endothelial dysfunction is caused by both immunological and non-immunological risk factors[11]. The immunological response is the principal initiating stimulus and results in endothelial injury and dysfunction and altered endothelial permeability, with consequent myo-intimal hyperplasia and extracellular matrix synthesis. Non-immunological events, including ischemia/reperfusion time, donor age, donor brain death, infections (*i.e.*, Cytomegalovirus, CMV) and traditional risk factors such as hypertension, dyslipidemia and diabetes, contribute to maintaining inflammatory responses and to extend vessel damage[12-14].

Immunological response

Alloimmune injury is initiated when donor major histocompatibility antigens expressed on the surface of graft endothelial cells interact with recipient dendridic cells, resulting in a chronic immune response[15]. Recipient CD4+ lymphocytes recognize donor major histocompatibility complex (MHC) class II antigens on the cell's surface (HLA-DR, DP and DQ) and are activated. This process leads to a cascade of cytokines, such as Interleukin-2 (IL-2), IL-4, IL-5, IL-6, interferon-γ (IFN-γ), and tumor necrosis factor α and β (TNF-α, TNF-β), which promote the proliferation of alloreactive T cells and stimulate the expression of other cytokines and adhesion molecules (*i.e.*, intercellular adhesion molecule-1, ICAM, and vascular cell adhesion molecule-1, VCAM) by the endothelium with leukocyte adhesion to the vessel wall. As a result, the activated macrophages and lymphocytes in the intima of the artery secrete platelet-derived growth factor and transforming growth factor, which stimulate the proliferation of smooth muscle cells (SMCs) and vascular remodeling[16]. Non-human leukocyte antigen (HLA) allo- and auto-antibodies are an increasingly recognized component of the immune response. They are often directed against angiotensin type-1 receptor and the endothelin-1 type A receptor and may alone induce endothelial activation, trigger proinflammatory, and both proproliferative and profibrotic responses[17-19].

Nitric oxide pathway

Cytokines and growth factors lead to coronary endothelial vasodilator dysfunction through the dysregulation of the L-arginine-nitric oxide pathway, resulting in the reduced synthesis and bioactivity of the vasodilators in favor of endothelium-derived vasoconstrictors such as endothelin (ET) and thromboxane. Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator known. It induces vasodilatation by stimulating soluble guanylate cyclase to produce cyclic guanosine monophosphate and inhibits platelet and leukocyte adherence to the vessel wall. IFN-γ is the determinant mediator, linking endothelial dysfunction to structural changes in transplanted human arteries through the down-regulation of endothelial NO synthase (eNOS) expression, inducible-NOS (iNOS) activation and potentiating growth-factor-induced SMC mitogenesis. The iNOS is not a normal constituent of quiescent healthy cells but is expressed in a wide variety of cell types that have been exposed to bacterial endotoxin or combinations of inflammatory cytokines. Under conditions of reduced availability of L-arginine (the NO precursor), the product of iNOS is the superoxide anion, which can increase local oxidative stress and exacerbate the inflammatory process[10,20,21]. The increased production of reactive oxygen species (ROS) is considered a major determinant of reduced levels of NO[22]. In human cardiac allografts, enhanced endomyocardial iNOS mRNA expression is accompanied by the expression of nitrotyrosine protein, suggesting peroxynitrite-mediated vessel damage. Importantly, dietary L-arginine has been shown to attenuate the structural changes of CAV *in vivo*

and has been associated with the down-regulation of insulin-like growth factor- I and IL-6[10]. Recently, great importance has been attributed to the ratio of L-arginine/asymmetric dimethylarginine (ADMA), which is an endogenous NO synthase inhibitor. ADMA is normally produced by the hydrolysis of proteins and degraded by the oxidant-sensitive enzyme dimethylarginine dimethyl aminohydrolase (DDAH)[23]. An increase in the ADMA levels of HT patients has been observed due to an oxidative impairment of the DDAH. The loss of endothelium-derived NO permits the increased activity of the proinflammatory transcription nuclear factor kappa B (NF-κB), resulting in the expression of leukocyte adhesion molecules[22].

Non-immunological mechanisms

Non-immunological risk factors for endothelial dysfunction are the same as those observed in non-transplanted patients, such as CMV infections, diabetes and dyslipidemia. CMV infection of seronegative HT recipients plays an important role in CAV development. It increases the ADMA levels, generates ROS and, through NF-κB activation and TNF-α production, induces proinflammatory cytokines and destabilizes the mRNA message for eNOS[24]. Donor- or recipient-related factors (*e.g.*, age/gender, pre-transplant diagnosis) and factors related to surgery (*e.g.*, ischemia-reperfusion injury) also increase the risk of CAV[25,26]. Diabetes mellitus is present in 28% of recipients at 1 year after HT and in 40% of patients at 5 years after HT[4]. Risk factors for new-onset diabetes include pre-transplant blood glucose of > 5.6 mmol/L, a family history of diabetes, being overweight, and the pre-transplant use of immunosuppressive drugs, particularly calcineurin inhibitors and corticosteroids[27].

Insulin resistance impedes the removal of triglycerides (TG) from very-low-density lipoproteins (VLDL) that are in circulation, resulting in hypertriglyceridemia and high VLDL concentrations. This impedance increases the transfer of cholesterol from high-density lipoproteins (HDL), thus decreasing the HDL concentrations and forming small cholesterol-depleted LDL[28]. These small dense LDL particles are rich in TG but contain relatively little cholesterol and are not readily cleared by the physiological LDL receptor; these particles are highly atherogenic[29]. Markers of metabolic syndrome such as a TG/HDL ratio of ≥ 3 and levels of C-reactive protein (CRP) > 3 mg/L are considered markers of insulin resistance and may lead to endothelial dysfunction and the development of CAV[28]. Hyperlipidemia occurs frequently in HT recipients, with pre-existing or similar conditions to treatment with calcineurin inhibitors and corticosteroids. Hyperlipidemia leads to an increased intimal thickening, but there is only limited evidence that shows its direct association with CAV development[28]. Importantly, the benefits from statin therapy are well documented. Early treatment has been reported to be beneficial to first-year survival and has helped reduce severe rejection, thereby decreasing the development of CAV[30]. Statins inhibit MHC II induction by IFN-γ on primary human endo-thelial cells and monocytes-macrophages and may exert a dampening effect on MHC II-mediated T lymphocyte activation[31].

HISTOPATHOLOGICAL FEATURES

The precise interaction between host and donor endothelium remains unclear, but there is a significant amount of data showing a partial re-endothelization from recipient-derived cells, possibly as a response to allogenic stimuli causing vascular injury[32-34]. Endothelial chimerism (the coexistence of both donor and recipient endothelial cells) has been shown to be much higher in the microcirculation than in larger vessels, with a predilection for small epicardial and intramyocardial vessels, which had a notable 3- to 5-fold-greater chimerism than their larger counterparts. The high degree of endothelial chimerism may have immune implications for myocardial rejection or graft vasculopathy[33-37]. It has been hypothesized that this replacement could lead to a decrease in alloreactivity with a positive influence on graft outcome, but further studies are needed[38].

A study conducted by our group investigated the correlation between levels of human endothelial circulating progenitor cells (EPCs) and microvascular dysfunction, as evaluated by CFR. We demonstrated that EPCs in both the circulation and the graft decrease significantly in HT recipients with microvascular damage. A possible explanation for this may involve humoral factors that occur in a chronic low-grade rejection and influence mobilization, migration, and cell survival[39,40].

Hiemann *et al*[41] established a grading system of microvasculopathy in post-transplantation biopsies by light microscopy. The endothelial layer was defined as the mono-cell layer at the inner part of the blood vessel wall. The presence of a thin layer of cells whose diameter was less than the diameter of the endothelial cell cores was considered normal. Endothelial cells were graded as thickened if the diameter of the cell layer was at least as thick as the endothelial cell cores. The wall layer (media) was defined as the poly-cell layer adjacent to the endothelium. The wall was graded as normal if its diameter was less than the luminal radius. Wall thickening was classified as non-stenotic if the ratio of the luminal radius to wall thickness was < 3 but ≥ 1, and stenotic wall thickening was graded if this ratio was < 1 (Table 1). Stenotic microvasculopathy was diagnosed if there was evidence of microvascular stenosis due to either endothelial thickening or wall thickening in at least one blood vessel per field of view on endomyocardial biopsies[41].

MICROVASCULOPATHY: DIAGNOSTIC TOOLS

Microvascular disease can be detected in HT recipients using both invasive and non-invasive techniques. The international society of heart and lung transplantation (ISHLT) guidelines has suggested CFR during coronary

Table 1 Different definitions of microvasculopathy		
Author	Microvessels diameter (μm)	Microvasculopathy assessment
Drakos *et al*[97]	< 60	Microvascular density (number of microvessels/total tissue analysis area)
Escaned *et al*[96]	< 100	Arteriolar density, capillary and arteriolar obliteration index
Hiemann *et al*[41]	10-20	Luminal radius/medial thickness < 1

angiography as an option for detecting microvascular disease in HT recipients who are suspected of having CAV, but its routine use has not yet been widely instituted[31,42]. CFR is the ratio of the maximum stress flow (during intravenous adenosine vasodilator stress) to the rest flow for a given arterial distribution with or without a stenosis or diffuse narrowing, and it could be performed in more quickly and less expensively using TDE[43,44]. Our group demonstrated that microvascular dysfunction, as evaluated by CFR measured in the distal portion of the left anterior descending coronary artery (LAD), correlates with intimal hyperplasia measured by IVUS in patients with physiologically normal epicardial coronary arteries[45-47].

Dobutamine stress echocardiography (DSE) is a useful technique for HT recipients unable to undergo an angiogram for CAV detection. For CAV detection, the sensitivity and specificity of DSE have been shown in different studies to vary from 67% to 95% and from 55% to 91%, respectively[48-50]. However, its ability in detecting microvascular graft disease is still uncertain[51].

Another noninvasive test is dual-source computed tomography, which showed a sensitivity of 100%, a specificity of 92%, a positive predictive value of 50%, a negative predictive value of 100%, and a global accuracy of 93% in detecting CAV. Similar to DSE, its predictive value in microvascular dysfunction is not well established[52].

Magnetic resonance perfusion imaging with myocardial perfusion reserve (MPR) analysis showed a significant correlation with CFR when invasively evaluated.

Muehling and colleagues analyzed the resting endomyocardial/epimyocardial perfusion ratio (Endo/Epi ratio), which is decreased in impaired coronary circulation. CAV can be excluded by an MPR of > 2.3 with a sensitivity and specificity of 100% and 85%, respectively, and an Endo/Epi ratio of > 1.3 with a sensitivity and specificity of 100% and 80%, respectively[53,54].

MEDICAL TREATMENT

CAV prevention requires a combination of immunosuppressant agents, the prevention of CMV infection and a reduction in common cardiovascular risk factors[25,42,55].

Endothelial dysfunction is an early marker and contributes to the development of CAV[6,56-58]. Standard immunosuppression after cardiac transplantation includes a calcineurin inhibitor (CNIs, such as cyclosporin or tacrolimus) in combination with an antiproliferative agent [mycophenolate mofetil (MMF) or azathioprine (AZA)] with or without corticosteroids[59]. Cyclosporin (Cy-A) was the first immunosuppressive drug that had an important impact on the result of clinical HT by reducing the incidence and severity of rejection. Cy-A is known to impair endothelial function by increasing the release and response to vasoconstrictors, impairing the synthesis of NO, and generating free radicals. It may also result in increased ET levels and an impaired vascular response to NO[60-63]. Kobashigawa *et al*[64] showed that the five-year survival and incidence of angiographic CAV were similar between groups treated with microemulsion Cy-A- or tacrolimus. In a study by Meiser *et al*[65], a more pronounced intimal proliferation was detected in the group treated with Cy-A and MMF than in the tacrolimus-MMF-treated group. Moreover, microvascular endothelial function deteriorates more in Cy-A-treated patients than in tacrolimus-treated patients, a finding that correlates with the enhanced ET-1 concentration and reduced vascular remodeling[65-67]. The progression of CAV is slower in patients randomized to receive MMF instead of AZA. The combination of Cy-A and MMF was associated with a 35% reduction in 3-year mortality or graft loss compared with patients treated with Cy-A and AZA[68]. MMF-treated HT patients, when compared to AZA-treated patients, both treated concurrently on Cy-A and corticosteroids, have significantly less progression of first-year intimal thickening[69]. In terms of CAV prevention, MMF is superior to AZA in both combinations. A trend toward improved survival in MMF patients was noted. The lower number of rejection episodes in the MMF groups may have contributed to these results.

MMF is associated with the reduction of leukocyte adhesion to the graft endothelium and inhibition of the proliferation of SMCs[70-72]. Rapamycin therapy has been associated with improved coronary artery physiology at the level of both the epicardial artery and the microvasculature soon after HT[73]. Proliferation signal inhibitors (PSIs), *e.g.*, sirolimus and everolimus, may have the potential to reduce the incidence of microvasculopathy and, later, of CAV. In a 2-year randomized clinical trial, the use of sirolimus was associated with fewer acute rejection episodes and a significant absence of the progression of intimal plus medial proliferation compared with the use of AZA[74,75]. These drugs were also associated with a lower rate of CMV infection[76,77]. The occurrence of malignancies after HT is a well-described consequence of immunosuppression that affects the long-term prognosis of HT recipients. Patients on mTOR inhibitors, a class of drugs that has been experimentally proven to have both immunosuppressive and potent antitumor effects, developed significantly fewer malignancies, as expected due to the drug's mechanism of action[78]. In a recent retrospective study, Fröhlich *et al*[79] demonstrated that statin use is also protective against malignancies. Hypercholesterolemia and hypertriglyceridemia may occur in HT recipients who are treated with sirolimus, but the presence of these side effects did not appear to impair its ability to slow the progression of CAV[80]. Everolimus is an analog

of sirolimus. Several studies demonstrated a decreased severity and incidence of CAV in HT recipients receiving immunosuppressive therapy with everolimus. It was compared with AZA in the largest trial conducted thus far for HT, which randomized 634 patients. This study showed that both average intimal thickening by IVUS and the incidence of acute rejection at 6 mo after HT were significantly lower in patients receiving everolimus[74,81,82]. Prophylaxis consisting of CMV hyperimmune globulin plus ganciclovir has been associated with decreased intimal thickening and reduced coronary artery disease[83].

Of the recommendations made by the ISHLT regarding CAV management, only statin therapy had a level of evidence A[42]. In several studies, cholesterol and TG have been proven to directly correlate with the development and progression of CAV[84]. It is currently advocated that statins should be given soon after HT, when the most rapid expansion of intimal hyperplasia occurs. Different statins have been associated with the reduced progression of CAV. Simvastatin improved the 8-year survival in HT recipients[85]. A one-year trial in 92 patients randomized to pravastatin or no 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor showed not only lower mean cholesterol levels but also less intimal thickening by IVUS as well as less frequent high-grade acute rejections and rejections with hemodynamic compromise[86].

The vasculoprotective effects of statins are likely mediated by multiple immunogenic effects. The immunomodulating effects of statins in the presence of Cy-A include the suppression of T-cell responses[87], the reduction of chemokine synthesis by mononuclear cells in the peripheral bloodstream, and the inhibition of the expression of *MHC-II* genes[88]. Simvastatin inhibits the proliferation of SMCs, which is an important process in the pathogenesis of the atherosclerotic lesion. Moreover, simvastatin has been shown to have a direct influence on the gene expression of ET-1 in cultivated endothelial cells, leading to improved endothelial function and thus protecting against atherosclerosis and microvasculopathy[89]. Another direct positive effect of simvastatin in the atherogenesis process is that it reduces monocyte adhesion to endothelial cells, which is one of the initial steps in the development of atherosclerotic plaques[90].

The use of calcium channel blockers or angiotensin-converting enzyme inhibitors (ACE-Is) decrease the incidence of CAV detected by IVUS[91]. Additionally, the use of calcium channel blockers decreases angiographically detected CAV 2-years after HT[92]. ACE-Is partially improve allograft microvascular endothelial dysfunction, reduce oxidative stress, and down-regulate endothelial ET-1 release[93], and their use has been associated with plaque regression[94] and improved graft survival[30]. The combined use of an ACE-I and a calcium-antagonist is more effective than the individual use of either drug alone on CAV development. Large randomized clinical trials are warranted to evaluate the possibility of this synergistic efficacy[95].

CONCLUSION

Coronary microvascular function has an impact on long-term graft survival after HT. Microvascular vessel disease has been demonstrated by histological findings of stenotic microvasculopathy and evaluated by non-invasive CFR[41,45,96]. The potential influence of combined immunosuppressive regimens, lipid-lowering agents, or ACE-Is and/or calcium-antagonists on microvessel response is therefore of major interest. More trials are needed for microvasculopathy prevention and/or CFR preservation and to reduce the negative prognostic impact on the survival of HT recipients.

REFERENCES

1   **Mehra MR**, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, Madsen J, Parameshwar J, Starling RC, Uber PA. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010; **29**: 717-727 [PMID: 20620917 DOI: 10.1016/j.healun.2010.05.017]

2   **Lund LH**, Edwards LB, Kucheryavaya AY, Dipchand AI, Benden C, Christie JD, Dobbels F, Kirk R, Rahmel AO, Yusen RD, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report--2013; focus theme: age. *J Heart Lung Transplant* 2013; **32**: 951-964 [PMID: 24054804 DOI: 10.1016/j.healun.2013.08.006]

3   **Stehlik J**, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. *J Heart Lung Transplant* 2012; **31**: 1052-1064 [PMID: 22975095 DOI: 10.1016/j.healun.2012.08.002]

4   **Stehlik J**, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dobbels F, Kirk R, Rahmel AO, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Heart Transplant Report--2011. *J Heart Lung Transplant* 2011; **30**: 1078-1094 [PMID: 21962016 DOI: 10.1016/j.healun.2011.08.003]

5   **Osto E**, Tona F, Angelini A, Montisci R, Ruscazio M, Vinci A, Tarantini G, Ramondo A, Gambino A, Thiene G, Caforio AL, Gerosa G, Iliceto S. Determinants of coronary flow reserve in heart transplantation: a study performed with contrast-enhanced echocardiography. *J Heart Lung Transplant* 2009; **28**: 453-460 [PMID: 19416773 DOI: 10.1016/j.healun.2009.02.008]

6   **Fish RD**, Nabel EG, Selwyn AP, Ludmer PL, Mudge GH, Kirshenbaum JM, Schoen FJ, Alexander RW, Ganz P. Responses of coronary arteries of cardiac transplant patients to acetylcholine. *J Clin Invest* 1988; **81**: 21-31 [PMID: 3121675 DOI: 10.1172/JCI113297]

7   **Mügge A**, Heublein B, Kuhn M, Nolte C, Haverich A, Warnecke J, Forssmann WG, Lichtlen PR. Impaired coronary dilator responses to substance P and impaired flow-dependent dilator responses in heart transplant patients with graft vasculopathy. *J Am Coll Cardiol* 1993; **21**: 163-170 [PMID: 7678019 DOI: 10.1016/0735-1097(93)90732-G]

8   **Benvenuti C**, Aptecar E, Mazzucotelli JP, Jouannot P, Loisance D, Nitenberg A. Coronary artery response to cold-pressor test is impaired early after operation in heart transplant recipients. *J Am Coll Cardiol* 1995; **26**: 446-451 [PMID: 7608449 DOI: 10.1016/0735-1097(95)80021-8]

9   **Preumont N**, Lenaers A, Goldman S, Vachiery JL, Wikler D, Damhaut P, Degré S, Berkenboom G. Coronary vasomotility and myocardial blood flow early after heart transplantation. *Am J Cardiol* 1996; **78**: 550-554 [PMID: 8806341 DOI: 10.1016/

S0002-9149(96)00363-3]

10   **Weis M**, Cooke JP. Cardiac allograft vasculopathy and dys-regulation of the NO synthase pathway. *Arterioscler Thromb Vasc Biol* 2003; **23**: 567-575 [PMID: 12649081 DOI: 10.1161/01. ATV.0000067060.31369.F9]

11   **Vassalli G**, Gallino A, Weis M, von Scheidt W, Kappenberger L, von Segesser LK, Goy JJ. Alloimmunity and nonimmunologic risk factors in cardiac allograft vasculopathy. *Eur Heart J* 2003; **24**: 1180-1188 [PMID: 12831811 DOI: 10.1016/ S0195-668X(03)00237-9]

12   **Rahmani M**, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. *Circ Res* 2006; **99**: 801-815 [PMID: 17038650 DOI: 10.1161/01.RES.0000246086.93555.f3]

13   **Caforio AL**, Tona F, Fortina AB, Angelini A, Piaserico S, Gambino A, Feltrin G, Ramondo A, Valente M, Iliceto S, Thiene G, Gerosa G. Immune and nonimmune predictors of cardiac allograft vasculopathy onset and severity: multivariate risk factor analysis and role of immunosuppression. *Am J Transplant* 2004; **4**: 962-970 [PMID: 15147431 DOI: 10.1111/ j.1600-6143.2004.00434.x]

14   **Valantine HA**. Cardiac allograft vasculopathy: central role of endothelial injury leading to transplant "atheroma". *Transplantation* 2003; **76**: 891-899 [PMID: 14508350 DOI: 10.1097/01.TP.0000080981.90718.EB]

15   **Rogers NJ**, Lechler RI. Allorecognition. *Am J Transplant* 2001; **1**: 97-102 [PMID: 12099369 DOI: 10.1034/j.1600-6143.2001.10201.x]

16   **Behrendt D**, Ganz P, Fang JC. Cardiac allograft vasculopathy. *Curr Opin Cardiol* 2000; **15**: 422-429 [PMID: 11198625 DOI: 10.1097/00001573-200011000-00009]

17   **Hiemann NE**, Meyer R, Wellnhofer E, Schoenemann C, Heidecke H, Lachmann N, Hetzer R, Dragun D. Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation. *Transplantation* 2012; **94**: 919-924 [PMID: 23034559 DOI: 10.1097/TP.0b013e3182692ad2]

18   **Schneider MP**, Boesen EI, Pollock DM. Contrasting actions of endothelin ET(A) and ET(B) receptors in cardiovascular disease. *Annu Rev Pharmacol Toxicol* 2007; **47**: 731-759 [PMID: 17002597 DOI: 10.1146/annurev.pharmtox.47.120505.105134. Contrasting]

19   **Reinsmoen NL**. Role of angiotensin II type 1 receptor-activating antibodies in solid organ transplantation. *Hum Immunol* 2013; **74**: 1474-1477 [PMID: 23831255 DOI: 10.1016/ j.humimm.2013.06.034]

20   **Wildhirt SM**, Weis M, Schulze C, Conrad N, Pehlivanli S, Rieder G, Enders G, von Scheidt W, Reichart B. Expression of endomyocardial nitric oxide synthase and coronary endothelial function in human cardiac allografts. *Circulation* 2001; **104**: I336-I343 [PMID: 11568079 DOI: 10.1161/hc37t1.094598]

21   **Wildhirt SM**, Weis M, Schulze C, Conrad N, Pehlivanli S, Rieder G, Enders G, von Scheidt W, Reichart B. Coronary flow reserve and nitric oxide synthases after cardiac transplantation in humans. *Eur J Cardiothorac Surg* 2001; **19**: 840-847 [PMID: 11404140 DOI: 10.1016/S1010-7940(01)00681-9]

22   **Osto E**, Tona F, De Bon E, Iliceto S, Cella G. Endothelial dysfunction in cardiac allograft vasculopathy: potential pharmacological interventions. *Curr Vasc Pharmacol* 2010; **8**: 169-188 [PMID: 19485920 DOI: 10.2174/157016110790886992]

23   **Cooke JP**. Does ADMA cause endothelial dysfunction? *Arterioscler Thromb Vasc Biol* 2000; **20**: 2032-2037 [PMID: 10978245 DOI: 10.1161/01.ATV.20.9.2032]

24   **Petrakopoulou P**, Kübrich M, Pehlivanli S, Meiser B, Reichart B, von Scheidt W, Weis M. Cytomegalovirus infection in heart transplant recipients is associated with impaired endothelial function. *Circulation* 2004; **110**: II207-II212 [PMID: 15364864 DOI: 10.1161/01.CIR.0000138393.99310.1c]

25   **Valantine H**. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. *J Heart Lung Transplant* 2004; **23**: S187-S193 [PMID: 15093804 DOI: 10.1016/j.healun.2004.03.009]

26   **Tilney NL**, Paz D, Ames J, Gasser M, Laskowski I,

Hancock WW. Ischemia-reperfusion injury. *Transplant Proc* 2001; **33**: 843-844 [PMID: 11267092 DOI: 10.1016/ S0041-1345(00)02341-1]

27   **Potena L**, Valantine HA. Cardiac allograft vasculopathy and insulin resistance--hope for new therapeutic targets. *Endocrinol Metab Clin North Am* 2007; **36**: 965-981; ix [PMID: 17983931 DOI: 10.1016/j.ecl.2007.07.012.Cardiac]

28   **Raichlin ER**, McConnell JP, Lerman A, Kremers WK, Edwards BS, Kushwaha SS, Clavell AL, Rodeheffer RJ, Frantz RP. Systemic inflammation and metabolic syndrome in cardiac allograft vasculopathy. *J Heart Lung Transplant* 2007; **26**: 826-833 [PMID: 17692787 DOI: 10.1016/j.healun.2007.05.008]

29   **Valantine H**, Rickenbacker P, Kemna M, Hunt S, Chen YD, Reaven G, Stinson EB. Metabolic abnormalities characteristic of dysmetabolic syndrome predict the development of transplant coronary artery disease: a prospective study. *Circulation* 2001; **103**: 2144-2152 [PMID: 11331254 DOI: 10.1161/01. CIR.103.17.2144]

30   **Kobashigawa JA**, Starling RC, Mehra MR, Kormos RL, Bhat G, Barr ML, Sigouin CS, Kolesar J, Fitzsimmons W. Multicenter retrospective analysis of cardiovascular risk factors affecting long-term outcome of de novo cardiac transplant recipients. *J Heart Lung Transplant* 2006; **25**: 1063-1069 [PMID: 16962467 DOI: 10.1016/j.healun.2006.05.001]

31   **Colvin-Adams M**, Agnihotri A. Cardiac allograft vasculopathy: current knowledge and future direction. *Clin Transplant* 2011; **25**: 175-184 [PMID: 21457328 DOI: 10.1111/ j.1399-0012.2010.01307.x]

32   **Ross R**. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; **362**: 801-809 [PMID: 8479518 DOI: 10.1038/362801a0]

33   **Minami E**, Laflamme MA, Saffitz JE, Murry CE. Extracardiac progenitor cells repopulate most major cell types in the transplanted human heart. *Circulation* 2005; **112**: 2951-2958 [PMID: 16275883 DOI: 10.1161/CIRCULATIONAHA.105.576017]

34   **Kapessidou Y**, Habran C, Buonocore S, Flamand V, Barvais L, Goldman M, Braun MY. The replacement of graft endothelium by recipient-type cells conditions allograft rejection mediated by indirect pathway CD4(+) T cells. *Transplantation* 2006; **81**: 726-735 [PMID: 16534475 DOI: 10.1097/01. tp.0000184444.93108.d1]

35   **Hillebrands JL**, Klatter FA, van den Hurk BM, Popa ER, Nieuwenhuis P, Rozing J. Origin of neointimal endothelium and alpha-actin-positive smooth muscle cells in transplant arteriosclerosis. *J Clin Invest* 2001; **107**: 1411-1422 [PMID: 11390423 DOI: 10.1172/JCI10233]

36   **Quaini F**, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med* 2002; **346**: 5-15 [PMID: 11777997 DOI: 10.1056/NEJMoa012081]

37   **Simper D**, Wang S, Deb A, Holmes D, McGregor C, Frantz R, Kushwaha SS, Caplice NM. Endothelial progenitor cells are decreased in blood of cardiac allograft patients with vasculopathy and endothelial cells of noncardiac origin are enriched in transplant atherosclerosis. *Circulation* 2003; **108**: 143-149 [PMID: 12835206 DOI: 10.1161/01.CIR.0000081703.34526.5D]

38   **Koopmans M**, Kremer Hovinga IC, Baelde HJ, de Heer E, Bruijn JA, Bajema IM. Endothelial chimerism in transplantation: Looking for needles in a haystack. *Transplantation* 2006; **82**: S25-S29 [PMID: 16829790 DOI: 10.1097/01. tp.0000231446.41051.98]

39   **Osto E**, Castellani C, Fadini GP, Baesso I, Gambino A, Agostini C, Avogaro A, Gerosa G, Thiene G, Iliceto S, Angelini A, Tona F. Impaired endothelial progenitor cell recruitment may contribute to heart transplant microvasculopathy. *J Heart Lung Transplant* 2011; **30**: 70-76 [PMID: 20817567 DOI: 10.1016/j.healun.2010.07.004]

40   **Angelini A**, Castellani C, Tona F, Gambino A, Caforio AP, Feltrin G, Della Barbera M, Valente M, Gerosa G, Thiene G. Continuous engraftment and differentiation of male recipient Y-chromosome-positive cardiomyocytes in do-

nor female human heart transplants. *J Heart Lung Transplant* 2007; **26**: 1110-1118 [PMID: 18022076 DOI: 10.1016/j.healun.2007.08.004]

41 **Hiemann NE**, Wellnhofer E, Knosalla C, Lehmkuhl HB, Stein J, Hetzer R, Meyer R. Prognostic impact of microvasculopathy on survival after heart transplantation: evidence from 9713 endomyocardial biopsies. *Circulation* 2007; **116**: 1274-1282 [PMID: 17709643 DOI: 10.1161/CIRCULATIONAHA.106.647149]

42 **Costanzo MR**, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010; **29**: 914-956 [PMID: 20643330 DOI: 10.1016/j.healun.2010.05.034]

43 **Caiati C**, Montaldo C, Zedda N, Bina A, Iliceto S. New noninvasive method for coronary flow reserve assessment: contrast-enhanced transthoracic second harmonic echo Doppler. *Circulation* 1999; **99**: 771-778 [PMID: 9989962 DOI: 10.1161/01.CIR.99.6.771]

44 **Caiati C**, Montaldo C, Zedda N, Montisci R, Ruscazio M, Lai G, Cadeddu M, Meloni L, Iliceto S. Validation of a new noninvasive method (contrast-enhanced transthoracic second harmonic echo Doppler) for the evaluation of coronary flow reserve: comparison with intracoronary Doppler flow wire. *J Am Coll Cardiol* 1999; **34**: 1193-1200 [PMID: 10520812 DOI: 10.1016/S0735-1097(99)00342-3]

45 **Tona F**, Caforio AL, Montisci R, Angelini A, Ruscazio M, Gambino A, Ramondo A, Thiene G, Gerosa G, Iliceto S. Coronary flow reserve by contrast-enhanced echocardiography: a new noninvasive diagnostic tool for cardiac allograft vasculopathy. *Am J Transplant* 2006; **6**: 998-1003 [PMID: 16611336 DOI: 10.1111/j.1600-6143.2006.01298.x]

46 **Tona F**, Caforio AL, Montisci R, Gambino A, Angelini A, Ruscazio M, Toscano G, Feltrin G, Ramondo A, Gerosa G, Iliceto S. Coronary flow velocity pattern and coronary flow reserve by contrast-enhanced transthoracic echocardiography predict long-term outcome in heart transplantation. *Circulation* 2006; **114**: I49-I55 [PMID: 16820624 DOI: 10.1161/CIRCULATIONAHA.105.001321]

47 **Tona F**, Osto E, Tarantini G, Gambino A, Cavallin F, Feltrin G, Montisci R, Caforio AL, Gerosa G, Iliceto S. Coronary flow reserve by transthoracic echocardiography predicts epicardial intimal thickening in cardiac allograft vasculopathy. *Am J Transplant* 2010; **10**: 1668-1676 [PMID: 20642688 DOI: 10.1111/j.1600-6143.2010.03160.x]

48 **Eroglu E**, D'hooge J, Sutherland GR, Marciniak A, Thijs D, Droogne W, Herbots L, Van Cleemput J, Claus P, Bijnens B, Vanhaecke J, Rademakers F. Quantitative dobutamine stress echocardiography for the early detection of cardiac allograft vasculopathy in heart transplant recipients. *Heart* 2008; **94**: e3 [PMID: 17916666 DOI: 10.1136/hrt.2007.119693]

49 **Spes CH**, Klauss V, Mudra H, Schnaack SD, Tammen AR, Rieber J, Siebert U, Henneke KH, Uberfuhr P, Reichart B, Theisen K, Angermann CE. Diagnostic and prognostic value of serial dobutamine stress echocardiography for noninvasive assessment of cardiac allograft vasculopathy: a comparison with coronary angiography and intravascular ultrasound. *Circulation* 1999; **100**: 509-515 [PMID: 10430765 DOI: 10.1161/01.CIR.100.5.509]

50 **Cai Q**, Rangasetty UC, Barbagelata A, Fujise K, Koerner MM. Cardiac allograft vasculopathy: advances in diagnosis.

Cardiol Rev 2011; **19**: 30-35 [PMID: 21135600 DOI: 10.1097/CRD.0b013e3181fbde2f]

51 **Jackson PA**, Akosah KO, Kirchberg DJ, Mohanty PK, Minisi AJ. Relationship between dobutamine-induced regional wall motion abnormalities and coronary flow reserve in heart transplant patients without angiographic coronary artery disease. *J Heart Lung Transplant* 2002; **21**: 1080-1089 [PMID: 12398873 DOI: 10.1016/S1053-2498(02)00444-8]

52 **Mastrobuoni S**, Bastarrika G, Ubilla M, Castaño S, Azcarate P, Barrero EA, Castellano JM, Herreros J, Rabago G. Dual-source CT coronary angiogram in heart transplant recipients in comparison with dobutamine stress echocardiography for detection of cardiac allograft vasculopathy. *Transplantation* 2009; **87**: 587-590 [PMID: 19307798 DOI: 10.1097/TP.0b013e318195a5a7]

53 **Lim YJ**, Nanto S, Masuyama T, Kodama K, Ikeda T, Kitabatake A, Kamada T. Visualization of subendocardial myocardial ischemia with myocardial contrast echocardiography in humans. *Circulation* 1989; **79**: 233-244 [PMID: 2914344 DOI: 10.1161/01.CIR.79.2.233]

54 **Muehling OM**, Wilke NM, Panse P, Jerosch-Herold M, Wilson BV, Wilson RF, Miller LW. Reduced myocardial perfusion reserve and transmural perfusion gradient in heart transplant arteriopathy assessed by magnetic resonance imaging. *J Am Coll Cardiol* 2003; **42**: 1054-1060 [PMID: 13678930 DOI: 10.1016/S0735-1097(03)00924-0]

55 **Gustafsson F**, Ross HJ. Proliferation signal inhibitors in cardiac transplantation. *Curr Opin Cardiol* 2007; **22**: 111-116 [PMID: 17284989 DOI: 10.1097/HCO.0b013e328012545e]

56 **Hollenberg SM**, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Oberoi M, Johnson MR, Costanzo MR. Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. *Circulation* 2001; **104**: 3091-3096 [PMID: 11748106 DOI: 10.1161/hc5001.100796]

57 **Davis SF**, Yeung AC, Meredith IT, Charbonneau F, Ganz P, Selwyn AP, Anderson TJ. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. *Circulation* 1996; **93**: 457-462 [PMID: 8565162 DOI: 10.1161/01.CIR.93.3.457]

58 **Tona F**, Marra MP, Fedrigo M, Famoso G, Bellu R, Thiene G, Gerosa G, Angelini A, Iliceto S. Recent developments on coronary microvasculopathy after heart transplantation: a new target in the therapy of cardiac allograft vasculopathy. *Curr Vasc Pharmacol* 2012; **10**: 206-215 [PMID: 22239627 DOI: 10.2174/157016112799304987]

59 **Raichlin E**, Prasad A, Kremers WK, Edwards BS, Rihal CS, Lerman A, Kushwaha SS. Sirolimus as primary immunosuppression is associated with improved coronary vasomotor function compared with calcineurin inhibitors in stable cardiac transplant recipients. *Eur Heart J* 2009; **30**: 1356-1363 [PMID: 19383734 DOI: 10.1093/eurheartj/ehp123]

60 **Sudhir K**, MacGregor JS, DeMarco T, De Groot CJ, Taylor RN, Chou TM, Yock PG, Chatterjee K. Cyclosporine impairs release of endothelium-derived relaxing factors in epicardial and resistance coronary arteries. *Circulation* 1994; **90**: 3018-3023 [PMID: 7994850 DOI: 10.1161/01.CIR.90.6.3018]

61 **Diederich D**, Skopec J, Diederich A, Dai FX. Cyclosporine produces endothelial dysfunction by increased production of superoxide. *Hypertension* 1994; **23**: 957-961 [PMID: 8206635 DOI: 10.1161/01.HYP.23.6.957]

62 **Jeanmart H**, Malo O, Carrier M, Nickner C, Desjardins N, Perrault LP. Comparative study of cyclosporine and tacrolimus vs newer immunosuppressants mycophenolate mofetil and rapamycin on coronary endothelial function. *J Heart Lung Transplant* 2002; **21**: 990-998 [PMID: 12231370 DOI: 10.1016/S1053-2498(02)00429-1]

63 **Barton M**, Haudenschild CC, d'Uscio LV, Shaw S, Münter K, Lüscher TF. Endothelin ETA receptor blockade restores NO-mediated endothelial function and inhibits atheroscle-

rosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA* 1998; **95**: 14367-14372 [PMID: 9826706 DOI: 10.1073/pnas.95.24.14367]

64   **Kobashigawa JA**, Patel J, Furukawa H, Moriguchi JD, Yeatman L, Takemoto S, Marquez A, Shaw J, Oeser BT, Subherwal S, Wu GW, Kawano J, Laks H. Five-year results of a randomized, single-center study of tacrolimus vs microemulsion cyclosporine in heart transplant patients. *J Heart Lung Transplant* 2006; **25**: 434-439 [PMID: 16563974 DOI: 10.1016/j.healun.2005.11.452]

65   **Meiser BM**, Groetzner J, Kaczmarek I, Landwehr P, Müller M, Jung S, Uberfuhr P, Fraunberger P, Stempfle HU, Weis M, Reichart B. Tacrolimus or cyclosporine: which is the better partner for mycophenolate mofetil in heart transplant recipients? *Transplantation* 2004; **78**: 591-598 [PMID: 15446320 DOI: 10.1097/01.TP.0000129814.52456.25]

66   **Petrakopoulou P**, Anthopoulou L, Muscholl M, Klauss V, von Scheidt W, Uberfuhr P, Meiser BM, Reichart B, Weis M. Coronary endothelial vasomotor function and vascular remodeling in heart transplant recipients randomized for tacrolimus or cyclosporine immunosuppression. *J Am Coll Cardiol* 2006; **47**: 1622-1629 [PMID: 16631000 DOI: 10.1016/j.jacc.2005.10.073]

67   **Tepperman ED**, Tumiati LC, Ramzy D, Badiwala MV, Sheshgiri R, Prodger JL, Ross HJ, Rao V. Tacrolimus preserves vasomotor function and maintains vascular homeostasis. *J Heart Lung Transplant* 2011; **30**: 583-588 [PMID: 21273094 DOI: 10.1016/j.healun.2010.11.022]

68   **Eisen HJ**, Kobashigawa J, Keogh A, Bourge R, Renlund D, Mentzer R, Alderman E, Valantine H, Dureau G, Mancini D, Mamelok R, Gordon R, Wang W, Mehra M, Constanzo MR, Hummel M, Johnson J. Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. *J Heart Lung Transplant* 2005; **24**: 517-525 [PMID: 15896747 DOI: 10.1016/j.healun.2005.02.002]

69   **Kobashigawa JA**, Tobis JM, Mentzer RM, Valantine HA, Bourge RC, Mehra MR, Smart FW, Miller LW, Tanaka K, Li H, Gjertson DW, Gordon RD. Mycophenolate mofetil reduces intimal thickness by intravascular ultrasound after heart transplant: reanalysis of the multicenter trial. *Am J Transplant* 2006; **6**: 993-997 [PMID: 16611335 DOI: 10.1111/j.1600-6143.2006.01297.x]

70   **Kobashigawa J**, Miller L, Renlund D, Mentzer R, Alderman E, Bourge R, Costanzo M, Eisen H, Dureau G, Ratkovec R, Hummel M, Ipe D, Johnson J, Keogh A, Mamelok R, Mancini D, Smart F, Valantine H. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate Mofetil Investigators. *Transplantation* 1998; **66**: 507-515 [PMID: 9734496 DOI: 10.1097/00007890-199808270-00016]

71   **Cantarovich M**, Giannetti N, Cecere R. Impact of cyclosporine 2-h level and mycophenolate mofetil dose on clinical outcomes in de novo heart transplant patients receiving anti-thymocyte globulin induction. *Clin Transplant* 2003; **17**: 144-150 [PMID: 12709082 DOI: 10.1034/j.1399-0012.2003.00036.x]

72   **Kaczmarek I**, Ertl B, Schmauss D, Sadoni S, Knez A, Daebritz S, Meiser B, Reichart B. Preventing cardiac allograft vasculopathy: long-term beneficial effects of mycophenolate mofetil. *J Heart Lung Transplant* 2006; **25**: 550-556 [PMID: 16678034 DOI: 10.1016/j.healun.2006.01.003]

73   **Sinha SS**, Pham MX, Vagelos RH, Perlroth MG, Hunt SA, Lee DP, Valantine HA, Yeung AC, Fearon WF. Effect of rapamycin therapy on coronary artery physiology early after cardiac transplantation. *Am Heart J* 2008; **155**: 889.e1-889.e6 [PMID: 18440337 DOI: 10.1016/j.ahj.2008.02.004]

74   **Eisen HJ**, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sørensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med* 2003; **349**: 847-858 [PMID: 12944570 DOI: 10.1056/NEJMoa022171]

75   **Keogh A**, Richardson M, Ruygrok P, Spratt P, Galbraith A, O'Driscoll G, Macdonald P, Esmore D, Muller D, Faddy S. Sirolimus in de novo heart transplant recipients reduces acute rejection and prevents coronary artery disease at 2 years: a randomized clinical trial. *Circulation* 2004; **110**: 2694-2700 [PMID: 15262845 DOI: 10.1161/01.CIR.0000136812.90177.94]

76   **Eisen H**, Kobashigawa J, Starling RC, Valantine H, Mancini D. Improving outcomes in heart transplantation: the potential of proliferation signal inhibitors. *Transplant Proc* 2005; **37**: 4S-17S [PMID: 15809102 DOI: 10.1016/j.transproceed.2005.02.118]

77   **Weill D**. Role of cytomegalovirus in cardiac allograft vasculopathy. *Transpl Infect Dis* 2001; **3** Suppl 2: 44-48 [PMID: 11926750 DOI: 10.1034/j.1399-3062.2001.00009.x]

78   **Doesch AO**, Müller S, Konstandin M, Celik S, Kristen A, Frankenstein L, Ehlermann P, Sack FU, Katus HA, Dengler TJ. Malignancies after heart transplantation: incidence, risk factors, and effects of calcineurin inhibitor withdrawal. *Transplant Proc* 2010; **42**: 3694-3699 [PMID: 21094840 DOI: 10.1016/j.transproceed.2010.07.107]

79   **Fröhlich GM**, Rufibach K, Enseleit F, Wolfrum M, von Babo M, Frank M, Berli R, Hermann M, Holzmeister J, Wilhelm M, Falk V, Noll G, Lüscher TF, Ruschitzka F. Statins and the risk of cancer after heart transplantation. *Circulation* 2012; **126**: 440-447 [PMID: 22761452 DOI: 10.1161/CIRCULATIONAHA.111.081059]

80   **Massy ZA**. Hyperlipidemia and cardiovascular disease after organ transplantation. *Transplantation* 2001; **72**: S13-S15 [PMID: 11585238 DOI: 10.1097/00007890-200109271-00005]

81   **Gurk-Turner C**, Manitpisitkul W, Cooper M. A comprehensive review of everolimus clinical reports: a new mammalian target of rapamycin inhibitor. *Transplantation* 2012; **94**: 659-668 [PMID: 22986894 DOI: 10.1097/TP.0b013e31825b411c]

82   **Patel JK**, Kobashigawa JA. Everolimus for cardiac allograft vasculopathy--every patient, at any time? *Transplantation* 2011; **92**: 127-128 [PMID: 21555972 DOI: 10.1097/TP.0b013e318220588f]

83   **Valantine HA**, Gao SZ, Menon SG, Renlund DG, Hunt SA, Oyer P, Stinson EB, Brown BW, Merigan TC, Schroeder JS. Impact of prophylactic immediate posttransplant ganciclovir on development of transplant atherosclerosis: a post hoc analysis of a randomized, placebo-controlled study. *Circulation* 1999; **100**: 61-66 [PMID: 10393682 DOI: 10.1161/01.CIR.100.1.61]

84   **Hoang K**, Chen YD, Reaven G, Zhang L, Ross H, Billingham M, Valantine H. Diabetes and dyslipidemia. A new model for transplant coronary artery disease. *Circulation* 1998; **97**: 2160-2168 [PMID: 9626177 DOI: 10.1161/01.CIR.97.21.2160]

85   **Wenke K**, Meiser B, Thiery J, Nagel D, von Scheidt W, Krobot K, Steinbeck G, Seidel D, Reichart B. Simvastatin initiated early after heart transplantation: 8-year prospective experience. *Circulation* 2003; **107**: 93-97 [PMID: 12515749 DOI: 10.1161/01.CIR.0000043241.32523.EE]

86   **Kobashigawa JA**, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA. Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 1995; **333**: 621-627 [PMID: 7637722 DOI: 10.1056/NEJM199509073331003]

87   **Kurakata S**, Kada M, Shimada Y, Komai T, Nomoto K. Effects of different inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, pravastatin sodium and simvastatin, on sterol synthesis and immunological functions in human lymphocytes in vitro. *Immunopharmacology* 1996; **34**: 51-61 [PMID: 8880225 DOI: 10.1016/0162-3109(96)00108-7]

88   **Kwak B**, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. *Nat Med* 2000; **6**: 1399-1402 [PMID: 11100127 DOI: 10.1038/82219]

89   **Hernández-Perera O**, Pérez-Sala D, Navarro-Antolín J, Sánchez-Pascuala R, Hernández G, Díaz C, Lamas S. Effects

of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest* 1998; **101**: 2711-2719 [PMID: 9637705 DOI: 10.1172/JCI1500]

90   **Teupser D**, Bruegel M, Stein O, Stein Y, Thiery J. HMG-CoA reductase inhibitors reduce adhesion of human monocytes to endothelial cells. *Biochem Biophys Res Commun* 2001; **289**: 838-844 [PMID: 11735122 DOI: 10.1006/bbrc.2001.6066]

91   **Mehra MR**, Ventura HO, Smart FW, Collins TJ, Ramee SR, Stapleton DD. An intravascular ultrasound study of the influence of angiotensin-converting enzyme inhibitors and calcium entry blockers on the development of cardiac allograft vasculopathy. *Am J Cardiol* 1995; **75**: 853-854 [PMID: 7717300 DOI: 10.1016/S0002-9149(99)80432-9]

92   **Schroeder JS**, Gao SZ, Alderman EL, Hunt SA, Johnstone I, Boothroyd DB, Wiederhold V, Stinson EB. A preliminary study of diltiazem in the prevention of coronary artery disease in heart-transplant recipients. *N Engl J Med* 1993; **328**: 164-170 [PMID: 8417382 DOI: 10.1056/NEJM199301213280303]

93   **Steinhauff S**, Pehlivanli S, Bakovic-Alt R, Meiser BM, Becker BF, von Scheidt W, Weis M. Beneficial effects of quinaprilat on coronary vasomotor function, endothelial oxidative stress, and endothelin activation after human heart transplantation. *Transplantation* 2004; **77**: 1859-1865 [PMID: 15223904 DOI: 10.1097/01.TP.0000131148.78203.B7]

94   **Bae JH**, Rihal CS, Edwards BS, Kushwaha SS, Mathew V, Prasad A, Holmes DR, Lerman A. Association of angiotensin-converting enzyme inhibitors and serum lipids with plaque regression in cardiac allograft vasculopathy. *Transplantation* 2006; **82**: 1108-1111 [PMID: 17060862 DOI: 10.1097/01.tp.0000230378.61437.a5]

95   **Erinc K**, Yamani MH, Starling RC, Crowe T, Hobbs R, Bott-Silverman C, Rincon G, Young JB, Feng J, Cook DJ, Smedira N, Tuzcu EM. The effect of combined Angiotensin-converting enzyme inhibition and calcium antagonism on allograft coronary vasculopathy validated by intravascular ultrasound. *J Heart Lung Transplant* 2005; **24**: 1033-1038 [PMID: 16102438 DOI: 10.1016/j.healun.2004.06.005]

96   **Escaned J**, Flores A, García-Pavía P, Segovia J, Jimenez J, Aragoncillo P, Salas C, Alfonso F, Hernández R, Angiolillo DJ, Jiménez-Quevedo P, Bañuelos C, Alonso-Pulpón L, Macaya C. Assessment of microcirculatory remodeling with intracoronary flow velocity and pressure measurements: validation with endomyocardial sampling in cardiac allografts. *Circulation* 2009; **120**: 1561-1568 [PMID: 19805652 DOI: 10.1161/CIRCULATIONAHA.108.834739]

97   **Drakos SG**, Kfoury AG, Hammond EH, Reid BB, Revelo MP, Rasmusson BY, Whitehead KJ, Salama ME, Selzman CH, Stehlik J, Clayson SE, Bristow MR, Renlund DG, Li DY. Impact of mechanical unloading on microvasculature and associated central remodeling features of the failing human heart. *J Am Coll Cardiol* 2010; **56**: 382-391 [PMID: 20650360 DOI: 10.1016/j.jacc.2010.04.019]

REVIEW

Transplant options for patients with type 2 diabetes and chronic kidney disease

Costas Fourtounas

Costas Fourtounas, Department of Internal Medicine-Nephrology, Patras University Hospital, 26500 Patras, Greece
Author contributions: Fourtounas C solely contributed to this paper.
Correspondence to: Costas Fourtounas MD, PhD, Associate Professor of Internal Medicine-Nephrology, Department of Internal Medicine-Nephrology, Patras University Hospital, Rio, Patras, 26500, Greece. cfourt@usa.net
Telephone: +30-69-44763781 Fax: +30-26-10999724
Received: November 28, 2013 Revised: March 20, 2014
Accepted: May 14, 2014
Published online: June 24, 2014

Abstract

Chronic kidney disease (CKD) has become a real epidemic around the world, mainly due to ageing and diabetic nephropathy. Although diabetic nephropathy due to type 1 diabetes mellitus (T1DM) has been studied more extensively, the vast majority of the diabetic CKD patients suffer from type 2 diabetes mellitus (T2DM). Renal transplantation has been established as a first line treatment for diabetic nephropathy unless there are major contraindications and provides not only a better quality of life, but also a significant survival advantage over dialysis. However, T2DM patients are less likely to be referred for renal transplantation as they are usually older, obese and present significant comorbidities. As pre-emptive renal transplantation presents a clear survival advantage over dialysis, all T2DM patients with CKD should be referred for early evaluation by a transplant center. The transplant center should have enough time in order to examine their eligibility focusing on special issues related with diabetic nephropathy and explore the best options for each patient. Living donor kidney transplantation should always be considered as the first line treatment. Otherwise, the patient should be listed for deceased donor kidney transplantation. Recent progress in transplantation medicine has improved the "transplant menu" for T2DM patients with diabetic nephropathy and there is an ongoing discussion about the place of simultaneous pancreas kidney (SPK) transplantation in well selected patients. The initial hesitations about the different pathophysiology of T2DM have been forgotten due to the almost similar short- and long-term results with T1DM patients. However, there is still a long way and a lot of ethical and logistical issues before establishing SPK transplantation as an ordinary treatment for T2DM patients. In addition recent advances in bariatric surgery may offer new options for severely obese T2DM patients with CKD. Nevertheless, the existing data for T2DM patients with advanced CKD are rather scarce and bariatric surgery should not be considered as a cure for diabetic nephropathy, but only as a bridge for renal transplantation.

Core tip: Kidney transplantation has been established as a first line treatment for patients with type 2 diabetes mellitus (T2DM) and diabetic nephropathy, as it is accompanied with a significant survival advantage over dialysis. Pre-emptive living donor kidney transplantation should be the ultimate goal unless there are obvious contraindications and all patients should be referred for early evaluation by a transplant center. There is an ongoing debate about the exact role of simultaneous pancreas kidney transplantation. At the moment it should be offered only in well selected T2DM patients. Bariatric surgery may serve as a bridge for renal transplantation for severely obese T2DM patients with chronic kidney disease.

Fourtounas C. Transplant options for patients with type 2 diabetes and chronic kidney disease. *World J Transplant* 2014; 4(2): 102-110 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/i2/102.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i2.102

INTRODUCTION

Chronic kidney disease (CKD) has become a real epidemic around the world, mainly due to ageing and diabetic nephropathy[1-3]. Although diabetic nephropathy due to type 1 diabetes mellitus (T1DM) has been studied more extensively, the vast majority (90%-95%) of the diabetic CKD patients suffer from type 2 diabetes mellitus (T2DM). Currently, about 40%-45% of the dialysis (hemodialysis or peritoneal dialysis) population is diabetics and present increased morbidity and mortality compared with other causes of CKD[1-6]. In addition diabetic patients comprise almost 40% of the transplant waiting lists nowadays[7].

Diabetic CKD patients undergoing dialysis present excessive morbidity and mortality mainly due to cardiovascular complications[3-6]. Several years ago, diabetic nephropathy was considered as a relative or absolute contraindication for renal transplantation, due to increased rates of cardiovascular and infectious complications and unacceptable morbidity and mortality. However, the landmark study of Wolfe et al[8] has shown that renal transplantation provided a clear survival advantage for diabetics with end-stage renal disease (ESRD) and reduced mortality by 73% compared with patients remaining on the waiting list. The projected life expectancy was more pronounced for younger diabetics (presumably T1DM) reaching a gain of 17 years, whereas the gain was also significant even for patients older than 60 years (presumably T2DM).

Pancreas transplantation and especially simultaneous pancreas kidney (SPK) transplantation outcomes have seen a dramatic improvement regarding both allograft and patient survival, mainly due to advances in immunosuppression and surgical techniques[9-12]. Historically, pancreas transplantation was considered as a relative if not absolute contraindication for T2DM[7] but current data provide evidence that it can also be offered to well selected T2DM patients with CKD with comparable outcomes[7,12-14]. So, the transplant menu for T2DM patients has been expanded, but the best transplant option is still uncertain[15].

This is an update regarding current trends in transplant medicine for T2DM patients with CKD and is based on the studies published in details in peer-reviewed journals, several previous review articles[4-7,16-18] and novel data[14,19-21] which may change our attitudes and policies regarding the management of this frail CKD population.

PRE-TRANSPLANT EVALUATION

There is hard evidence that pre-emptive renal transplantation presents a clear survival advantage over dialysis and all T2DM patients with CKD should be referred for early evaluation by a transplant center[6]. The goal of the pre-transplant risk evaluation is to determine whether the T2DM candidate is eligible for transplantation and discuss all the potential transplant options which may include: (1) kidney transplantation. The kidney allograft may origin from a deceased donor (DDKT) or by a living donor (LDKT). If the operation takes place before the need of dialysis it is referred as pre-emptive KT[22]; (2) simultaneous pancreas kidney (SPK) transplantation, where there is a combined transplantation of both organs, coming usually from the same donor, during a single operation. The origin of the grafts is usually from deceased donors, but there are also reports of segmental pancreatic grafts from living donors[23].

The pros and the cons of both options will be discussed in details below in separate sections of this review.

The contraindications include the general contraindications for any organ transplantation, such as the presence of malignancy, active infection, psychiatric disease, drug/alcohol dependence, morbid obesity and untreated or end-stage organ damage with special emphasis on cardiovascular comorbidities[24,25]. Age should not be considered as an absolute contraindication for renal transplantation[8] but the increased rates of medical and surgical complications and the lower graft survival rates[5,6,8,25], should be clearly explained in elderly diabetic candidates although some other studies did not confirm these results. Most transplant centers do not accept diabetic patients older than 45-50 years for SPK[13,15] although there are reports of SPK transplantation in patients over this limit. In 2010 the international pancreas transplant registry (IPTR) reported that 2% of pancreas transplant recipients were older than 60 years at the time of transplantation[12].

Obesity [body mass index (BMI) > 30-35 kg/m^2] has also been considered as a relative contraindication for transplantation in diabetic patients as it is accompanied with inferior outcomes for both KT[26,27] and SPK[28] mainly due to surgical complications. However, only morbid obesity (BMI > 40 kg/m^2) should be considered as an absolute contraindication. Recent advances in bariatric surgery can ameliorate this contraindication and make even obese T2DM patients eligible for transplantation[29,30]. This important issue will be discussed in the end of this review.

As T2DM patients with diabetic nephropathy present increased cardiovascular morbidity and mortality, the pre-transplant evaluation should focus on the presence and the severity of coronary and peripheral artery disease. Although, there is no consensus regarding the optimal protocol for cardiovascular risk stratification, most transplant centers refer the candidates for cardiac stress testing and/or coronary angiography, especially in ages older than 55-60 years as well as dyslipidemia, history of smoking and presence of cerebrovascular or peripheral vascular disease[6,24]. However, the provocative study of Patel et al[31] has challenged this approach reporting that aggressive pre-transplant testing and coronary interventions did not translate into better outcomes post transplantation in high risk patients.

Peripheral arterial occlusive disease and carotid arteries stenosis examination by ultrasound examination are also mandatory in the pre-transplant evaluation. Many centers suggest a more thorough examination in high risk patients by CT or MR angiography or even intra-arterial angiography[6,24].

All possible advantages of transplantation should be

carefully balanced against the potential complications of the surgical procedure and the long-term side-effects of immunosuppression[15]. A state of the art approach is to refer the patient with advanced diabetic nephropathy to the transplant center early, when his estimated glomerular filtration rate is about 25-30 mL/min in order to provide enough time for evaluation of both the transplant candidate and any potential living donors[6]. However, most T2DM patients with CKD are not referred early even to a nephrologist and the above policy remains rather elusive. Nevertheless, by an early referral, the transplant team can evaluate more thoroughly the diabetic candidate and order more complicated investigations such as coronary angiography, without increasing the risk of premature start of dialysis[6]. In addition, early referral will also provide time to search for LDKT with the most suitable donor or even alternative options for pre-emptive transplantation in cases of immunologically incompatible but still qualified donors, such as kidney paired donation[32].

As SPK transplantation may also be an option for selected T2DM patients with CKD, all available data should be discussed with the transplant candidate. It should be emphasized that SPK transplantation is surgically more challenging compared with kidney transplantation, is accompanied with increased rates of complications and the short- and long-term outcomes should be reported in an unbiased way. However, it is not a standard procedure for most transplant centers and the patient may need to be referred to a more experienced center.

Regarding T2DM patients eligible for transplantation the United Network for Organ Sharing (UNOS) has defined the following criteria for SPK: (1) insulin therapy and C-peptide level < 2 ng/mL; or (2) insulin therapy with C-peptide level > 2 ng/mL and BMI < 28 kg/m^2[13,14,19,33]. The initial concern regarding pancreas transplantation in T2DM patients was insulin resistance that prevails in this type of DM and may result in lower pancreas allograft survival due to β cell exhaustion from the increased insulin demands[15,21]. These concerns and the discussion about the pros and the cons of SPK transplantation are discussed later in this review.

KIDNEY TRANSPLANTATION FOR T2DM PATIENTS WITH CKD

Kidney transplantation is not a "panacea" for T2DM patients with CKD. Although pre-emptive renal transplantation[34], offers a significant survival advantage for all (diabetics and non diabetics) CKD patients, diabetic CKD patients present inferior survival rates compared with other populations. Becker et al[22] have reported that the patient survival benefits of pre-emptive transplantation are more pronounced in LDKT than in DDKT (RR = 0.685; P = 0.001). However renal graft survival did not present significant differences in pre-emptive transplantation except LDKT (RR = 0.81, P = 0,09)[22]. The main reason for these poor outcomes is the accumulated cardiovascular burden during the era before reaching

ESRD. Cosio et al[35] has shown that diabetic patients who have undergone renal transplantation have significantly increased rates of post-transplant cardiovascular events, cardiovascular mortality and all-cause mortality. It is also noteworthy that most cardiovascular events or deaths usually appear during the first three post-transplant months when the most important complications such as rejection or infections present a peak[8,35,36]. All these data highlight the importance of a thorough pre-transplant evaluation, which may detect early and potentially reversible abnormalities. In addition elderly T2DM patients with advanced CKD may present significantly decreased survival after renal transplantation rising ethical issues regarding allocation policies in an era of graft shortage and increased demand around the world.

Immunosuppressive regimens for T2DM patients do not show any difference compared with other populations. However, there is a current trend for steroid free or steroid avoidance protocols which may not aggravate glycemic control. These policies have not yet been translated into better long-term outcomes.

Hypertension and hyperlipidemia are also highly prevalent in diabetic patients post-transplantation and they should be treated aggressively. However, diabetic transplant recipients present higher rates of hyperkalemia after renin angiotensin system inhibition[4-6].

Glycemic control should also be intensified as hyperglycemia has been associated with worse outcome. However, optimal targets for renal transplantation have not been set yet and transplant physicians usually follow the guidelines for the general population.

SPK TRANSPLANTATION FOR T2DM PATIENTS WITH CKD

By the end of 2010 more than 35000 pancreas transplantations had been reported to the IPTR with the vast majority (24000) performed in the United States[12].

Historically, pancreas transplantation was considered as a relative if not absolute contraindication for T2DM[7]. This concept relied on the pathophysiology of T2DM where insulin resistance has been considered as the prevailing disorder and these patients do not seem to need extra insulin but a better responsiveness of the peripheral tissues to it. However, the classification of diabetes is not always so simple and many patients present with overlapping clinical syndromes. In addition, even in the long-run, not a few T2DM patients may become dependent on exogenous insulin due to pancreatic b-cells exhaustion. Although the classical phenotype of T2DM with CKD is characterized by advanced age and obesity, there are many patients who do not fit on this model and may be seen as candidates for pancreas transplantation.

Initial reports about SPK transplantation in T2DM were based on cases of "unrecognized" T2DM[7]. The IPTR started to be record data about the type of diabetes since 1994. The overall rate of pancreas transplantation in T2DM patients has shown an increase from 2% in 1995 up to 7%

Table 1 Selected data from pancreas transplantation single-center and database studies in type 2 diabetes mellitus patients

Ref.	PTx Era	Number of PTx (n)	Age at PTx (yr)	BMI (kg/m^2)	Follow-up (yr)	Pancreas survival rates (yr)	Patient survival rates (yr)
Light et al[37]	1989-1999	30 SPK	40 ± 9.3^1	24.8 ± 5.4^1	3.8	82% (1)[1]	82% (1)[1]
			41.7 ± 6.6^2	25.5 ± 4^2		95% (1)[2]	100% (1)[2]
						82% (5)	82% (5)
						95% (5)	95% (5)
Light et al[38]	1989-2004	38 SPK	40 ± 9.3^1	24.8 ± 5.4^1	> 10	67% (5)[1]	73% (5)[1]
			37.9 ± 8.7^2	23 ± 4.5^2		56% (10)[2]	70% (10)[2]
Light et al[20]	1989-2008	58 SPK	42.8 ± 8.4	26.1 ± 4.4	> 15-20	58.60% (> 10)	75.8% (> 10)
Nath et al[39]	1994-2002	7 SPK	52.5 ± 8.4	27.2 ± 5	4.3	65% (3.3)	94% (1)
		4 PAK					71% (3.3)
		6 PTA					
Singh et al[40]	2002-2007	7 SPK	51 ± 2.9	ND	3.3	71% (3.3)	86% (1)
							71% (3.3)
Chakkera et al[41]	2003-2008	10 SPK	51.9 ± 9	27 ± 3	1.3	100% (1)	100% (1)
Margreiter et al[21]	2000-2009	21 SPK	53.6 ± 5.9	25.1 ± 3.3	7.3 ± 3	81.8 (1)	90.5 (1)
						75.9 (5)	80.1 (5)
Sampaio et al[14]	2000-2007	582 SPK	47 (40-52)	< 18.5 = 2.8%	3.7	-	67% (5)
				18.5 to 25 = 43.9%			
				25 to 30 = 36.2%			
				> 30 = 17.15			
Wiseman et al[19]	2000-2008	424 SPK	18-34 = 6.1%	24.7 ± 2.8	5	87.7 (1)	82% (5)
			35-49 = 54%			83.6 (5)	
			50-59 = 39.9%				

[1]Data for non African-Americans; [2]Data for African-Americans. PTx: Pancreas transplantation; PAK: Pancreas after kidney; PTA: Pancreas transplant alone; SPK: Simultaneous pancreas kidney.

in 2010. According to the same database, in 2010 approximately 8% of SPK, 5% of pancreas after kidney (PAK) and 1% of pancreas transplant alone (PTA) were performed in T2DM patients. T2DM patients who underwent PAK or SPK were older than T1DM patients, whereas there were no age differences between the two groups for PTA. As expected, T2DM patients had a longer duration of DM (22 $\pm$ 8 years) and significantly higher BMI[12].

The usefulness of SPK in T2DM patients with CKD can not be justified by evidence from randomized controlled studies and is based on several single center[20,21,37-41] and two recent database studies[14,19] which will be analyzed in details (Table 1). The main problem of all these studies is that they rely on different approaches regarding the classification of diabetes, which are based on several clinical or laboratorial criteria not validated in CKD and different demographics. There is an ongoing debate about the usefulness of C-peptide for the diagnosis of diabetes as there is evidence that not a few T1DM patients may present measurable serum levels[42] and many T2DM patients may also present with undetectable serum levels after many years post-diagnosis. Covic et al[43] have confirmed these data in CKD patients making the situation even more complicated.

In addition, traditional exclusion criteria for SPK such as age > 50 years and BMI > 30 kg/m^2 which were applied in the first studies, tend to be ignored in the more recent reports, making the interpretation of the short and long-term outcomes not so easy.

Single-center studies

Light et al[37] were the first who attempted to publish

pooled data about outcomes of T2DM patients who underwent SPK transplantation. In 2001 they presented data for 30 patients classified as T2DM according to C-peptide levels > 0.8 ng/mL and compared them with a group of 89 patients with lower C-peptide levels over a 10 years period[37]. C-peptide levels were not crucial for the decision to proceed with SPK transplantation in their center. There were no differences between the two groups regarding patient and graft survival rates, although T2DM patients tended to be older and heavier (not statistically significant differences). In 2005 the same group extended their follow-up period and reported outcomes in 38 SPK recipients[38]. Outcomes at 5 and 10 years post transplant did not show significant differences and the authors suggested that decisions about SPK transplants should not be based on C-peptide levels, but on general acceptance criteria. In 2013 they reported a 20 years experience of SPK transplantation based on data from 173 patients[20]. The T2DM group included 58 patients who underwent transplantation from 1989 through 2008 with the same inclusion criteria (C-peptide levels > 0.8 ng/mL). According to this analysis T2DM patients presented better pancreatic graft survival ($P = 0.064$) but lower patient survival (0.019) during the extended follow-up period. There are no definite explanations for these results, but it is noteworthy that T2DM patients presented lower rejection rates. Moreover, the T2DM group included more African-American and was older, heavier and had a shorter duration of insulin dependence. The authors concluded that C-peptide should not be a marker for SPK candidacy and transplant centers should base their decisions on general criteria which prove whether the diabetic

patient can tolerate the surgical procedure and adhere to the complex follow-up post-transplant.

Nath et al[39] reported a cohort of 17 T2DM patients who underwent pancreas transplantation from 1994 through 2002. Seven patients underwent SPK, 4 patients PAK and 6 patients PTA. The authors adopted the american diabetes association and World Health Organization criteria for T1DM and T2DM and did not rely on C-peptide levels[39]. Three patients were on oral hypoglycemic agents at the time of transplantation. Although 1 patient died during the peri-operative period (aspiration pneumonia) the other pancreas recipients presented excellent graft survival rates (94%). Long-term follow up (4.3 years) showed a patient survival rate of 71% and a pancreas survival rate of 63%.

Singh et al[40] stratified a cohort of 74 SPK transplants from 2002 through 2007 into two groups according to C-peptide cut-off levels of 2 ng/mL. They wisely did not use the terms T1DM or T2DM but they isolated a subgroup of SPK recipients of "insulin requiring diabetic patients with C-peptide production" for further analysis. So, they reported short- and long-term outcomes in 67 patients with "no" C-peptide (mean 0.2 ± 0.4, range 0-1.9 ng/mL) and 7 patients with C-peptide production (mean 5.7 ± 2.7, range 2.5-9.5 ng/mL). Their selection criteria for SPK transplantation included insulin requirement for at least 5 years, daily dose < 1 U/kg, age < 60 years, and absence of severe comorbid conditions, but not C-peptide levels. Patient survival was better in the "no" C-peptide group at 3 mo, 1 year and last follow-up (40 mo), whereas death-censored kidney and pancreas graft survivals did not present significant differences between the two groups. However, there were significant differences between the two groups before SPK transplantation, which have definitely influenced outcomes. The group with the C-peptide production included more African-Americans, was older, heavier and had a shorter duration of diabetes and a longer dialysis vintage.

Chakkera et al[41] reported a cohort study of 80 patients who underwent SPK transplantation from 2003 until 2008. Among them, 10 patients were identified as T2DM patients according to a composite metric which included clinical criteria (absence of ketoacidosis and use of oral antidiabetics), presence of measurable C peptide levels and negative glutamic acid decarboxylase antibodies (anti-GAD65). Patients were eligible for SKP, if BMI was lower than 30 kg/m^2 and needed < 1 U/kg of insulin per day. T2DM patients presented excellent (100%) 1 year pancreas survival as well as T1DM patients (96%) and equal renal graft survival rates after a 16 mo follow-up period. The authors also commented on the usual value of the C-peptide cutoffs in the diagnosis of T1DM (< 0.8 ng/mL) and highlighted that there was a significant overlap of C-peptide levels among T1DM (almost 15% had detectable levels and 8% > 0.8 ng/mL), whereas 30% of the T2DM patients presented low C-peptide levels (< 2 ng/mL) and could be misclassified as T1DM.

Margreiter et al[21] have recently reported their experience from 195 T1DM and 21 T2DM patients who underwent SPK transplantation during a nine years period (2000-2009) in Austria. The vast majority (30/32) of the T2DM patients were on exogenous insulin therapy and had a history of oral antidiabetic agents for at least 6 months. Only 2 patients were receiving oral antidiabetics at the time of transplantation. The main criteria for the diagnosis of T2DM were measurable fasting C peptide levels and absence of autoantibodies for diabetes. All patients presented a low cardiovascular risk profile and were eligible for SPK if BMI was lower than 32 kg/m^2. The authors compared outcomes with T1DM patients who underwent SPK transplantation (n = 195) and T2DM patients who underwent DDKT alone (n = 32) during the same period. Although pancreas allograft survival was lower in T2DM patients, it did not reach statistical significance. In a univariate analysis, the T1DM group presented better patient and kidney survival compared with the other groups. However, in a multivariate analysis model the statistical significance was lost, when data were adjusted for various important confounding variables such as donor and recipient age, secondary complications of diabetes, waiting time, delayed graft function etc.

Selected data for comparison from all these studies are shown in Table 1.

Database studies

Sampaio et al[14] studied outcomes of SPK transplantation during the period between 2000 and 2007 using data from the UNOS database. Among 6756 SPK transplants there were 582 T2DM cases (8.6%). T2DM patients presented higher rates of delayed kidney graft function and primary kidney non function and inferior rates of 5 year overall (73.5% vs 77.8%, P = 0.007) and death censored kidney graft survival 82.9% vs 85.3%, P = 0.04) compared with T1DM patients. However, this group included more African-American and Hispanics and the patients were older at diabetes onset and at the time of transplantation, were more often obese and had a higher pre-transplant dialysis time. All these parameters are known to impact transplant outcomes and when data were analyzed after adjustment for confounders, diabetes type could not be identified as a risk factor for all outcomes. In details, hazard ratios were 1.10 (95%CI: 0.86-1.42) for patient death, 1.08 (95%CI: 0.91-1.28) for pancreas allograft failure and 1.16 (95%CI: 0.95-1.39) for kidney allograft failure with T1DM values as reference. Further analysis revealed that increased recipients' age, time spend on dialysis pretransplant and higher BMI were associated with worse outcomes in T2DM patients. However, the study carried a significant limitation regarding the definition of diabetes type which relied mainly on clinical history data and not specified criteria.

Wiseman et al[19] analyzed data from 424 SPK transplants in T2DM from 2000 through 2008, using the Scientific Registry of Transplant Recipients database and compared outcomes with patients who underwent LDKT or DDKT. They included in their analysis only recipients aged from 18 to 59 years with a BMI index ranging from 18-30 kg/m^2. Although there were no reliable definitions

of diabetes type in this study, the selection criteria have probably eliminated the percentage of misclassification. In this study the authors reported several very interesting and important results. Although SPK outcomes were excellent even after 5 years post-transplant and looked superior to DDKT, this difference was not due to the pancreas allograft *per se* but to other important factors such as younger allograft kidney donors, younger recipient age and less waiting time for transplantation. In addition the analysis provided a clear 5 year survival advantage in favor of LDKT over SPK. However, the authors acknowledge that the possible advantages of SPK (euglycemia) regarding patient and kidney survival may become clearer after a longer follow-up and patients who undergo SPK may represent a special and probably pre-selected population of T2DM patients. In addition quality of life issues (insulin injections, hypoglycemia, *etc.*) may be more important for several T2DM patients with ESRD than survival. Nevertheless, these data provide clear evidence that LDKT should be considered as a first choice treatment for T2DM patients with CKD and SPK should be seen as a second choice for well selected patients.

Data overview

The results from all these single center and database studies do not provide a clear message about the pros and the cons of SPK in T2DM with CKD and many physicians remain skeptic about its definite role, as it carries significant surgical challenges and it is not an immediately life saving procedure[13,17]. The recently applied UNOS criteria for eligibility of T2DM patients for SPK include only C-peptide levels cut-offs and BMI values (see above), although there is no solid data about this policy[15]. Theoretically, T2DM patients who are eligible for listing for both DDKT and SKT transplantation may be transplanted faster if listed for SPK according to the priority criteria for kidney and pancreas allocation. Nevertheless, it should be emphasized that this theoretical concern may not be proven correct in the real clinical practice, as SPK transplantation is performed only in selected transplant centers and its rates tend to fall over the last years[12].

ISLET TRANSPLANTATION AND T2DM

Islet Transplantation refers to the transplantation of isolated pancreatic islets, which have been harvested from one or more deceased donors. It is not a classic surgical procedure and the islets are infused percutaneously into the portal vein[44].

Allogeneic islet transplantation in humans become popular after the landmark study of the Edmonton group in 2000[44] which showed insulin independence in seven T1DM patients with a steroid free regimen. Nevertheless, these first encouraging results could not be fully reproduced by other centers and patients needed multiple islet transfusions with a long-term success below 10%[45-47]. In addition, the immunosuppressive protocols are potentially nephrotoxic and may be accompanied with a deterioration of the renal function[48,49] whereas the

failed islet grafts may lead to recipients' alloimmunization (sensitization) by the production of *de novo* anti-HLA antibodies in titers ranging between 10.8%-31%[48-50]. These poor results have raised skepticism in the transplant community[51] and today only a few centers continue islet transplants on a regular basis in T1DM patients[46,47]. Although the ultimate goal of islet transplantation would be to achieve insulin independence, this remains an exemption and the current goals focus mainly on protection from hypoglycemia, reduction of the daily dose of insulin and correction of HbA1c[47].

Islet transplantation has not been widely applied in T2DM patients. In the literature there is only one report regarding islet transplantation in 5 insulin treated T2DM patients[52]. However these patients were undergoing liver transplantation and islet were given as a possible treatment for coexisting T2DM. Three of them presented normalization of HBA1c and no need for insulin therapy. However, although hypothetical, if clinical data for T1DM patients improve in the future, it would not be a surprise to see islet transplantation applied in T2DM patients, following the example of SPK[17,19].

BARIATRIC SURGERY FOR T2DM PATIENTS WITH CKD

The term "diabesity" has been introduced in the current literature in order to describe the frequent co-existence of T2DM and obesity[53]. Although bariatric surgery procedures tend to increase around the world, there is a debate about its place in the treatment of diabetes[53,54]. Current standards suggest that it has a role in patients with BMI > 35 kg/m^2 with one at least comorbid condition including T2DM[54]. Its theoretical advantages for T2DM patients with lower BMI values remain unproven[53-55]. In addition, there is an ongoing interest regarding the impact of obesity on the pathogenesis and the progression of CKD[56]. However, there is no solid data regarding the beneficial effects of bariatric surgery in CKD, except some small observational single-center studies focusing mainly on the regression of micro- or macro-albuminuria[56,57].

As most transplant centers include obesity (BMI > 30-35 kg/m^2) in the contraindications for renal or SPK transplantation due to excessive surgical complications, many obese T2DM patients may not qualify. So, bariatric surgery has been recently introduced, not as a cure for diabetic nephropathy *per se*, but as a "bridge" for transplantation. There are a few reports about this alternative in patients with advanced CKD, but the complication rates were substantially higher than in non CKD patients[18,58,59]. However these data came from open surgical procedures and currently applied laparoscopic approaches may reduce complications and improve outcomes. Nevertheless, although promising, bariatric surgery in CKD patients or more especially in T2DM patients with CKD has not been studied in depth and should be still considered as experimental[56,60]. If applied, this must be done in specialized and experienced centers under a multidisciplinary

approach.

Nevertheless, a recent analysis of the United States Renal Data System has questioned the current BMI thresholds, as it has shown that even obese diabetic renal transplant recipients may show a survival benefit compared to treatment with dialysis, except patients with BMI > 40 kg/m^2 and obese African Americans[61].

CONCLUSION

Although during the first era of transplant medicine T2DM patients with CKD were considered non eligible for kidney transplantation, recent progress in transplantation medicine has improved their "transplant menu". As pre-emptive kidney transplantation provides a clear survival advantage over dialysis, all patients with no obvious contraindications, should be referred for early evaluation by a transplant center.

There are data that SPK transplantation may be offered in T2DM patients with acceptable long-term outcomes, but it should be noted that the decision is not so easy, as these results come from retrospective studies from very experienced centers and these patients carry particular characteristics (younger ages, no obesity, minimal cardiovascular risk, *etc.*) that may not apply to the average T2DM patient with CKD.

Bariatric surgery may also be considered as a "bridge" to transplantation for very obese T2DM candidates, but at the moment there are no clear data about its outcomes and possible complication rates in this population. Prospective multi-center studies are warranted in order to clarify all these issues. Until then, the most appropriate transplant option for T2DM patients with diabetic nephropathy should always be individualized, taking under consideration the patient's wills, his overall medical condition and the transplant center's experience with all these procedures.

The transplant menu looks delicious, but we must be a bit more patient.

REFERENCES

1   **Coresh J**, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA* 2007; **298**: 2038-2047 [PMID: 17986697 DOI: 10.1001/jama.298.17.2038]
2   **Burrows NR**, Li Y, Geiss LS. Incidence of treatment for end-stage renal disease among individuals with diabetes in the U.S. continues to decline. *Diabetes Care* 2010; **33**: 73-77 [PMID: 20040673 DOI: 10.2337/dc09-0343]
3   **Dousdampanis P**, Trigka K, Fourtounas C. Diagnosis and management of chronic kidney disease in the elderly: a field of ongoing debate. *Aging Dis* 2012; **3**: 360-372 [PMID: 23185717]
4   **Luan FL**, Samaniego M. Transplantation in diabetic kidney failure patients: modalities, outcomes, and clinical management. *Semin Dial* 2010; **23**: 198-205 [PMID: 20374550 DOI: 10.1111/j.1525-139X.2010.00708.x]
5   **Guerra G**, Ilahe A, Ciancio G. Diabetes and kidney transplantation: past, present, and future. *Curr Diab Rep* 2012; **12**: 597-603 [PMID: 22872422 DOI: 10.1007/s11892-012-0306-3]
6   **Gaston RS**, Basadonna G, Cosio FG, Davis CL, Kasiske BL, Larsen J, Leichtman AB, Delmonico FL. Transplantation in the diabetic patient with advanced chronic kidney disease: a task force report. *Am J Kidney Dis* 2004; **44**: 529-542 [PMID: 15332226 DOI: 10.1053/j.ajkd.2004.05.030]
7   **Orlando G**, Stratta RJ, Light J. Pancreas transplantation for type 2 diabetes mellitus. *Curr Opin Organ Transplant* 2011; **16**: 110-115 [PMID: 21150617 DOI: 10.1097/MOT.0b013e3283424d1f]
8   **Wolfe RA**, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; **341**: 1725-1730 [PMID: 10580071 DOI: 10.1056/NEJM199912023412303]
9   **Boggi U**, Amorese G, Marchetti P. Surgical techniques for pancreas transplantation. *Curr Opin Organ Transplant* 2010; **15**: 102-111 [PMID: 20010103 DOI: 10.1097/MOT.0b013e32833553de]
10   **Boggi U**, Vistoli F, Egidi FM, Marchetti P, De Lio N, Perrone V, Caniglia F, Signori S, Barsotti M, Bernini M, Occhipinti M, Focosi D, Amorese G. Transplantation of the pancreas. *Curr Diab Rep* 2012; **12**: 568-579 [PMID: 22828824 DOI: 10.1007/s11892-012-0293-4]
11   **White SA**, Shaw JA, Sutherland DE. Pancreas transplantation. *Lancet* 2009; **373**: 1808-1817 [PMID: 19465236 DOI: 10.1016/S0140-6736(09)60609-7]
12   **Gruessner AC**. 2011 update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the International Pancreas Transplant Registry (IPTR). *Rev Diabet Stud* 2011; **8**: 6-16 [PMID: 21720668 DOI: 10.1900/RDS.2011.8.6]
13   **Sener A**, Cooper M, Bartlett ST. Is there a role for pancreas transplantation in type 2 diabetes mellitus? *Transplantation* 2010; **90**: 121-123 [PMID: 20562677 DOI: 10.1097/TP.0b013e3181e5b7e6]
14   **Sampaio MS**, Kuo HT, Bunnapradist S. Outcomes of simultaneous pancreas-kidney transplantation in type 2 diabetic recipients. *Clin J Am Soc Nephrol* 2011; **6**: 1198-1206 [PMID: 21441123 DOI: 10.2215/CJN.06860810]
15   **Cohen DJ**, Ratner LE. Type 2 diabetes: the best transplant option is still uncertain. *Clin J Am Soc Nephrol* 2012; **7**: 530-532 [PMID: 22442186 DOI: 10.2215/CJN.02120212]
16   **Fourtounas C**, Dousdampanis P. Kidney, Pancreas and Islet Transplant Options for Patients with Diabetic Nephropathy. *J Diabetes Metab* 2013; **S9**: 001 [DOI: 10.4172/2155-6156.S9-001]
17   **Wiseman AC**. Kidney transplant options for the diabetic patient. *Transplant Rev* (Orlando) 2013; **27**: 112-116 [PMID: 23927899 DOI: 10.1016/j.trre.2013.07.002]
18   **Scalea JR**, Cooper M. Surgical strategies for type II diabetes. *Transplant Rev* (Orlando) 2012; **26**: 177-182 [PMID: 22115951 DOI: 10.1016/j.trre.2011.07.002]
19   **Wiseman AC**, Gralla J. Simultaneous pancreas kidney transplant versus other kidney transplant options in patients with type 2 diabetes. *Clin J Am Soc Nephrol* 2012; **7**: 656-664 [PMID: 22344508 DOI: 10.2215/CJN.08310811]
20   **Light J**, Tucker M. Simultaneous pancreas kidney transplants in diabetic patients with end-stage renal disease: the 20-yr experience. *Clin Transplant* 2013; **27**: E256-E263 [PMID: 23480129 DOI: 10.1111/ctr.12100]
21   **Margreiter C**, Resch T, Oberhuber R, Aigner F, Maier H, Sucher R, Schneeberger S, Ulmer H, Bösmüller C, Margreiter R, Pratschke J, Öllinger R. Combined pancreas-kidney transplantation for patients with end-stage nephropathy caused by type-2 diabetes mellitus. *Transplantation* 2013; **95**: 1030-1036 [PMID: 23407544 DOI: 10.1097/TP.0b013e3182861945]
22   **Becker BN**, Rush SH, Dykstra DM, Becker YT, Port FK. Pre-emptive transplantation for patients with diabetes-related kidney disease. *Arch Intern Med* 2006; **166**: 44-48 [PMID: 16401809 DOI: 10.1001/archinte.166.1.44]

23   **Gruessner RW**, Sutherland DE. Simultaneous kidney and segmental pancreas transplants from living related donors - the first two successful cases. *Transplantation* 1996; **61**: 1265-1268 [PMID: 8610429 DOI: 10.1097/00007890-199604270-00025]

24   **Kasiske BL**, Cangro CB, Hariharan S, Hricik DE, Kerman RH, Roth D, Rush DN, Vazquez MA, Weir MR. The evaluation of renal transplantation candidates: clinical practice guidelines. *Am J Transplant* 2001; **1** Suppl **2**: 3-95 [PMID: 12108435]

25   **EBPG (European Expert Group on Renal Transplantation)**, European Renal Association (ERA-EDTA), European Society for Organ Transplantation (ESOT). European Best Practice Guidelines for Renal Transplantation (part 1). *Nephrol Dial Transplant* 2000; **15** Suppl 7: 1-85 [PMID: 11286185 DOI: 10.1093/ndt/15.suppl_7.2]

26   **Meier-Kriesche HU**, Ojo AO, Port FK, Arndorfer JA, Cibrik DM, Kaplan B. Survival improvement among patients with end-stage renal disease: trends over time for transplant recipients and wait-listed patients. *J Am Soc Nephrol* 2001; **12**: 1293-1296 [PMID: 11373354]

27   **Meier-Kriesche HU**, Arndorfer JA, Kaplan B. The impact of body mass index on renal transplant outcomes: a significant independent risk factor for graft failure and patient death. *Transplantation* 2002; **73**: 70-74 [PMID: 11792981 DOI: 10.1097/00007890-200201150-00013]

28   **Sampaio MS**, Reddy PN, Kuo HT, Poommipanit N, Cho YW, Shah T, Bunnapradist S. Obesity was associated with inferior outcomes in simultaneous pancreas kidney transplant. *Transplantation* 2010; **89**: 1117-1125 [PMID: 20164819 DOI: 10.1097/TP.0b013e3181d2bfb2]

29   **Pham PT**, Danovitch GM, Pham PC. Obesity and its impact on transplantation and alloimmunity. *Transplantation* 2013; **96**: e71-e72 [PMID: 24247903 DOI: 10.1097/01.TP.0000436929.53768.93]

30   **Porubsky M**, Powelson JA, Selzer DJ, Mujtaba MA, Taber T, Carnes KL, Fridell JA. Pancreas transplantation after bariatric surgery. *Clin Transplant* 2012; **26**: E1-E6 [PMID: 22050266 DOI: 10.1111/j.1399-0012.2011.01559.x]

31   **Patel RK**, Mark PB, Johnston N, McGeoch R, Lindsay M, Kingsmore DB, Dargie HJ, Jardine AG. Prognostic value of cardiovascular screening in potential renal transplant recipients: a single-center prospective observational study. *Am J Transplant* 2008; **8**: 1673-1683 [PMID: 18510627 DOI: 10.1111/j.1600-6143.2008.02281.x]

32   **Pavlakis M**, Kher A. Pre-emptive kidney transplantation to improve survival in patients with type 1 diabetes and imminent risk of ESRD. *Semin Nephrol* 2012; **32**: 505-511 [PMID: 23062992 DOI: 10.1016/j.semnephrol.2012.07.014]

33   **Kaufman DB**, Sutherland DE. Simultaneous pancreas-kidney transplants are appropriate in insulin-treated candidates with uremia regardless of diabetes type. *Clin J Am Soc Nephrol* 2011; **6**: 957-959 [PMID: 21527647 DOI: 10.2215/CJN.03180411]

34   **Mange KC**, Joffe MM, Feldman HI. Effect of the use or non-use of long-term dialysis on the subsequent survival of renal transplants from living donors. *N Engl J Med* 2001; **344**: 726-731 [PMID: 11236776 DOI: 10.1056/NEJM200103083441004]

35   **Cosio FG**, Hickson LJ, Griffin MD, Stegall MD, Kudva Y. Patient survival and cardiovascular risk after kidney transplantation: the challenge of diabetes. *Am J Transplant* 2008; **8**: 593-599 [PMID: 18294155 DOI: 10.1111/j.1600-6143.2007.02101.x]

36   **Lentine KL**, Brennan DC, Schnitzler MA. Incidence and predictors of myocardial infarction after kidney transplantation. *J Am Soc Nephrol* 2005; **16**: 496-506 [PMID: 15615820 DOI: 10.1681/ASN.2004070580]

37   **Light JA**, Sasaki TM, Currier CB, Barhyte DY. Successful long-term kidney-pancreas transplants regardless of C-peptide status or race. *Transplantation* 2001; **71**: 152-154 [PMID: 11211183 DOI: 10.1097/00007890-200101150-00025]

38   **Light JA**, Barhyte DY. Simultaneous pancreas-kidney trans-

39   **Nath DS**, Gruessner AC, Kandaswamy R, Gruessner RW, Sutherland DE, Humar A. Outcomes of pancreas transplants for patients with type 2 diabetes mellitus. *Clin Transplant* 2005; **19**: 792-797 [PMID: 16313327 DOI: 10.1111/j.1399-0012.2005.00423.x]

40   **Singh RP**, Rogers J, Farney AC, Hartmann EL, Reeves-Daniel A, Doares W, Ashcraft E, Adams PL, Stratta RJ. Do pretransplant C-peptide levels influence outcomes in simultaneous kidney-pancreas transplantation? *Transplant Proc* 2008; **40**: 510-512 [PMID: 18374116 DOI: 10.1016/j.transproceed.2008.01.048]

41   **Chakkera HA**, Bodner JK, Heilman RL, Mulligan DC, Moss AA, Mekeel KL, Mazur MJ, Hamawi K, Ray RM, Beck GL, Reddy KS. Outcomes after simultaneous pancreas and kidney transplantation and the discriminative ability of the C-peptide measurement pretransplant among type 1 and type 2 diabetes mellitus. *Transplant Proc* 2010; **42**: 2650-2652 [PMID: 20832562 DOI: 10.1016/j.transproceed.2010.04.065]

42   **Wang L**, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. *Diabetes Care* 2012; **35**: 465-470 [PMID: 22355018 DOI: 10.2337/dc11-1236]

43   **Covic AM**, Schelling JR, Constantiner M, Iyengar SK, Sedor JR. Serum C-peptide concentrations poorly phenotype type 2 diabetic end-stage renal disease patients. *Kidney Int* 2000; **58**: 1742-1750 [PMID: 11012908 DOI: 10.1046/j.1523-1755.2000.00335.x]

44   **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238 [PMID: 10911004 DOI: 10.1056/NEJM200007273430401]

45   **Ludwig B**, Ludwig S, Steffen A, Saeger HD, Bornstein SR. Islet versus pancreas transplantation in type 1 diabetes: competitive or complementary? *Curr Diab Rep* 2010; **10**: 506-511 [PMID: 20830612 DOI: 10.1007/s11892-010-0146-y]

46   **Shapiro AM**. State of the art of clinical islet transplantation and novel protocols of immunosuppression. *Curr Diab Rep* 2011; **11**: 345-354 [PMID: 21830042 DOI: 10.1007/s11892-011-0217-8]

47   **Vardanyan M**, Parkin E, Gruessner C, Rodriguez Rilo HL. Pancreas vs. islet transplantation: a call on the future. *Curr Opin Organ Transplant* 2010; **15**: 124-130 [PMID: 20009930 DOI: 10.1097/MOT.0b013e32833553f8]

48   **Senior PA**, Zeman M, Paty BW, Ryan EA, Shapiro AM. Changes in renal function after clinical islet transplantation: four-year observational study. *Am J Transplant* 2007; **7**: 91-98 [PMID: 17227560 DOI: 10.1111/j.1600-6143.2006.01573.x]

49   **Ferrari-Lacraz S**, Berney T, Morel P, Marangon N, Hadaya K, Demuylder-Mischler S, Pongratz G, Pernin N, Villard J. Low risk of anti-human leukocyte antigen antibody sensitization after combined kidney and islet transplantation. *Transplantation* 2008; **86**: 357-359 [PMID: 18645502 DOI: 10.1097/TP.0b013e31817ba628]

50   **Cantarovich D**, Vistoli F, Bignon JD. Anti-human leukocyte antigen antibodies after islet transplantation: what do they really mean? *Transplantation* 2008; **86**: 204-205 [PMID: 18645479 DOI: 10.1097/TP.0b013e31817ba6e5]

51   **Bromberg JS**, LeRoith D. Diabetes cure--is the glass half full? *N Engl J Med* 2006; **355**: 1372-1374 [PMID: 17005956 DOI: 10.1056/NEJMe068183]

52   **Ricordi C**, Alejandro R, Angelico MC, Fernandez LA, Nery J, Webb M, Bottino R, Selvaggi G, Khan FA, Karatzas T, Olson L, Mintz DH, Tzakis AG. Human islet allografts in patients with type 2 diabetes undergoing liver transplantation. *Trans-*

plants in type I and type II diabetic patients with end-stage renal disease: similar 10-year outcomes. *Transplant Proc* 2005; **37**: 1283-1284 [PMID: 15848696 DOI: 10.1016/j.transproceed.2004.12.215]

plantation 1997; **63**: 473-475 [PMID: 9039943 DOI: 10.1097/00 007890-199702150-00025]

53 **Dixon JB**, le Roux CW, Rubino F, Zimmet P. Bariatric surgery for type 2 diabetes. *Lancet* 2012; **379**: 2300-2311 [PMID: 22683132 DOI: 10.1016/S0140-6736(12)60401-2]

54 **American Diabetes Association**. Standards of medical care in diabetes--2009. *Diabetes Care* 2009; **32** Suppl 1: S13-S61 [PMID: 19118286 DOI: 10.2337/dc09-S013]

55 **Dixon JB**, Zimmet P, Alberti KG, Rubino F. Bariatric surgery: an IDF statement for obese Type 2 diabetes. *Diabet Med* 2011; **28**: 628-642 [PMID: 21480973 DOI: 10.1111/j.1464-5491.2011.03306.x]

56 **Neff KJ**, Frankel AH, Tam FW, Sadlier DM, Godson C, le Roux CW. The effect of bariatric surgery on renal function and disease: a focus on outcomes and inflammation. *Nephrol Dial Transplant* 2013; **28** Suppl 4: iv73-iv82 [PMID: 24071659 DOI: 10.1093/ndt/gft262]

57 **Heneghan HM**, Cetin D, Navaneethan SD, Orzech N, Brethauer SA, Schauer PR. Effects of bariatric surgery on diabetic nephropathy after 5 years of follow-up. *Surg Obes Relat Dis* 2013; **9**: 7-14 [PMID: 23211651 DOI: 10.1016/j.soard.2012.08.016]

58 **Koshy AN**, Coombes JS, Wilkinson S, Fassett RG. Laparoscopic gastric banding surgery performed in obese dialysis patients prior to kidney transplantation. *Am J Kidney Dis* 2008; **52**: e15-e17 [PMID: 18617303 DOI: 10.1053/j.ajkd.2008.05.016]

59 **Alexander JW**, Goodman HR, Gersin K, Cardi M, Austin J, Goel S, Safdar S, Huang S, Woodle ES. Gastric bypass in morbidly obese patients with chronic renal failure and kidney transplant. *Transplantation* 2004; **78**: 469-474 [PMID: 15316378 DOI: 10.1097/01.TP.0000128858.84976.27]

60 **Modanlou KA**, Muthyala U, Xiao H, Schnitzler MA, Salvalaggio PR, Brennan DC, Abbott KC, Graff RJ, Lentine KL. Bariatric surgery among kidney transplant candidates and recipients: analysis of the United States renal data system and literature review. *Transplantation* 2009; **87**: 1167-1173 [PMID: 19384163 DOI: 10.1097/TP.0b013e31819e3f14]

61 **Gill JS**, Lan J, Dong J, Rose C, Hendren E, Johnston O, Gill J. The survival benefit of kidney transplantation in obese patients. *Am J Transplant* 2013; **13**: 2083-2090 [PMID: 23890325 DOI: 10.1111/ajt.12331]

REVIEW

Human amniotic membrane transplantation: Different modalities of its use in ophthalmology

Chintan Malhotra, Arun K Jain

Chintan Malhotra, Arun K Jain, Advanced Eye Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Author contributions: Malhotra C and Jain AK solely contributed to this paper in terms of the research for this review and the drafting of the article as well as revising it critically for important intellectual content.

Correspondence to: Arun K Jain, MD, DNB, Advanced Eye Centre, Post Graduate Institute of Medical Education and Research, Sector12, Room No 110, Chandigarh 160012, India. aronkjain@yahoo.com

Telephone: +91-931-6131944 Fax: +91-172-2744401
Received: November 23, 2013 Revised: March 1, 2014
Accepted: May 8, 2014
Published online: June 24, 2014

Abstract

The amniotic membrane (AM) is the inner layer of the fetal membranes and consist of 3 different layers: the epithelium, basement membrane and stroma which further consists of three contiguous but distinct layers: the inner *compact layer*, middle *fibroblast layer* and the outermost *spongy layer*. The AM has been shown to have anti-inflammatory, anti-fibrotic, anti-angiogenic as well as anti-microbial properties. Also because of its transparent structure, lack of immunogenicity and the ability to provide an excellent substrate for growth, migration and adhesion of epithelial corneal and conjunctival cells, it is being used increasingly for ocular surface reconstruction in a variety of ocular pathologies including corneal disorders associated with limbal stem cell deficiency, surgeries for conjunctival reconstruction, as a carrier for *ex vivo* expansion of limbal epithelial cells, glaucoma surgeries and sceral melts and perforations. However indiscriminate use of human AM needs to be discouraged as complications though infrequent can occur. These include risk of transmission of bacterial, viral or fungal infections to the recipient if the donors are not adequately screened for communicable diseases, if the membrane is not processed under sterile conditions or if storage is improper. Optimal outcomes can be achieved only with meticulous case selection. This review explores the ever expanding ophthalmological indications for the use of human AM.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Human amniotic membrane; Limbus; Stem cells; Ocular surface; Cornea

Core tip: Amniotic membrane transplantation is a very useful armamentarium in the hands of the ophthalmic surgeons for treating a variety of ocular surface disorders. Because of its transparent structure, anti- inflammatory, anti-fibrotic and anti-angiogenic properties and ability to provide a substrate for growth of corneal and conjunctival epithelial cells, it forms an ideal material for ocular surface reconstruction.

INTRODUCTION

The ocular surface is an extremely sensitive and dynamic structure, the health of which is crucial for the optimal functioning of the eye. Any mechanical or chemical insult to it either from exogenous sources, *i.e.*, chemical injuries by substances like acids and alkalis, or from endogenous factors, *i.e.*, change in the amount and composition of the tear film due to severe dry eye states associated with conditions like Stevens Johnson syndrome (SJS), rheumatoid arthritis and other collagen vascular diseases ,can result in anatomic, physiologic and optical dysfunction of the eye as a whole.

Layers of human amniotic membrane
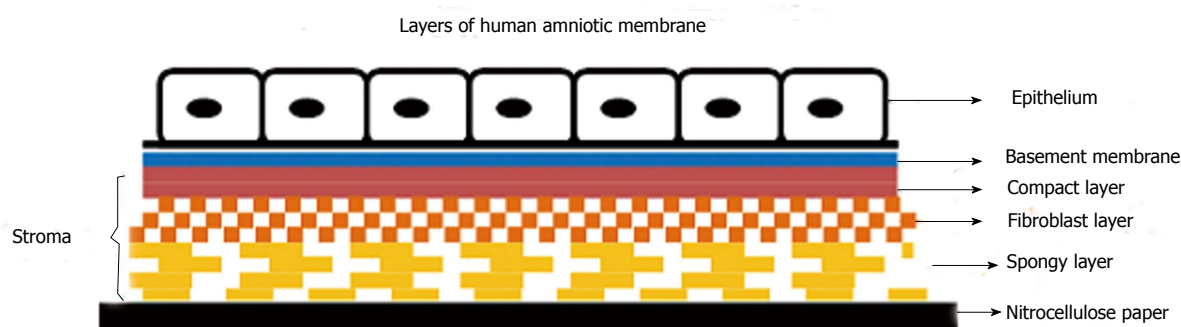
Figure 1 Line diagram showing layers of cryopreserved human amniotic membrane, oriented with the stromal side in contact with the nitrocellulose filter paper and epithelial side facing up.

Various biological tissues have been attempted to be used as donor tissue to repair and reconstruct the ocular surface or to decrease the inflammation in instances where the conjunctiva and cornea get significantly damaged. These include among others oral, labial and vaginal mucous membranes and rabbit peritoneum. Amniotic membrane (AM) was first used therapeutically by Davis for skin transplantation in 1910[1]. De Roth however is the first person credited with having used fetal membranes in ophthalmic surgery in an attempt to reconstruct the ocular surface in patients with symblepharon[2]. The initial enthusiasm for use of this tissue however disappeared from documented ophthalmic literature, till the early nineties, when Batlle *et al*[3] used it to repair conjunctival defects and reconstruct the fornices.

STRUCTURE OF THE FETAL MEMBRANES

The fetal membranes consist of two layers: the outer chorion which is vascular and in contact with the uterine wall, and the amnion which is avascular, lies inner to the chorion and is in contact with amniotic fluid. The AM is 0.02-0.05 mm thick and is classically considered to be composed of three layers (Figure 1).

Epithelium

Which is a monolayer of metabolically active cuboidal cells with microvilli present on its apical surface.

Basement membrane

Made up of type IV, V and VII collagen(also found in conjunctival and corneal basement membranes) in addition to fibronectin and laminin[4], It is one of the thickest membranes in the human body and can withstand current cryopreservation techniques.

Stroma

This is further divided into three contiguous but distinct layers: the inner *compact layer* which is in contact with the basement membrane and contributes to the tensile strength of the membrane, middle *fibroblast layer* which is thick and made up of a loose fibroblast network and the outermost *spongy layer*.

MECHANISM OF ACTION

Several mechanisms of action are attributed to the AM's ability to help in healing and reconstruction of the ocular surface.

Mechanical

The AM acts as a biological bandage and shields the regenerating epithelium from the frictional forces generated by the blinking movements of the eyelids[5]. This is especially of significance in cases where entropion, trichiasis, keratinization of lid margin/palpebral conjunctiva or other such lid pathology exists which can damage the fragile epithelium, *e.g.*, trachoma, SJS, ocular cicatricial pemphigoid (OCP), *etc*. Use of the AM in addition to tilting the balance of the ocular surface towards healing, also dramatically decreases the subjective symptoms of pain and discomfort experienced by these patients, especially when implanted on deepithelized areas of the cornea. This has been attributed to a purely mechanical effect and not because of the biological mediators present in the membrane, as elegantly demonstrated by Lee *et al*[6] in experimental studies on rabbits where application of amniotic fluid to denuded corneas (created by subjecting the animals to excimer laser photo keratectomy) increased the corneal sensitivity and upregulated regeneration of nerves.

Promotion of epithelialization

The basement membrane of the AM closely resembles that of the conjunctiva and cornea especially with regards to its collagen composition. It thus serves as a substrate on which epithelial cells can grow easily. Four main effects on the regenerating corneal epithelium have been described: (1) facilitation of epithelial cell migration[7,8]; (2) reinforcement of basal epithelial cell adhesion[9-11]; (3) promotion of epithelial cell differentiation[12-14]; and (4) Prevention of apoptosis[15,16]. These properties render it suitable for use in cases of nonhealing or persistent epithelial defects of the ocular surface, especially that of the cornea.

Anti-fibrotic and anti-inflammatory properties

Fetal hyaluronic acid is an important constituent of the

stromal matrix of the AM. This helps to suppress TGF β signaling with reduced expression of TGF β-1, β-2, and β-3 isoforms in addition to reduced expression of TGF-Receptor Ⅱ. This inhibits proliferation of corneal, limbal and conjunctival fibroblasts. Differentiation of fibroblasts into myofibroblasts is also inhibited, thus reducing scarring after pterygium surgery and ocular surface reconstruction[17]. Anti-inflammatory effect of AM is driven by inhibition of expression of pro inflammatory cytokines from the damaged ocular surface, *e.g.*, interleukin (IL) 1a, IL-2, IL-8, interferon-γ, tumor necrosis factor-β, basic fibroblast growth factor and platelet derived growth factor[18]. In addition to the chemically mediated anti-inflammatory effect, Shimmura *et al*[19] also demonstrated a more mechanical effect by showing that inflammatory cells get trapped and undergo apoptosis in the matrix of the AM.

Anti-angiogenic properties
In addition to the anti-inflammatory properties which retard new vessel proliferation, a specific anti-angiogenic effect has also been ascribed to the AM. This has been demonstrated to be due to the production of several potent anti angiogenic chemicals including thrombospondin -1, endostatin and all four tissue inhibitors of metalloproteases (TIMP-1, 2, 3 and 4)[20]. Though beneficial in most situations the anti-angiogenic effect of AM needs to be kept in mind and balanced against its other potential benefits when using it in limbal stem cell deficiency associated with limbal ischaemia, *i.e.*, in chemical injuries of the ocular surface.

Anti- microbial properties
A literature review reveals conflicting reports about the anti-microbial properties of AM. Burn patients treated with AM have been shown to have decreased bacterial counts and control of infections[21,22]. Antibacterial effects effects have been demonstrated against both gram positive cocci including streptococci and *Staphylococcus aureus* as well as gram negative bacilli including *Escherichia coli* and *Pseudomonas aeruginosa*[23,24]. These antibacterial effects have been attributed to the presence of several anti-microbial factors in the amniotic fluid including bactricidin, beta-lysin , lysozyme, transferrin and 7S immunoglobulin[25,26]. Other investigators however believe that the AM does not per se contain any chemical antimicrobial substances, but rather just constitutes an effective physical barrier against infection because of its ability to adhere closely to the underlying surface[24,27].

In addition to the above properties another important characteristic of the human AM is a lack of expression of the major histocompatibility antigens HLA-A, B, or DR antigens[28,29]. Hence immunological rejection after its transplantation does not occur and obviates the need for any immune suppression. This feature along with the transparent structure and ability to be preserved for prolonged periods make the AM an ideal substrate for ocular surface transplantation.

PROCURING, PROCESSING AND PRESERVING THE AM

AM is retrieved under strict aseptic conditions from donors undergoing elective cesarean section and who have been previously screened serologically for potentially communicable diseases including human immunodeficiency virus, hepatitis B and C viruses and syphilis. Placenta obtained after vaginal delivery are not used for this purpose because of the potential for contamination with bacteria from the vagina. It is recommended that the maternal donor should undergo repeat serological screening after 6 mo (to cover the window period for transmission of communicable diseases) before the AM is released for use[30]. Tissue is used for transplantation only when both the samples are negative.

Antibiotics covering both gram positive and gram negative bacteria as well as fungi (50 μg/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL of neomycin, 2.5 μg/mL of amphotericin B) are used to wash the placenta under sterile conditions. Blunt dissection is then used to separate the amnion from the chorion. The AM may be preserved by means of cryopreservation (cryopreserved human amniotic membrane, CHAM) or in a dry deepithelialized form (dry human amniotic membrane, DHAM). To prepare CHAM Kim *et al*[31,32] and Lee *et al*[33] recommended using 50% glycerol in Dulbecco's modified Eagle Medium (DMEM) in a ratio of 1:1 to store the membrane. The membrane is cut into multiple pieces and placed on nitrocellulose paper strips with epithelial side up. It is then placed in vials containing the glycerol/DMEM storage medium and cryopreserved at -80 ℃. Just prior to use the membrane should be taken out and warmed to room temperature for 10 min. CHAM stored in glycerol may be safely and effectively used for over a year with the added advantage of antiviral and antibacterial properties of glycerol[34]. One drawback with using CHAM is the need of a -80 ℃ refrigerator. This precludes its use outside big institutions.

DHAM does not require to be attached to nitrocellulose paper and is free standing. DHAM is prepared by subjecting the amniotic membrane to sterilization using low energy electron beam radiation and then preserving it using low heat and air vaccum .DHAM can be stored at room temperature for upto 2-5 years and is rehydrated prior to use. It is usually 35-40 microns thick but a third generation, 110-μm-thick, freeze dried, and freestanding human AM allograft *(Ambio 5; IOP Inc, Costa Mesa, California)* is commercially available. This has an additional thick layer of retained collagen from the chorionic membrane, which makes it thicker and confers greater tectonic function.

Both fresh and preserved AM have been found to be equally effective when transplanted onto the ocular surface[35]. Use of freshly acquired AM however is associated with certain disadvantages including the risk of transmission of communicable diseases as the donor cannot undergo repeat serological testing, and wastage of
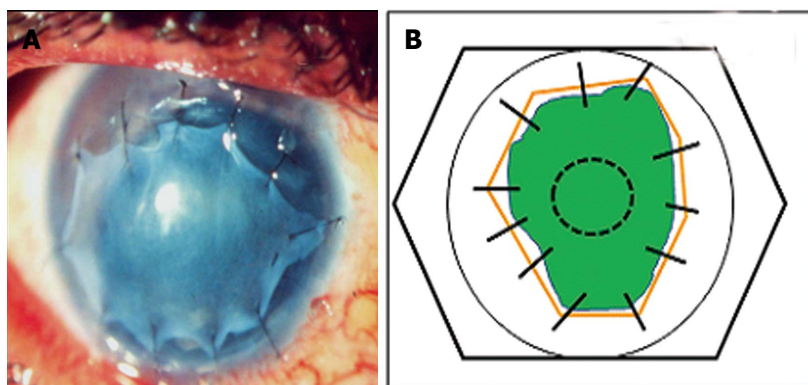
Figure 2 **(A) Amniotic membrane used as an inlay graft (B) line diagram showing amniotic membrane (solid orange lines) used as an Inlay graft for a non-healing epithelial defect (green).** The membrane is trimmed to fit the size of the underlying defect and sutured to the cornea using interrupted 10-0 nylon mono-filament sutures.

unused tissue (with preserved AM, up to 30 grafts can be prepared with one placenta). Preservation of the AM by any means has been shown to adversely affect the viability and proliferative capacity of its epithelial cells[36,37]. Kruse *et al*[36] proposed that AM grafts function primarily as a matrix and not by virtue of transplanted functional cells. Other literature on the subject also supports the view that viability of cellular components of the AM is not essential for its biological effectiveness[38].

SURGICAL PRINCIPLES AND METHODS OF IMPLANTATION

Rationale for determining orientation of AM on ocular surface

This is important as the indication for which the AM is being used and the endpoint desired determines the preferred orientation with which it is used on the ocular surface. Histopathological analysis has revealed that after application of AM the re-epithelialization of the ocular surface by the host epithelium (*i.e.*, by the host corneal or conjunctival epithelium) occurs preferentially on the basement membrane side of the epithelium[39], though Seitz *et al*[40] have also demonstrated that corneal epithelial cells do possess the ability to grow on the stromal side of the membrane. Hence where the membrane is used with the aim of providing conjunctival or corneal cells a substrate to grow on, the AM is used epithelial/basement side up. The stromal matrix of the AM on the other hand has the ability to trap inflammatory cells and induce their apoptosis, thus down regulating the inflammatory response[38]. Thus in the presence of acute inflammation, the membrane may be used to protect the ocular surface from the deleterious effects of the pro inflammatory cells and mediators- here it is used epithelial side down, so that the stromal side faces the palpebral aperture.

Determining the orientation of the AM

The AM supplied on the nitrocellulose filter paper is usually oriented epithelial side up, with the stromal side in direct contact with the paper. The stromal surface can be identified by the presence of vitreous-like strands that can be raised with a sponge or a fine forceps. This may need to be performed at a few points for confirmation.

Depending on the indication for which it is used there are three surgical techniques by which the AM can be used over the ocular surface.

Graft or inlay technique: In this technique the AM is intended to act as a substrate or scaffold for epithelial cells to grow. The AM is placed epithelial/basement membrane side up and is trimmed to fit the size of the underlying epithelial or stromal defect. It is usually sutured to the cornea using non absorbale 10-0 nylon sutures and to the episclera and conjunctiva using 9-0 or 10-0 vicryl (Figure 2). It is preferred to keep the epithelial/basement membrane side up in this technique because the basement membrane of the amnion acts as an excellent substrate for growth of the progenitor epithelial cells by prolonging their lifespan, maintaining clonigenecity and preventing apoptosis[41]. The surrounding 1-2 mm of the host corneal epithelium is debrided. This ensures that the regenerating epithelium grows over the basement membrane of the AM, and consequently the AM stroma gets incorporated into the host tissue ("graft"). Depending on the depth of the underlying defect this technique may be used as a *single layer graft inlay* where a single layer of AM is used, or *multilayer graft inlay* where multiple layers of the AM are placed into the ground of the ulcer, which is filled without sutures before a superficial graft is sutured to the periphery of the ulcer, again after depithelialization of a ring-shaped area around the cornea ulcer. The epithelium is expected to grow over the uppermost layer of this multilayer graft[40]. This is also referred to as the *layered or fill in technique.* The layering may be done either by cutting the AM into multiple pieces and placing them one on top of one another or by using a larger piece of AM which is repeatedly folded (*blanket fold*) upon itself.

Patch or overlay technique: Here the AM is sutured to the ocular surface keeping it larger than the underlying defect so that host epithelium is present below the membrane. The membrane is sutured to the surrounding conjunctiva or episclera using 9-0 vicryl suture. An additional 10-0 nylon suture may be applied to the peripheral cornea in a purse string manner to ensure prolonged retention (Figure 3). The AM may be used epithelial side up or stromal side up as the host epithelium is expected to grow under the membrane which basically acts only as a "biological bandage contact lens" to protect the fragile
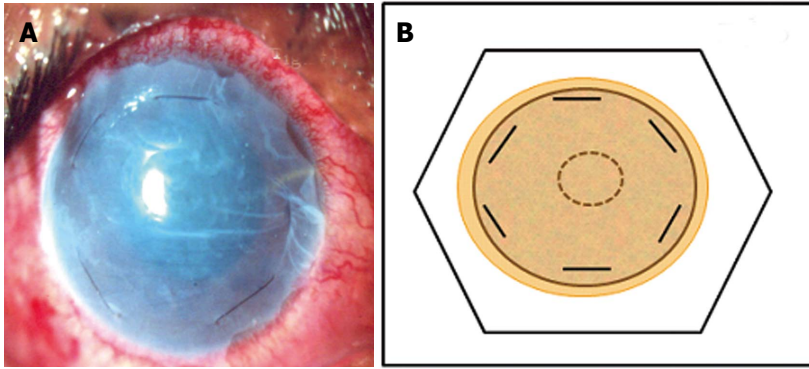
Figure 3 Amniotic membrane used as an overlay patch. A: Clinical photograph; B: Line diagram. The amniotic membrane is trimmed to cover the whole of the corneal surface and fixed by 10-0 nylon monofilamemt sutures at the corneal periphery parallel to the limbus.

new epithelium from the frictional forces generated due to eyelid movements. In this situation the AM is expected to fall off or be removed after a certain time.

Combined (inlay and overlay) technique: Two or more layers of AM are used in this technique, with the inner smaller layer/layers acting as a graft and the outer larger layer acting as a patch. Also known as the "*sandwich technique*" a single-layer(Figure 4A) or multilayer inlay (Figure 4B) is combined with an onlay[40]. The epithelium is expected to grow under the patch but over the uppermost inlay graft.

The availability of fibrin glue for ophthalmic use has in many cases supplanted the use of sutures, and the AM may be adhered to the ocular surface using the recombitant fibrin glue. This reduces the surgical time and also increases patient comfort.

OPHTHALMOLOGICAL INDICATIONS FOR USE OF AM

The list of indications for which AM is used in ophthalmology is expanding with each passing year. Broadly its use can be classified into (1) corneal surface disorders, without limbal stem cell deficiency (LSCD); (2) corneal surface disorders with associated LSCD; (3) conjunctival surface reconstruction, *e.g.*, pterygium removal, after removal of large lesions other than pterygium, after symblepharon lysis; (4) as a carrier for ex vivo expansion of corneal epithelial cells; (5) glaucoma; (6) treatment of sclera melts and perforations; and (7) other miscellaneous indications.

Persistent epithelial defects and Non Healing corneal ulcers

Persistent epithelial defects (PED's) may occur due to a variety of mechanisms including innervations deficits of the cornea (*e.g.*, neurotrophic keratopathy following Herpes Zoster keratitis, after penetrating keratoplasty), chronic inflammation or mechanical factors. These factors may act individually or in synergy, and lead to epithelial defects which are unresponsive to conventional management strategies, *e.g.*, lubrication, bandage contact lenses, tarsorrahaphy, *etc*. Untreated these PED's can progress to stromal collagenolysis, ulceration, perforation or scarring.

In these situations AM may be used as a single layer or multilayer graft (inlay) depending on the depth of the lesion providing a substrate for the epithelial cells to migrate and adhere to the basement membrane. The inlay may also be combined with an epithelial side down onlay patch graft especially if significant ocular surface inflammation co-exists as stromal surface of the onlay graft will help mop up the inflammatory cells and mediators on the palpebral surface. Success rates of using AM for PED's have been reported as varying from 64% to 91%[33,42]. Early detachment of the membrane however remains a major problem despite the use of multiple sutures or a protective bandage contact lens (BCL)[42].

AM has also been used successfully in nonhealing infective ulcers due to bacteria, fungi, viruses and protozoa. The nonhealing of the ulcer inspite of adequate antimicrobial therapy in these situations may be because of release of proinflammatory mediators, proteolytic enzymes and collagenazes by the microorganisms, stromal keratocytes and polymorphonuclear cells[43,44]. Single or multilayer AM has an inhibitory effect on these proteolytic enzymes and also provides a healthy basement membrane, thus tilting the ocular surface milieu in favour of rapid healing.

Corneal perforations and descemetoceles

Corneal perforations and descemetoceles are globe and sight threatening complications associated with loss of tectonic strength of corneal stroma as well as associated underlying inflammation. A majority of the methods used to treat these conditions including tissue adhesives, lamellar keratoplasty, penetrating keratoplasty, bandage contact lenses and conjunctival flaps provide tectonic support, but do not directly address the inflammatory component. Multilayer AM has been used to treat non traumatic microperforations and descemetoceles with upto 72.7% to 82.3% success rate being reported[45,46]. AM in this situation provides tectonic support, collagen substitution for corneal stroma and anti- inflammatory and antifibrotic actions which halt progressive tissue degradation. Depending on the underlying severity and extent of the disease process it may be used as a permanent surgical therapy or as a temporizing measure till a more definitive surgical procedure can be performed. One of the authors of this review (AKJ) has reported successful management of a case of perforated peripheral corneal ulcer in
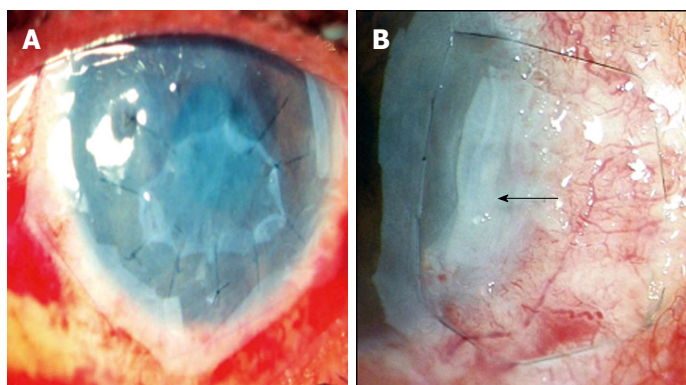
Figure 4 Single layer inlay covered by a larger "patch" or "onlay" (A), multi-layer inlay (amniotic membrane folded upon itself in form of a blanket fold- black arrow) covered by a larger "patch" which is fixed to underlying episclera by a purse string suture in a case of deep, non-resolving peripheral ulcerative keratitis (B).

a patient of acne rosacea with amniotic membrane after three applications of cyanoacrylate glue with bandage contact lens failed to seal the perforation successfully[47]. A final best corrected visual acuity of 6/6 was achieved in this patient. Though used most commonly for small perforations, Kim *et al*[48] have reported successful outcomes even in patients with perforations > 2 mm in size using fibrin glue to augment the thickness of the AM-a procedure they termed "fibrin glue assisted augmented AMT".

Symptomatic bullous keratopathy

In symptomatic patients with good visual potential and intolerant to a BCL, AM may be used as a temporizing measure till a definitive treatment for the bullous keratopathy, *i.e.*, endothelial or penetrating keratoplasty is undertaken. It may also be used as an alternative to anterior stromal puncture in patients with a poor visual potential with the objective of providing longer pain free periods. Espana *et al*[49] have reported a mean follow up of 25 mo and noted that 88 % patients were able to obtain a pain free status.

Band keratopathy

Band keratopathy due to abnormal deposition of calcium on the corneal surface results in ocular irritation and epithelial surface breakdown. Primary treatment involves removal of calcium deposits by ethylene diamine tetra acetic acid chelation or superficial keratectomy. AM transplantation has been used as an adjunct after primary surgical treatment in band keratopathy with pain relief being reported in 93% cases and visual acuity improvement in 44% of sighted eyes[50].

Corneal disorders with associated LSCD: Any acute or chronic insult to the the limbal epithelial stem cells can lead to a state of partial or total LSCD which may manifest as conjunctivalization of the cornea, neovascularization, PED's and chronic inflammation. This may be seen after thermal or chemical burns, cicatrizing disorders like SJS and OCP, aniridia, chronic contact lens wear, untreated vernal keratoconjunctivitis (VKC) and multiple surgeries involving the limbal area. Successful long term outcome in these eyes after lamellar or penetrating keratoplasty requires prior optimization of the ocular surface

and restoration of the stem cell population. Success of AM in these scenarios depends on the severity of the LSCD. In cases of partial LSCD amniotic membrane alone can be an effective therapy to restore the ocular surface as by promoting epithelialization and reducing inflammation it restores a normal stroma which maximizes functioning of the remaining limbal stem cells[51,52]. In cases of total LSCD however AM transplantation has only an adjunct role to limbal stem cell transplantation which is the definitive modality of treatment, as AM can only optimize functioning of existent limbal stem cells (LSC's) as is seen in partial LSCD, but it cannot cause repopulation of the affected eye with LSC's in cases of advanced total LSCD. In these situations use of de-epithelized AM as a carrier for ex vivo expansion of limbal autologus or allolimbal stem cells is another good option as it combines the advantages of both techniques, *i.e.,* simultaneous optimization of the ocular surface by the AM and replacement of the stem cells.

Conjunctival reconstruction: AM can be used for reconstruction of the conjunctival surface as a substitute for conjunctival grafts in situations where availability of autologous conjunctival tissue is limited, *i.e.,* after removal of large conjunctival lesions, patients having undergone repeated conjunctival surgery leading to a scarred conjunctiva, or where the conjunctiva needs to be preserved, *i.e.*, patients with glaucoma who may require filtering surgery in the future. Use of AM in these situations is helpful as in addition to providing a healthy basement membrane for growth of conjunctival epithelial cells it also helps in maintaining the normal goblet cell containing phenotype of these cells[53]. However as the AM is only a temporary substitute, to provide long term reepithelialization of the conjunctival surface, the surrounding conjunctival tissue must be healthy with an intact vascular bed.

Pterygium: Use of AM as an alternative to autologous conjunctival grafts was described by Prabhasawat *et al*[54]. They reported higher recurrence rates (10.9%) for primary pterygia with the use of AM as compared to recurrence with use of autologous conjunctival autografts (2.6%). However later studies which emphasized extensive removal of fibrovascular tissue adjacent to the pterygium have reported that recurrence rates with use of AM
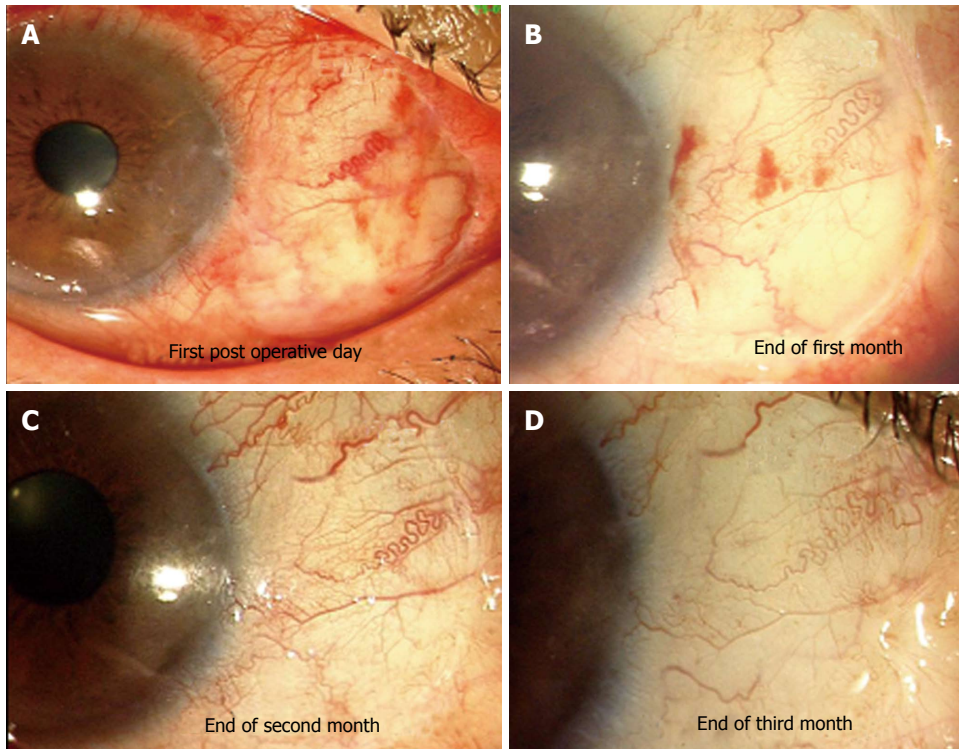
Figure 5 Amniotic membrane used to cover bare sclera after excision of primary pterygium. A-D: Serial photographs showing appearance of amniotic membrane graft. At end of 3 mo excellent integration and cosmetic appearance was achieved.

transplantation (3%-3.8% in primary pterygia and 9.5% in recurrent pterygia) were similar to those reported after conjunctival autografting and intra operative mitimycin C use[55,56]. Jain *et al*[57] have described the use of AM transplantation using fibrin glue in primary pterygia using a 'tuck in technique' where the edges of the AM graft were tucked underneath the adjacent free margin of conjunctiva on 3 sides and reported no recurrences in 11 out of 12 patients after a follow up of one year (Figure 5).

However conjunctival autograft is still considered to be the gold standard for treatment of primary pterygia and AM may be a reserved as a reasonable option in cases with diffuse conjunctival involvement, *i.e.*, primary extensive biheaded pterygia, in previous multiple failed surgeries and in patients in whom the bulbar conjunctiva must be preserved for a prospective glaucoma filtering procedure[58].

Conjunctival tumours and ocular surface squamous neoplasias: AM has been used for conjunctival reconstruction after excision of both benign and malignant tumours including ocular surface squamous neoplasias(OSSN), melanomas, lymphomas and complex choristomas. The AM provides a substrate for migration of the conjunctival epithelial cells. Advantages of using amniotic membrane as compared to conjunctival autografts in these situations include a lack of donor site morbidity and the ability to clinically monitor local tumour recurrence beneath the transparent AM graft[59].

After symblepharon lysis: AM can be used both in the prevention as well as treatment of symblepharon. In the acute phases of chemical injury the AM can be used as a patch to cover the entire ocular surface and sutured to the fornices through the eyelids to prevent symblepharon formation as well as simultaneously reduce ocular surface inflammation. The AM should be a continuous sheet devoid of buttonholes. A large sheet is placed on the ocular surface and it is first anchored to the inner surface of the everted lower lid close to the lid margin using multiple interrupted 10-0 vicryl sutures. To anchor the sheet to the fornices two sets of double armed 4-0 chromic gut sutures on a cutting needle are used and the needles are passed from the AM surface through the inferior fornix, *via* the full-thickness of the eyelid and are made to exit through the eyelid skin. The two needles of each of the two sets of sutures are passed through two segments of an encircling band and then tied[60]. A sutureless amniotic patch (ProKeras; Bio-Tissue Inc., Miami,Florida) is also available for this purpose. Another modification suggested by Rahman *et al*[61] is the use of a conformer on which the AM is sutured and placed on the ocular surface, with the AM acting as a patch and the conformer maintaining the fornices because of its rigidity. Though the AM has also been used for symblephera associated with SJS and OCP the outcomes are usually not as successful as compared to stable non progressive cicatrization because of the chronic ongoing inflammation associated with these diseases[62].

As a carrier for *ex vivo* expansion of epithelial cells: Progenitor stem cells for the conjunctiva and cornea have been established to reside in the conjunctival fornices

and limbal area respectively. In the eye, *i.e.*, under in-vivo conditions these migrate onto the ocular surface and differentiate into daughter cells to continuously regenerate the conjunctival and corneal surface epithelia[63]. Expansion of these cell populations on basement membrane side of AM (with amniotic epithelium intact or de-epithelized AM) as well as on the stromal side of AM have been demonstrated previously. The corneal epithelial cells have been shown to migrate rapidly when limbal explants are placed on AM denuded of amniotic epithelial cells but with the basement membrane intact, relatively slowly when the amniotic epithelium is left behind and slowest when the membrane has been flipped over and the cells are grown on the stromal surface. Culturing the explants on an intact AM with devitalized epithelium favors expansion of an epithelial phenotype that closely resembles limbal stem cells[38].

Clinically in cases of LSCD, limbal biopsies can be used to harvest corneal epithelial stem cells for *ex vivo* expansion on AM, which can then be transplanted onto the eye after appropriate preparation of the host bed by resecting the vascularized pannus or any other procedure which may be required. The main advantage of this approach of expanding corneal epithelial cells *ex vivo* on AM is that only a small amount of limbal tissue is required to be harvested from the contralateral eye as compared to conventional limbal allografts which require up to 12-clock hours of limbal tissue and have the potential risk for limbal deficiency developing in the donor eye. Another advantage is that the AM is a natural substrate and when transplanted onto the corneal surface gets integrated into it. Excellent outcomes have been reported after transplantation of cultivated limbal stem cell on denuded AM for LSCD[64-66].

Glaucoma: AM has been used in glaucoma to reduce scarring at the time of filtering surgery, to repair early or late leaks, and act as a cover for valve procedures. Fujishima *et al*[67] attempted to reduce scarring in filtering surgery by incorporating a layer of amnion between the scleral flap and bed to prevent an adhesion between the two layers, but achieved only limited success. Use of AM to repair bleb leaks is controversial with some authors reporting good results[68] while others have reported it as being ineffective[69].

Treatment of corneo scleral melts and perforations: Small sclera perforations or melts can be treated by multilayerd AM alone while for larger scleral defects AM has been used over the sclera patch, basement side up so as to facilitate epithelialization of the scleral patch graft as well as to reduce the inflammation[45,70]. It has been used with success for both infectious scleral ulcerations after appropriate antimicrobial therapy[71]. as well as after non-infectious scleral melts. Tay *et al*[72] have reported using a double layer of freeze dried AM (*Ambio 5*; *IOP Inc, Costa Mesa, California*) in a crescentric manner to manage a case of carrier graft melt in a patient with Boston Keratoprosthesis Type 1.

Miscellaneous indications: Severe shield ulcers due to VKC which do not heal with conservative management respond well to surgical debridement of the mucous plaque and debri followed by using the AM as a patch to promote epithelialization. Using this technique Sridhar *et al*[73] achieved a success rate of 94.7% with shield ulcers. AM has been occasionally in oculoplasty for lid reconstruction, for treatment of punctual occlusion by applying it as a patch over the denuded punctual orifice[74] as a cover for ocular prosthesis at the time of insertion and to cover the tarsal plate in lid split procedure for correction of cicatricial entropion[75].

Complications and limitations of am use: Though the AM is finding ever expanding uses in ophthalmology, it must not be used indiscriminately as complications though infrequent can occur. Risk of transmission of bacterial, viral or fungal infections to the recipient exists if the donors are not adequately screened for communicable diseases, if the membrane is not processed under sterile conditions or if storage is improper. Incidence rates of 1.6%-8.0% have been reported post AM transplantation with gram positive isolates being reported most frequently[76-78]. Premature degradation of the membrane and cheese wiring may need frequent repeat transplantations. Occasionally, a residual subepithelial membrane may persist in some cases and inadvertently opacify the visual axis[38].

CONCLUSION

The AM is proving to be a very versatile tool in the hands of the ophthalmologist, and the indications for its use are rapidly expanding as there is a better understanding of its properties. However a judicious use and appropriate patient selection is important for achieving optimal outcomes.

REFERENCES

1   **Davis JW**. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Johns Hopkins Med J* 1910; **15**: 307
2   **deRoth A**. Plastic repair of conjunctival defects with fetal membrane. *Arch Ophthalmol* 1940; **23**: 522-525 [DOI: 10.1001/archopht.1940.00860130586006]
3   **Batlle JF**, Perdomo FJ. Placental membranes as a conjunctival substitute. *Ophthalmol* 1993: **100**: A107
4   **Fukuda K**, Chikama T, Nakamura M, Nishida T. Differential distribution of sub-chains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea and conunctiva. *Cornea* 1999; **18**: 73-79 [DOI: 10.1097/00003226-199901000-00013]
5   **Baum J**. Thygeson lecture. Amniotic membrane transplantation: why is it effective? *Cornea* 2002; **21**: 339-341 [PMID: 11973378 DOI: 10.1097/00003226-200205000-00001]
6   **Lee HS**, Kim JC. Effect of amniotic fluid in corneal sensitivity and nerve regeneration after eximer laser ablation. *Cornea* 1996;**15**: 517-524
7   **Meller D**, Pires RT, Tseng SC. Ex vivo preservation and expansion of human limbal epithelial stem cells on amniotic membrane cultures. *Br J Ophthalmol* 2002; **86**: 463-471 [PMID:

11914219]

8   **Meller D**, Tseng SC. Conjunctival epithelial cell differentia-
     tion on amniotic membrane. *Invest Ophthalmol Vis Sci* 1999;
     40: 878-886 [PMID: 10102284]

9   **Keene DR**, Sakai LY, Lunstrum GP. Type VII collagen forms
     an extended network of anchoring fibrils. *J Cell Biol* 1987;
     104: 611–621 [DOI: 10.1083/jcb.104.3.611]

10   **Sonnenberg A**, Calafat J, Janssen H, Daams H, van der
     Raaij-Helmer LM, Falconi R, Kennel SJ, Aplin JD, Baker J,
     Loizidou M. Integrin alpha 6/beta 4 complex is located in
     hemidesmosomes, suggesting a major role in epidermal cell-
     basement membrane adhesion. *J Cell Biol* 1991; **113**: 907-917
     [PMID: 2026654 DOI: 10.1083/jcb.113.4.907]

11   **Terranova VP**, Lyall RM. Chemotaxis of human gingival ep-
     ithelial cells to laminin. A mechanism for epithelial cell api-
     cal migration. *J Periodonto* 1986; **57**: 311–317 [DOI: 10.1902/
     jop.1986.57.5.311]

12   **Guo M**, Grinnell F. Basement membrane and human epider-
     mal differentiation in vitro. *J Invest Dermatol* 1989; **93**: 372-378
     [PMID: 2475548 DOI: 10.1111/1523-1747.ep12280275]

13   **Kurpakus MA**, Stock EL, Jones JC. The role of the basement
     membrane in differential expression of keratin proteins in
     epithelial cells. *Dev Biol* 1992; **150**: 243-255 [PMID: 1372569
     DOI: 10.1016/0012-1606(92)90239-D]

14   **Streuli CH**, Bailey N, Bissell MJ. Control of mammary epi-
     thelial differentiation: basement membrane induces tissue-
     specific gene expression in the absence of cell-cell interaction
     and morphological polarity. *J Cell Biol* 1991; **115**: 1383-1395
     [PMID: 1955479 DOI: 10.1083/jcb.115.5.1383]

15   **Boudreau N**, Sympson CJ, Werb Z, Bissell MJ. Suppression
     of ICE and apoptosis in mammary epithelial cells by extra-
     cellular matrix. *Science* 1995; **267**: 891-893 [PMID: 7531366
     DOI: 10.1126/science.7531366]

16   **Boudreau N**, Werb Z, Bissell MJ. Suppression of apoptosis
     by basement membrane requires three-dimensional tis-
     sue organization and withdrawal from the cell cycle. *Proc
     Natl Acad Sci USA* 1996; **93**: 3509-3513 [PMID: 8622967 DOI:
     10.1073/pnas.93.8.3509]

17   **Lee SB**, Li DQ, Tan DT, Meller DC, Tseng SC. Suppression
     of TGF-beta signaling in both normal conjunctival fibroblasts
     and pterygial body fibroblasts by amniotic membrane. *Curr
     Eye Res* 2000; **20**: 325-334 [PMID: 10806447]

18   **Solomon A**, Rosenblatt M, Monroy D, Ji Z, Pflugfelder SC,
     Tseng SC. Suppression of Interleukin 1 alpha and Interleukin
     1 beta in the human limbal epithelial cells cultured on the
     amniotic membrane stromal matrix. *Br J Ophthalmol* 2001; **85**:
     444-449 [DOI: 10.1136/bjo.85.4.444]

19   **Shimmura S**, Shimazaki J, Ohashi Y, Tsubota K. Antiinflam-
     matory effects of amniotic membrane transplantation in
     ocular surface disorders. *Cornea* 2001; **20**: 408-413 [PMID:
     11333331]

20   **Hao Y**, Ma DH, Hwang DG, Kim WS, Zhang F. Identifica-
     tion of antiangiogenic and antiinflammatory proteins in hu-
     man amniotic membrane. *Cornea* 2000; **19**: 348-352 [PMID:
     10832697 DOI: 10.1097/00003226-200005000-00018]

21   **Rao TV**, Chandrasekharam V. Use of dry human and
     bovine amnion as a biological dressing. *Arch Surg*
     1981; **116**: 891-896 [PMID: 7259490 DOI: 10.1001/arch-
     surg.1981.01380190029007]

22   **Robson MC**, Krizek TJ. The effect of human amniotic mem-
     branes on the bacteria population of infected rat burns. *Ann
     Surg* 1973; **177**: 144-149 [PMID: 4633388 DOI: 10.1097/000006
     58-197302000-00003]

23   **Kjaergaard N**, Hein M, Hyttel L, Helmig RB, Schønheyder
     HC, Uldbjerg N, Madsen H. Antibacterial properties of
     human amnion and chorion in vitro. *Eur J Obstet Gynecol
     Reprod Biol* 2001; **94**: 224-229 [PMID: 11165729 DOI: 10.1016/
     S0301-2115(00)00345-6]

24   **Kjaergaard N**, Helmig RB, Schønheyder HC, Uldbjerg N,
     Hansen ES, Madsen H. Chorioamniotic membranes consti-
     tute a competent barrier to group b streptococcus in vitro.
     Eur J Obstet Gynecol Reprod Biol 1999; **83**: 165-169 [PMID:
     10391527 DOI: 10.1016/S0301-2115(99)00009-3]

25   **Gudson J**. A Bactericidin for bacillus subtilis in pregnancy. *J
     Immunol* 1962; **88**: 494–499

26   **Galask RP**, Snyder IS. Antimicrobial factors in amniotic
     fluid. *Am J Obstet Gynecol* 1970; **106**: 59-65 [PMID: 4983127]

27   **Talmi YP**, Sigler L, Inge E, Finkelstein Y, Zohar Y. Antibacte-
     rial properties of human amniotic membranes. *Placenta* 1991;
     12: 285-288 [PMID: 1754577]

28   **Adinolfi M**, Akle CA, McColl I, Fensom AH, Tansley L, Con-
     nolly P, Hsi BL, Faulk WP, Travers P, Bodmer WF. Expres-
     sion of HLA antigens, beta 2-microglobulin and enzymes by
     human amniotic epithelial cells. *Nature* 1982; **295**: 325-327
     [PMID: 6173762 DOI: 10.1038/295325a0]

29   **Akle CA**, Adinolfi M, Welsh KI, Leibowitz S, McColl I. Im-
     munogenicity of human amniotic epithelial cells after trans-
     plantation into volunteers. *Lancet* 1981; **2**: 1003-1005 [PMID:
     6118474 DOI: 10.1016/S0140-6736(81)91212-5]

30   **Dua HS**, Azuara-Blanco A. Amniotic membrane transplan-
     tation. *Br J Ophthalmol* 1999; **83**: 748-752 [PMID: 10340988]

31   **Kim JC**, Tseng SC. Transplantation of preserved human am-
     niotic membrane for surface reconstruction in severely dam-
     aged rabbit corneas. *Cornea* 1995; **14**: 473-484 [PMID: 8536460
     DOI: 10.1097/00003226-199509000-00006]

32   **Kim JC**, Tseng SC. The effects on inhibition of corneal neo-
     vascularization after human amniotic membrane transplan-
     tation in severely damaged rabbit corneas. *Korean J Ophthal-
     mol* 1995; **9**: 32-46 [PMID: 7674551]

33   **Lee SH**, Tseng SC. Amniotic membrane transplantation for
     persistent epithelial defects with ulceration. *Am J Ophthalmol*
     1997; **123**: 303-312 [PMID: 9063239]

34   **Maral T**, Borman H, Arslan H, Demirhan B, Akinbingol
     G, Haberal M. Effectiveness of human amnion preserved
     long-term in glycerol as a temporary biological dressing.
     Burns 1999; **25**: 625-635 [PMID: 10563689 DOI: 10.1016/
     S0305-4179(99)00072-8]

35   **Adds PJ**, Hunt CJ, Dart JK. Amniotic membrane grafts,
     "fresh" or frozen? A clinical and in vitro comparison. *Br J
     Ophthalmol* 2001; **85**: 905-907 [PMID: 11466241 DOI: 10.1136/
     bjo.85.8.905]

36   **Kruse FE**, Joussen AM, Rohrschneider K, You L, Sinn B,
     Baumann J, Völcker HE. Cryopreserved human amniotic
     membrane for ocular surface reconstruction. *Graefes Arch
     Clin Exp Ophthalmol* 2000; **238**: 68-75 [PMID: 10664056 DOI:
     10.1007/s004170050012]

37   **Kubo M**, Sonoda Y, Muramatsu R, Usui M. Immunogenicity
     of human amniotic membrane in experimental xenotrans-
     plantation. *Invest Ophthalmol Vis Sci* 2001; **42**: 1539-1546
     [PMID: 11381058]

38   **Dua HS**, Gomes JA, King AJ, Maharajan VS. The amniotic
     membrane in ophthalmology. *Surv Ophthalmol* 2004; **49**:
     51-77 [PMID: 14711440]

39   **Hu DJ**, Basti S, Wen A, Bryar PJ. Prospective comparison of
     corneal re-epithelialization over the stromal and basement
     membrane surfaces of preserved human amniotic mem-
     brane. *ARVO* 2003; 3151

40   **Seitz B**, Resch MD, Schlötzer-Schrehardt U, Hofmann-Rum-
     melt C, Sauer R, Kruse FE. Histopathology and ultrastruc-
     ture of human corneas after amniotic membrane transplanta-
     tion. *Arch Ophthalmol* 2006; **124**: 1487-1490 [PMID: 17030720
     DOI: 10.1001/archopht.124.10.1487]

41   **Grueterich M**, Tseng SC. Human limbal progenitor cells
     expanded on intact amniotic membrane ex vivo. *Arch Oph-
     thalmol* 2002; **120**: 783-790 [PMID: 12049584 DOI: 10.1001/ar-
     chopht.120.6.783]

42   **Letko E**, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR,
     Samson CM, Nguyen QD, Harper SL, Primack JD, Azar DT,
     Gruterich M, Dohlman CH, Baltatzis S, Foster CS. Amniotic
     membrane inlay and overlay grafting for corneal epithe-

lial defects and stromal ulcers. *Arch Ophthalmol* 2001; **119**: 659-663 [PMID: 11346392 DOI: 10.1001/archopht.119.5.659]

43   **Brown SI**, Bloomfield SE, Tam W. The cornea-destroying enzyme of Pseudomonas aeruginosa. *Invest Ophthalmol* 1974; **13**: 174-180 [PMID: 4273282]

44   **Buxton JN**, Fox ML. Conjunctival flaps in the treatment of refractory pseudomonas corneal abscess. *Ann Ophthalmol* 1986; **18**: 315-318 [PMID: 3504115]

45   **Hanada K**, Shimazaki J, Shimmura S, Tsubota K. Multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. *Am J Ophthalmol* 2001; **131**: 324-331 [PMID: 11239864 DOI: 10.1016/S0002-9394(00)00825-4]

46   **Solomon A**, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC. Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers. *Ophthalmology* 2002; **109**: 694-703 [PMID: 11927426 DOI: 10.1016/S0161-6420(01)01032-6]

47   **Jain AK**, Sukhija J. Amniotic membrane transplantation in ocular rosacea. *Ann Ophthalmol* (Skokie) 2007; **39**: 71-73 [PMID: 17914210 DOI: 10.1007/BF02697331]

48   **Kim HK**, Park HS. Fibrin glue-assisted augmented amniotic membrane transplantation for the treatment of large noninfectious corneal perforations. *Cornea* 2009; **28**: 170-176 [PMID: 19158560 DOI: 10.1097/ICO.0b013e3181861c54]

49   **Espana EM**, Grueterich M, Sandoval H, Solomon A, Alfonso E, Karp CL, Fantes F, Tseng SC. Amniotic membrane transplantation for bullous keratopathy in eyes with poor visual potential. *J Cataract Refract Surg* 2003; **29**: 279-284 [PMID: 12648638 DOI: 10.1016/S0886-3350(02)01525-0]

50   **Anderson DF**, Prabhasawat P, Alfonso E, Tseng SC. Amniotic membrane transplantation after the primary surgical management of band keratopathy. *Cornea* 2001; **20**: 354-361 [PMID: 11333320 DOI: 10.1097/00003226-200105000-00004]

51   **Tseng SC**, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol* 1998; **116**: 431-441 [PMID: 9565039 DOI: 10.1001/archopht.116.4.431]

52   **Anderson DF**, Ellies P, Pires RT, Tseng SC. Amniotic membrane transplantation for partial limbal stem cell deficiency. *Br J Ophthalmol* 2001; **85**: 567-575 [PMID: 11316719 DOI: 10.1136/bjo.85.5.567]

53   **Prabhasawat P**, Tseng SC. Impression cytology study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. *Arch Ophthalmol* 1997; **115**: 1360-1367 [PMID: 9366664 DOI: 10.1001/archopht.1997.011100160530001]

54   **Prabhasawat P**, Barton K, Burkett G, Tseng SC. Comparison of conjunctival autografts, amniotic membrane grafts, and primary closure for pterygium excision. *Ophthalmology* 1997; **104**: 974-985 [PMID: 9186439 DOI: 10.1016/S0161-6420(97)30197-3]

55   **Solomon A**, Pires RTF, Tseng SCG: Amniotic membrane transplantation after extensive removal of primary and recurrent pterygia. *Ophthalmol* 2001; **108**: 449-460 [DOI: 10.1016/S0161-6420(00)00567-4]

56   **Ma DH**, See LC, Liau SB, Tsai RJ. Amniotic membrane graft for primary pterygium: comparison with conjunctival autograft and topical mitomycin C treatment. *Br J Ophthalmol* 2000; **84**: 973-978 [PMID: 10966947]

57   **Jain AK**, Bansal R, Sukhija J. Human amniotic membrane transplantation with fibrin glue in management of primary pterygia: a new tuck-in technique. *Cornea* 2008; **27**: 94-99 [PMID: 18245974 DOI: 10.1097/ICO.0b013e318158b47f]

58   **Sangwan VS**, Murthy SI, Bansal AK, Rao GN. Surgical treatment of chronically recurring pterygium. *Cornea* 2003; **22**: 63-65 [PMID: 12502952 DOI: 10.1097/00003226-200301000-00015]

59   **Paridaens D**, Beekhuis H, van Den Bosch W, Remeyer L, Melles G. Amniotic membrane transplantation in the management of conjunctival malignant melanoma and primary acquired melanosis with atypia. *Br J Ophthalmol* 2001; **85**: 658-661 [PMID: 11371482 DOI: 10.1136/bjo.85.6.658]

60   **John T**. Human amniotic membrane transplantation: past, present, and future. *Ophthalmol Clin North Am* 2003; **16**: 43-65; vi [PMID: 12683248 DOI: 10.1016/S0896-1549(02)00110-4]

61   **Rahman I**, Said DG, Maharajan VS, Dua HS. Amniotic membrane in ophthalmology: indications and limitations. *Eye* (Lond) 2009; **23**: 1954-1961 [PMID: 19169225 DOI: 10.1038/eye.2008.410]

62   **Maharajan VS**, Shanmuganathan V, Currie A, Hopkinson A, Powell-Richards A, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction: indications and outcomes. *Clin Experiment Ophthalmol* 2007; **35**: 140-147 [PMID: 17362455 DOI: 10.1111/j.1442-9071.2006.01408.x]

63   **Lavker RM**, Tseng SC, Sun TT. Corneal epithelial stem cells at the limbus: looking at some old problems from a new angle. *Exp Eye Res* 2004; **78**: 433-446 [PMID: 15106923 DOI: 10.1016/j.exer.2003.09.008]

64   **Schwab IR**, Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea* 2000; **19**: 421-426 [PMID: 10928750 DOI: 10.1097/00003226-200007000-00003]

65   **Tsai RJ**, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000; **343**: 86-93 [PMID: 10891515 DOI: 10.1056/NEJM200007133430202]

66   **Koizumi N**, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. *Ophthalmology* 2001; **108**: 1569-1574 [PMID: 11535452]

67   **Fujishima H**, Shimazaki J, Shinozaki N, Tsubota K. Trabeculectomy with the use of amniotic membrane for uncontrollable glaucoma. *Ophthalmic Surg Lasers* 1998; **29**: 428-431 [PMID: 9599369]

68   **Nagai-Kusuhara A**, Nakamura M, Fujioka M, Negi A. Long-term results of amniotic membrane transplantation-assisted bleb revision for leaking blebs. *Graefes Arch Clin Exp Ophthalmol* 2008; **246**: 567-571 [PMID: 18071730 DOI: 10.1007/s00417-007-0727-x]

69   **Budenz DL**, Barton K, Tseng SC. Amniotic membrane transplantation for repair of leaking glaucoma filtering blebs. *Am J Ophthalmol* 2000; **130**: 580-588 [PMID: 11078836 DOI: 10.1016/S0002-9394(00)00600-0]

70   **Oh JH**, Kim JC. Repair of scleromalacia using preserved scleral graft with amniotic membrane transplantation. *Cornea* 2003; **22**: 288-293 [PMID: 12792468 DOI: 10.1097/00003226-200305000-00002]

71   **Ma DH**, Wang SF, Su WY, Tsai RJ. Amniotic membrane graft for the management of scleral melting and corneal perforation in recalcitrant infectious scleral and corneoscleral ulcers. *Cornea* 2002; **21**: 275-283 [PMID: 11917176]

72   **Tay E**, Utine CA, Akpek EK. Crescenteric amniotic membrane grafting in keratoprosthesis-associated corneal melt. *Arch Ophthalmol* 2010; **128**: 779-782 [PMID: 20547957 DOI: 10.1001/archophthalmol.2010.95]

73   **Sridhar MS**, Sangwan VS, Bansal AK, Rao GN. Amniotic membrane transplantation in the management of shield ulcers of vernal keratoconjunctivitis. *Ophthalmology* 2001; **108**: 1218-1222 [PMID: 11425678 DOI: 10.1016/S0161-6420(01)00622-4]

74   **Murube J**, Olivares C, Murube E: Treatment of dry eye by punctum patch. *Orbit* 1995; **14**: 1–7 [DOI: 10.3109/01676839509149156]

75   **Ti SE**, Tow SL, Chee SP. Amniotic membrane transplantation in entropion surgery. *Ophthalmology* 2001; **108**: 1209-1217 [PMID: 11425677 DOI: 10.1016/S0161-6420(01)00599-1]

76   **Marangon FB**, Alfonso EC, Miller D, Remonda NM, Muallem MS, Tseng SC. Incidence of microbial infection after amniotic membrane transplantation. *Cornea* 2004; **23**: 264-269 [PMID: 15084859 DOI: 10.1097/00003226-200404000-00008]

77   **Khokhar S**, Sharma N, Kumar H, Soni A. Infection after

use of nonpreserved human amniotic membrane for the reconstruction of the ocular surface. *Cornea* 2001; **20**: 773-774 [PMID: 11588437 DOI: 10.1097/00003226-200110000-00023]

78   **Messmer EM**. Hypopyon after amniotic membrane transplantation. *Ophthalmology* 2001; **108**: 1714-1715 [PMID: 11581029 DOI: 10.1016/S0161-6420(00)00167-6]

ORIGINAL ARTICLE

Multiple indications for everolimus after liver transplantation in current clinical practice

Itxarone Bilbao, Cristina Dopazo, Jose Lazaro, Lluis Castells, Mireia Caralt, Gonzalo Sapisochin, Ramon Charco

Itxarone Bilbao, Cristina Dopazo, Jose Lazaro, Mireia Caralt, Gonzalo Sapisochin, Ramon Charco, Hepatobiliopancreatic Surgery and Liver Transplant Unit of the Department of General Surgery, Hospital Vall Hebrón, Universidad Autónoma Barcelona, 08035 Barcelona, Spain
Lluis Castells, Hepatology Unit of the Department of Internal Medicine, Hospital Vall Hebrón, Universidad Autónoma Barcelona, 08035 Barcelona, Spain
Itxarone Bilbao, Lluis Castells, Ramon Charco, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD o Ciberehd), Hospital Vall d´Hebron, 08035 Barcelona, Spain
Author contributions: Bilbao I, Castells L and Charco R contributed to study design and data analysis; Dopazo C, Lazaro J, Caralt M and Sapisochin G contributed to data collection; Bilbao I contributed to drafting of manuscript.
Correspondence to: Itxarone Bilbao, MD, PhD, Hepatobiliopancreatic Surgery and Liver Transplant Unit of the Department of General Surgery, Hospital Vall Hebrón, Universidad Autónoma Barcelona, Paseo Vall d´Hebron, 119-129, 08035 Barcelona, Spain. ibilbao@vhebron.net
Telephone: +34-93-2746113 Fax: +34-93-2746112
Received: February 23, 2014 Revised: April 14, 2014
Accepted: May 29, 2014
Published online: June 24, 2014

Abstract

AIM: To assess our experience with the use and management of everolimus-based regimens post-liver transplantation and to redefine the potential role of this drug in current clinical practice.

METHODS: From October 1988 to December 2012, 1023 liver transplantations were performed in 955 patients in our Unit. Seventy-four patients (7.74%) received immunosuppression with everolimus at some time post-transplantation. Demographic characteristics, everolimus indication, time elapsed from transplantation to the introduction of everolimus, doses and levels administered, efficacy, side effects, discontinuation and post-conversion survival were analyzed.

RESULTS: Mean age at the time of conversion to everolimus was 57.7 ± 10 years. Indications for conversion were: refractory rejection 31.1%, extended hepatocellular carcinoma in explanted liver 19%, post-transplant hepatocellular carcinoma recurrence 8.1%, *de novo* tumour 17.6%, renal insufficiency 8.1%, severe neurotoxicity 10.8%, and others 5.4%. Median time from transplantation to introduction of everolimus was 6 mo (range: 0.10-192). Mean follow-up post-conversion was 22 ± 19 mo (range: 0.50-74). The event for which the drug was indicated was resolved in 60.8% of patients, with the best results in cases of refractory rejection, renal insufficiency and neurotoxicity. Results in patients with cancer were similar to those of a historical cohort treated with other immunosuppressants. The main side effects were dyslipidemia and infections. Post-conversion acute rejection occurred in 14.9% of cases. The drug was discontinued in 28.4% of patients.

CONCLUSION: Everolimus at low doses in combination with tacrolimus is a safe immunosuppressant with multiple early and late indications post-liver transplantation.

Key words: Everolimus; Liver transplantation; Indications; Off-protocol; Outcome

Core tip: Everolimus has a completely different mechanism of action to that of current basal calcineurine inhibitors used worldwide in liver transplantation. This immunosuppressant has a good profile for patients with pre- and post transplant renal dysfunction, one of the main concerns nowadays. It has also a promising role in cancer patients which is common in liver transplantation, either as an underlying disease (hepatocarcinoma in cirrhosis), or as de novo developing tumors. We

present our off-protocol experience with partial/total and early/late conversion to everolimus, highlighting its efficacy and safety in fitting with the different emerging scenarios after liver transplantation.

INTRODUCTION

Over the last thirty years, immunosuppression protocols in liver transplant patients have been based on calcineurine inhibitors (CNI) - cyclosporine in the eighties and tacrolimus in the nineties. Both were administered in combination with steroids. In the late nineties, monoclonal antibodies and mycophenolate mofetil (MMF), an antimetabolite with a different mechanism of action, were widely used. In the year 2000, sirolimus was the first inhibitor of the mammalian target of rapamycin (mTORi) launched into clinical practice as a primary immunosuppressant to replace the widespread use of CNI. However, its use declined due to severe adverse events and the warning issued on the risk of arterial thrombosis[1]. A few years later, everolimus (EVER) another mTORi was approved for use after acute rejection in heart[2] and kidney[3] transplantation. In 2012, EVER was approved for liver transplantation[4] by the EMA. In Spain, EVER was also approved for liver transplantation and obtained full registration at the end of 2012. In non-transplant areas, it has been approved for the treatment of advanced renal cell carcinoma[5].

mTORi reduce the expression of vascular endothelial growth factor and transforming growth factor-B, which are associated with tumour angiogenesis[6,7]. In solid organ transplantation, efficacy and safety can be achieved by targeting EVER trough levels at 3-8 ng/mL in combination with CNI. EVER is dosed twice daily and yields a steady state by day four.

The use of EVER is gaining acceptance in adult[8-10] and paediatric[11] liver transplant recipients. It has been used as maintenance[12-14], in *de novo* liver transplant patients[15], in cases of renal dysfunction as a CNI-sparing regimen[16-18], and in recipients with cancer[19-21]. The most common adverse events are leucopoenia, hyperlipidemia, gastrointestinal disorders, delayed wound healing, stomatitis, angioneurotic oedema, proteinuria and interstitial lung disease[22-24].

EVER was introduced into clinical practice at our centre in 2005, when some of the medical community had lost confidence in mTORi and had relegated the drug to compassionate use and to sporadic and desperate cases when other drugs failed. However, experience with sirolimus, especially the weak points of the drug,

prompted us to use EVER in order to optimise and redefine the true role of mTORi. The principal aim of this single-centre retrospective study was to study the current indications for total or partial conversion to EVER in liver transplant patients treated off-protocol.

MATERIALS AND METHODS

From October 1988 to October 2012, 1023 liver transplants were performed in 955 patients in our centre. We reviewed the prospectively collected data bases and medical records of these patients, focusing on the patients who received EVER for immunosuppression at some point post-transplantation. We recorded the demographic characteristics of these patients, the causes of conversion to EVER, the pre- and post-conversion immunosuppression regimens, the time elapsed between liver transplantation and the start of EVER treatment, doses and trough levels, efficacy, side effects, causes of discontinuation and mean follow-up post-conversion. Efficacy was assessed overall and according to the time elapsed from liver transplantation to the introduction of EVER. All patients receiving EVER gave their signed informed consent and met all the requirements for compassionate use of the drug.

Demographic characteristics
The following information on the demographic characteristics of the patients was obtained: age at time of transplantation and at time of conversion; gender; hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) status; indication for transplantation; Child and United Network for Organ Sharing classification status; body mass index (BMI) > 30; presence or absence of hypertension, diabetes mellitus (DM) and renal dysfunction at time of transplant; donor age; donor cause of death; donor time spent in the intensive care unit; presence or absence of graft steatosis > 20%; type of graft; presence or absence of portal thrombosis; type of biliary anastomosis; mean intraoperative red blood cells; and mean cold ischemia time. Renal dysfunction at time of transplant was defined as serum creatinine > 1.5 mg/dL or hepato-renal syndrome or need for dialysis.

Definition of the causes of conversion
Refractory rejection was defined as an incomplete response to treatment with steroid pulses with or without MMF. Patients outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver were considered advanced hepatocellular carcinoma (HCC). HCC recurrence was defined as tumour recurrence at any time during the follow-up period after liver transplantation. Diagnosis was based on radiologic images and assessed by a pathologist in either hepatic or extrahepatic specimens. *De novo* tumour was defined as the development of a malignant tumour (excluding HCC) during post-transplantation follow-up. Post-transplant neurological disorders were diagnosed by a neurologist

based on clinical symptoms, electroencephalograms, craneoencephalic computed tomography, cerebral magnetic resonance imaging, lumbar punctuation and viral serological testing. Renal dysfunction was defined as the presence of serum creatinine > 1.5 mg/dL. Amelioration of renal function was defined as a statistically significant (P < 0.05) difference between mean serum creatinine levels at two different points of follow-up.

Doses and trough levels

Doses and trough levels of EVER were assessed on the day of conversion and at 15 d and 1, 3, 6 and 12 mo post-conversion. Tacrolimus levels were also assessed at the same times.

Assessment of efficacy

The variables analysed at the time of conversion and thereafter were: total bilirubin and transaminases; serum creatinine; amelioration or resolution of neurotoxicity or other causes for which EVER was introduced. Serum creatinine was assessed on the day of conversion and at 3, 6 and 12 mo post-conversion. Acute rejection post-conversion was suspected based on enzymatic alteration of liver function, assessed by liver biopsy, and defined according to the Banff criteria.

Patients converted to prevent HCC recurrence were compared with a historical cohort not receiving EVER and matched for MELD status, year of transplantation ± 18 mo, presence or absence of vascular invasion, tumour type and size. We found appropriate matches for all the variables except vascular invasion due to a scarcity of receptors. Efficacy was assessed by comparing patient survival and the time elapsed from liver transplant to recurrence in the patients receiving EVER and those in the historical cohort.

Patients with HCC recurrence after transplantation were also compared with a historical cohort not receiving EVER and matched for the time elapsed from liver transplantation to tumour recurrence, site of recurrence, and Milan criteria. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Patients who developed *de novo* tumours were compared with a historical cohort of patients not receiving EVER and matched for tumour histology, time elapsed from liver transplantation to tumour, and type of treatment post-diagnosis. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Other efficacy variables were glucose levels and the need for anti-diabetic therapy post-conversion and blood pressure and the need for antihypertensive drugs. These variables were evaluated qualitatively as "amelioration or resolution", "worsening" and "no change".

Time elapsed from liver transplantation to conversion

Early conversion was defined as conversion during the first year post-transplantation, and late conversion as conversion after the first year post-transplantation.

Side effects and discontinuation

Possible side effects assessed were: hematologic toxicities; diarrhoea; proteinuria (though not assessed in all patients); levels of serum cholesterol and triglycerides and the need for hypolipidemic therapy; infections; and any other post-conversion adverse event.

Discontinuation was defined as stopping the drug when the patient was alive. The reason for EVER discontinuation was recorded.

Survival post-conversion

All patients were followed up until December 2012, death or drug withdrawal. Patient survival post-conversion and cause of death were analyzed according to the reason for conversion and EVER-related deaths.

Statistical analysis

The student's *t*-test or the Mann-Whitney *U* test were used for quantitative data and Pearson's χ^2 or Fisher's exact test for categorical data. Significance was set at P < 0.05. Data are expressed as mean ± SD, or as percentages. The Kaplan-Meier method was used for survival analysis. All analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Data on the demographic characteristics of recipients, donors and surgery are shown in Table 1. Mean patient age at the time of conversion was 57.7 ± 10 years and median age was 60 years (range: 27-74); nine patients (12.2%)were over 70 years of age.

Reasons for conversion to EVER are shown in Table 2. Pre-conversion therapy was based on tacrolimus in 69 patients, neoral cyclosporine (CyA) in four, and MMF in one. Post-conversion therapy consisted of: tacrolimus in 54 patients, CyA in three, and a CNI-free regimen in 17. Pre- and post-conversion drug combinations are specified in Table 3.

Median time between transplantation and introduction of EVER was 6 mo (range: 0.10-192 mo). Forty-two patients (56.8%) were converted during the first year post-transplantation and the remaining 32 patients (43.2%) after the first year. Median time between event onset and conversion was 1 mo (range: 0.1-19) (Table 1).

Doses and trough levels

Conversion to EVER was managed differently according to the reason for conversion; however, loading doses were never used. In cases of refractory rejection, EVER was administered at an initial dose of 1 mg every 12 h, with subsequent doses adjusted to obtain trough levels between 3 and 5 ng/mL. At the same time, CNI was maintained at high doses. When the reason for conversion was CNI-related adverse events, EVER was started at 0.5 mg once or twice a day and the CNI dose was reduced to half or completely withdrawn, depending on the severity of the adverse events. When the reason for conversion was cancer (extended tumour in the explant, can-

Table 1 Characteristics of recipients, donors, surgery and post-transplant evolution in 74 patients receiving everolimus *n* (%)

Recipient	Mean age (yr)	55.5 ± 9 r (25-69)
	Patients > 65 yr	10 (13.5)
	Male/female	55 (74.3)/19 (25.7)
	Diagnosis	
	HCC with cirrhosis	35 (47.2)
	Alcoholic cirrhosis	18 (24.3)
	HCV cirrhosis	16 (21.6)
	Cholostatic cirrhosis	3 (4.1)
	Liver insufficiency	2 (2.8)
	HCV - HBV	40 (54)-3 (4)
	ETOH	38 (51.4)
	HIV	4 (5.4)
	Child-Pugh A/B/C (%)	35-30-35
	UNOS (home/Hosp/ICU (%)	90.5-6.8-2.7
	Pre-LT associated disease	
	Renal insufficiency	11 (14.9)
	Diabetes mellitus	18 (24.3)
	Arterial hypertension	14 (18.9)
	Cardiopathy	3 (4.1%)
	Previous surgery	15 (20.3)
Donor	Mean age (yr)	48 ± 19 r (14-81)
	Patients > 70 yr	14 (19)
	Male/female (%)	49 (66)/25 (34)
	Graft steatosis > 20%	11 (15)
	Death (CET, CVA, Other) (%)	43-43-14
Surgery	E-E/E-E + Kehr/C-Y (%)	84-8-8
	Previous portal thrombosis	10 (13.6)
	Median RBC units	4 (r: 0-40)
	Cold ischaemia time (min)	378 ± 97
Post-transplant evolution	Ischaemia-reperfusion injury (ALT > 1000 IU, Quick < 60%)	14 (19)
	Biliary complication	7 (9.5)
	Postoperative arterial complication	2 (2.7)
Median time from event to conversion		1 mo (r: 0.1-19)
Median time from LT to conversion		6 mo (r: 0.1-192)
Early/late conversion	< 1 yr/ ≥ 1 yr	42 (56.8)/32 (43.2)
Mean follow-up post-conversion		22 ± 19 mo (r: 0.5-74)
Median follow-up post-conversion		17.5 mo

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBV: Hepatitis B virus; ETOH: Cirrhosis due to alcohol; HIV: Human immunodeficiency virus; UNOS: United Network for Organ Sharing classification; ICU: Intensive care unit; LT: Liver transplantation; CET: Cranioencephalic trauma; CVA: Cerebrovascular accident; E-E: End-to-end choledoco-choledostomy; E-E + K: End-to-end choledoco-choledostomy + kehr; C-Y: Choledoco-jejunostomy; RBC: Red blood cells; IU: International units; r: Range.

cer recurrence during follow-up, or *de novo* tumor), EVER was introduced at a dose of 0.5 mg/d, with trough levels adjusted to under 3 ng/mL, and CNI was drastically reduced to half or completely withdrawn. Doses and levels of EVER for the entire series of patients and tacrolimus levels for patients receiving this drug post-conversion are shown in Figure 1.

Efficacy
The cause of conversion to EVER was resolved in 60.8%

Table 2 Causes of conversion and other comorbidities at the time of conversion to everolimus in 74 liver transplant patients *n* (%)

Cause of conversion			
Refractory rejection	23 (31.1)	Resolution	17 (73.9)
Extended HCC in explanted liver	14 (19)	Prevention of recurrence	7 (50)
HCC recurrence during follow-up	6 (8.1)	Stabilization	0 (0)
De novo tumour	13 (17.6)	Prevention of recurrence	8 (61.5)
CNI-related neurotoxicity	8 (10.8)	Resolution or Stabilization	8 (100)
Renal dysfunction	6 (8.1)	Resolution or Amelioration	3 (50)
Other causes	4 (5.4)	Resolution	2 (50)
Comorbidity at time of conversion			
Chronic renal insufficiency	22 (29.8)	Resolution or Amelioration	15 (68.2)
Diabetes mellitus	21 (28.4)	Resolution or Amelioration	8 (38)
Arterial hypertension	25 (33.8)	Resolution or Amelioration	3 (12)
Dyslipidemia	30 (40.5)	Resolution or Amelioration	2 (6.7)

Outcome to everolimus shown as resolution, stabilization or amelioration of the cause or comorbidity. In 45 of 74 patients (60.8%), the cause was resolved, stabilized or ameliorated. HCC: Hepatocellular carcinoma; CNI: Calcineurin inhibitors. Other causes include: 1 chronic biliary cirrhosis recurrence plus chronic rejection, 1 sinusoidal hepatic fibrosis, 1 graft-versus-host disease, 1 chronic cholostatic liver dysfunction in the postoperative period.

of patients.

Refractory rejection: When EVER was used to treat refractory rejection (*n* = 23), the event was resolved correctly in 17 patients (73.9%) (Table 2). The remaining six patients failed to respond: four progressed to severe chronic refractory rejection finally requiring retransplantation and two died, one due to sepsis and one from concomitant severe hepatitis C recurrence.

Prevention of HCC recurrence: When EVER was indicated for prevention of HCC recurrence (*n* = 14), seven patients (50%) remained recurrence-free for a mean post-conversion follow-up of 33.8 ± 27 mo (Table 2). Three patients suffered recurrence at a mean post-conversion follow-up of 33.7 ± 33 mo, and four patients died due to HCC recurrence at a mean post-conversion follow-up of 15.1 ± 11 mo. When these 14 patients were compared with the historical cohort matched for MELD status, year of transplantation, and some pathological characteristics of the explanted liver, no differences either in survival or in time to recurrence were observed between the two groups (Table 4).

Patients with HCC recurrence: Six patients were converted to EVER due to HCC recurrence after liver transplantation. Types of post-transplant recurrences were: intra-abdominal at 122 mo; pulmonary at 6 mo; bone metastasis at 42 mo; liver recurrence at 46 mo; brain metastasis at 10 mo, and peritoneum-pulmonary metastasis

Table 3 Type of immunosuppression pre- and post-conversion to everolimus

Pre-conversion	*n* = 74	Post-conversion	*n* = 74
FK + MMF + ST	16	FK + EVER	38
FK + MMF	20	FK + EVER + MMF	1
FK + ST	12	FK + EVER + ST	11
FK	21	FK + EVER + MMF + ST	4
CyA + MMF + ST	1	CyA + EVER	3
CyA + MMF	1		
CyA	2	EVER	2
		EVER + ST	5
MMF + ST	1	EVER + MMF	2
		EVER + MMF + ST	8

FK: Tacrolimus; MMF: Mycophenolate mofetil; ST: Steroids; CyA: Neoral cyclosporine; EVER: Everolimus.

at 3 mo. Two patients were within the Milan criteria and four outside. All died at a mean time post-conversion of 14 ± 10.9 mo (3-31). When these six patients were compared with the historical cohort matched for recurrence site (1 suprarenal, 2 lung, 1 liver, 1 brain, 1 bone), time to recurrence and Milan criteria, survival post-recurrence was similar in those receiving EVER and those receiving other, non-mTORi immunosuppressants (Table 4).

Patients with *de novo* tumour: In thirteen patients, the reason for conversion to EVER was the appearance of a *de novo* tumor: 4 colon, 2 prostate, 2 esophagus, 2 larynx, 1 lung, 1 anus, and 1 breast. After onco surgical treatment of the tumor, eight patients remained alive and tumor-free at a mean follow-up post-tumor treatment of 37.7 ± 14.5 mo, four died at a mean follow-up post-tumor treatment of 21.5 ± 12.3 mo, and one (with colon cancer) is alive but with liver metastasis at 40 mo post-tumor treatment. In patients undergoing surgery, EVER was introduced as soon as healing was completed - 2-4 wk post-surgery. When these 13 patients were compared with the historical cohort matched for tumor type, time to development of the *de novo* tumor, and type of treatment, survival post-tumor treatment was similar in those receiving EVER and in those receiving other, non-mTORi immunosuppressants (Table 5).

Neurotoxicity: EVER was indicated in three patients with seizures, two with akinetic mutism, one with a cerebrovascular stroke plus multifocal progressive leukoencephalopathy, one with Guillain-Barré syndrome, and one with generalized tremor. Acompanying symptoms were different levels of speech disorders, including dysarthria, expressive dysphasia and apraxia. In all patients, EVER-based immunosuppression allowed a CNI-free period of time to reverse or ameliorate neurotoxicity.

Renal dysfunction: In the six patients in whom EVER was indicated due to renal insufficiency, serum creatinine changed from 2.54 ± 1.11 mg/dL pre-conversion to 1.63 ± 0.86 mg/dL at 3 mo post-conversion, 1.69 ± 0.91 mg/dL at 6 mo post-conversion, and 2 ± 1.45 mg/dL at 12
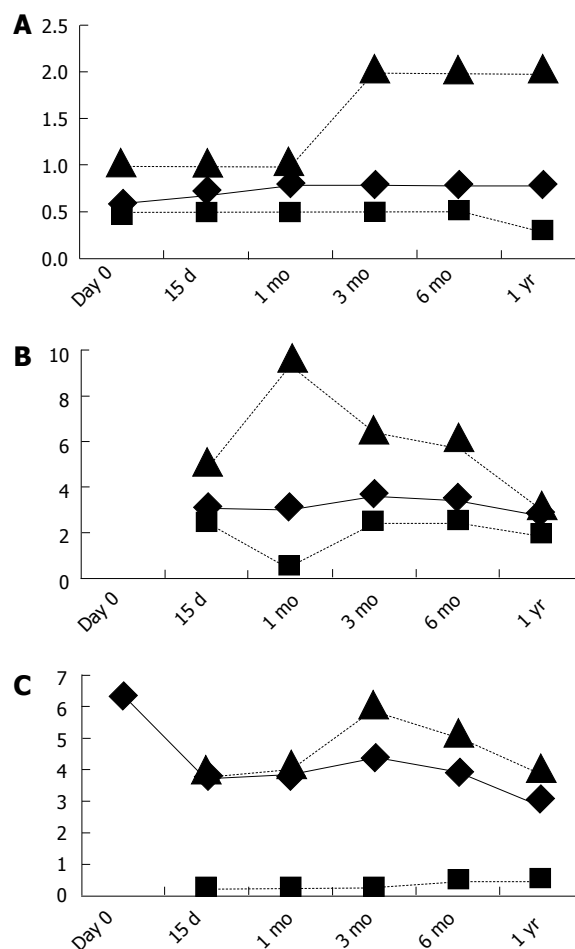
Figure 1 (A) Doses and (B) trough levels of everolimus for the entire series and (C) trough levels of tacrolimus for patients receiving this drug in the post-conversion regimen. Mean values and range (minimum-maximum). A: Doses of everolimus (ng/d); B: Trough levels of everolimus (ng/mL); C: Trough levels of tacrolimu (ng/mL).

mo post-conversion. In the three patients who converted within the first year post-transplantation, renal function ameliorated, while two patients with established chronic renal insufficiency for more than five years post-transplantation remained unchanged, and one patient with an episode of acute renal insufficiency in the immediate postoperative period failed to improve. If we consider all the patients suffering from renal insufficiency at the time of EVER introduction, whatever the reason for conversion, the improvement was statistically significant: serum creatinine was 2.5 ± 1.01 pre-conversion, 1.59 ± 0.62 at 3 mo post-conversion, 1.62 ± 0.56 at 6 mo post-conversion, and 1.74 ± 0.76 at 12 mo post-conversion.

Other causes: One patient was converted to EVER owing to liver dysfunction with cholostasis starting at 7 mo post-transplantation and progressing to severe cholostasis three months later (Table 2). Two liver biopsies at 8 and 10 mo post-transplantation revealed sinusoidal fibrosis and undetermined hepatitis. After conversion, liver function was completely restored within 1 mo. Another patient with a similar cholostatic syndrome one year af-

Table 4 Comparison between patients with hepatocellular carcinoma outside Milan criteria in the explanted liver receiving everolimus and a historical cohort not receiving mTOR inhibitors, and liver-transplanted patients with recurrence of hepatocellular carcinoma receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors *n* (%)

HCC outside Milan criteria in explanted livers	Patients receiving everolimus *n* = 14	Historical controls without mTORi *n* = 14	*P*
Recipient age at transplant (yr)	55.5 ± 11.3	56.38 ± 7.1	NS
Recipient sex (male-female) (%)	86-14	79 - 21	NS
Child–Pugh status	6.7 ± 1.8	6.5 ± 1.4	NS
MELD score	13.6 ± 5	11.4 ± 3.4	NS
Size of largest tumour on pathologic exam	3.43 ± 1.50	3.152 ± 1.05	NS
Nº of tumours at pathologic exam	2.70 ± 1.7	2.74 ± 1.7	NS
Microvascular invasion	10 (78)	4 (29)	0.02
Macrovascular invasion	5 (39)	0	0.01
Satellitosis	7 (50)	3 (21.4)	NS
Well-moderately differentiated tumour (%)	31-69	50-50	NS
Mean alpha-fetoprotein	366 ± 771	55 ± 125	NS
Median alpha-fetoprotein	12 (3-2571)	8 (2-445)	NS
HCC treatment while on waiting list	9 (64.3)	8 (57)	NS
Mean donor age in years	59 ± 14.9	58 ± 12.6	NS
Mean and median patient survival post-LT (mo)	56 ± 8.5 (59)	67 ± 11 (54)	NS
HCC recurrence in post-LT follow-up	*n* = 6	*n* = 6	*P*
Recipient age at transplant (yr)	53.6 ± 10	46.5 ± 13	NS
Recipient sex (male-female) (%)	100-0	83-17	NS
Milan criteria in explanted liver (yes-no) (%)	33-67	33-67	NS
Mean donor age (yr)	52.1 ± 16	41 ± 12.8	NS
Months from LT to recurrence	37.9 ± 45	28.5 ± 30	NS
Immunosuppression at recurrence (CyA-FK) (%)	17-83	17-83	NS
Type of recurrence (intra–extrahepatic) (%)	17-83	17-83	NS
Survival after recurrence (mo)	14.1 ± 11	16.6 ± 12.5	NS

HCC: Hepatocellular carcinoma; mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; CyA: Neoral cyclosporine; FK: Tacrolimus; NS: No significant.

Table 5 Comparison between liver-transplanted patients with *de novo* tumour receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors

	Patients receiving everolimus *n* =13	Historical controls without mTORi *n* =13	*P*
Recipient age at transplant (yr)	60.8 ± 5.8	59.5 ± 6.6	NS
Recipient sex (male-female) (%)	77-23	75-25	NS
Indication for LT (%)			NS
Postnecrotic-HCC in cirrhosis	68%	70%	NS
Mean time from LT to diagnosis of *de novo* tumour (mo)	67 ± 50	65.9 ± 37	NS
Tumour site and histology			NS
Colon ADK	4	4	
Prostate ADK	2	2	
Lung SCC	1	1	
Larynx SCC (4)	2	2	
Esophagus SCC(3) + ADK(1)	2	2	
Anus SCC	1	1	
Breast IDC	1	1	
Type of treatment			NS
Surgery ± QT ± RT	10	10	
QT ± RT	3	3	NS
Immunosuppression at diagnosis			
Cyclosporine-tacrolimus (%)	8-92	24-76	NS
Mean patient survival from diagnosis of tumour (mo)	32.9 ± 15	30.7 ± 20.6	NS

mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; HCC: Hepatocellular carcinoma; ADK: Adenocarcinoma; SCC: Squamous cell carcinoma; IDC: Infiltrative ductal carcinoma; QT: Chemotherapy; RT: Radiotherapy; NS: No significant.

ter transplantation did not improve and finally died. A third patient converted to EVER due to graft-versus-host disease one month post-transplantation. Immunosuppression was changed from tacrolimus to low doses of EVER to reduce any hypersensitivity to tacrolimus and counterbalance the steroid bolus administered. This patient was maintained on EVER monotherapy at 2-3 ng/mL and did well for two months but finally died from sepsis due to bone marrow aplasia as progression of his graft-versus-host disease. A fourth patient converted to EVER suffered long-lasting primary dysfunction of the liver. Two liver biopsies confirmed cholostatic preservation injury. Total bilirubin was normalized after introduction of EVER in combination with tacrolimus and steroids.

Efficacy for other comorbidities: Although EVER was never indicated for arterial hypertension and dia-

betes mellitus, 25 patients had high blood pressure at the time of conversion and 21 had insulin-dependent diabetes mellitus (Table 2). Blood pressure improved in three patients (12%), as shown by lower blood pressure or by a reduced need for antihypertensive drugs. One of them was converted to CNI free regimen (EVER and steroids). Glucose values or insulin doses improved in eight patients (38%). Three of them were converted to CNI free regimen (EVER and mycophenolate mofetil). Dyslipidemia was present in 30 patients and serum values improved in only two (6.7%), whose regimen were CNI low dose and EVER.

Efficacy according to the time elapsed between transplantation and conversion to EVER
In general, conversion to EVER was successful in a greater percentage of patients when the conversion occurred during the first year post-transplantation (Table 6). Success rates in cases of early conversion were higher than in those of late conversion, especially in cases of refractory rejection (84.6% *vs* 60%), neurotoxicity (100%) and renal dysfunction (75% *vs* 0%).

Side effects and discontinuation
Liver graft function after conversion was well preserved in all cases except in 11 patients (14.9%) who presented acute cellular rejection (4 moderate and 7 mild) requiring the reintroduction of CNI (Table 7). Ten of these

Table 6 Efficacy in cases of early (within one year post-transplantation) and late (after one year post-transplantation) conversion to everolimus *n* (%)		
Early conversion		
Cause of conversion	42 (56.8)	Resolution/stabilization or prevention of recurrence in 29 patients (69)
Refractory rejection	13 (17.6)	Resolution in 11 (84.6)
Advanced HCC in explanted liver	12 (16.3)	Prevention of recurrence in 6 (50)
HCC recurrence during follow-up	3 (4.1)	-
De novo tumour	0	-
CNI-related neurotoxicity	8 (10.8)	Resolution or amelioration in 8 (100)
Renal dysfunction	4 (5.4)	Resolution in 3 (75)
Other causes	2 (2.6)	Resolution in 1 (50)
Late conversion		
Cause of conversion	32 (43.2)	Resolution/stabilization or prevention of recurrence in 16 patients (50)
Refractory rejection	10 (13.5)	Resolution in 6 (60)
Advanced HCC in ex planted liver	2 (2.7)	Prevention of recurrence in 1 (50)
HCC recurrence during follow-up	3 (4.1)	-
De novo tumour	13 (17.6)	Prevention of recurrence in 8 (61.5)
CNI-related neurotoxicity	0	-
Renal dysfunction	2 (2.7)	Resolution in none (0)
Other causes	2 (2.7)	Resolution in 1 (50)

HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

Table 7 Adverse events, causes of discontinuation and mortality *n* (%)	
	Patients receiving everolimus (*n* = 74)
Adverse events	27 (36.5)
Dyslipidemia	27 (36.5)
Infections	9 (12.2)
Mucositis	3 (4.1)
Diarrhoea	1 (1.4)
Proteinuria	1 (1.4)
Acute rejections post-conversion	11 (14.9)
Causes of discontinuation	21 (28.4)
Resolution of the cause of conversion	6 (8.1)
Non-responding rejection and retransplantation	6 (8.1)
Drug-related adverse events	5 (6.7)
Intercurrent surgery	4 (5.5)
Causes of mortality	25 (33)
HCC recurrence during follow-up	10
De novo tumour	4
HCV recurrence	4
Chronic rejection	4
Sepsis	1
Graft-*vs*-host disease	1
Other causes	1

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

patients experiencing acute rejection had converted to EVER without CNI within the first year post-transplant.

EVER-related side effects occurred in 27 patients (36.5%), some of whom experienced more than one (Table 7). Dyslipidemia was managed with the introduction of hypolipemic drugs. Infections included severe hepatitis C recurrence in four cases, bacterial pneumonia in two, pulmonary tuberculosis in one, CMV infection, pulmonary aspergillosis and sepsis in graft-versus-host disease in one, and bacteriemia in one. Infections were treated according to the cause and by reducing the total amount of immunosuppression. Twenty-one patients (28.4%) stopped taking EVER (Table 7): six owing to resolution of the cause (acute rejection in four, convulsions in one, renal dysfunction in one); six because of inefficacy in resolving chronic rejection; five due to adverse events (infections in four, proteinuria in one); and four due to intercurrent surgery, with reintroduction of EVER two to three weeks after surgery.

Patient survival and follow-up
Mean follow-up post-conversion for the entire series was 22 ± 19.33 mo (range: 0.5-74), with a median of 17.5 mo. Actuarial patient survival post-conversion was 54%, 46% and 23% at 1, 3 and 5 years, respectively. Mean and median follow-up differed according to the reason for conversion: refractory rejection, 15.10 ± 15.96 mo (range: 0.5-54) and 9 mo; HCC outside Milan criteria, 29.10 ±

24.72 mo (range: 6-74) and 21.50 mo; post-transplant HCC recurrence, 14.16 ± 10.94 mo (range: 3-31) and 13 mo; *de novo* tumor, 32.92 ± 15 mo (range: 5-54) and 32 mo; renal dysfunction, 14.85 ± 13.58 (range: 0.5-41) and 18 mo; and other CNI-related adverse events, 25.87 ± 21.53 mo. Causes of death are shown in Table 7. There were no EVER-related deaths.

DISCUSSION
The principal aim of this retrospective study was to study the real use and management of EVER in patients treated off-protocol and help redefine the true role of mTORi in clinical practice. In the field of liver transplantation, we are faced with clear challenges for the 21st century, one of which is establishing patient profiles for individualising immunosuppression strategies. Sirolimus, the first mTORi introduced into clinical practice some years ago, was largely unsuccessful[1], but it has provided sufficient experience to help improve the use and management of EVER, another mTORi.

Reasons for introducing EVER
The most frequent indication for introducing EVER in our series was a high risk of tumour recurrence. So, our first experience with EVER was at low doses within a dual regimen while minimizing CNI. This experience provided evidence of the safety and efficacy of EVER, and we were able to avoid the adverse events associated with high doses of sirolimus. Furthermore, the pharmacokinetic differences between EVER and sirolimus permitted a 12-h administration and offered the possibility of providing much greater accuracy in trough levels and dose calculation.

We have used EVER in all types of transplant patients, regardless of age, sex, cause, the severity of liver disease, or concomitant diseases. Advanced age, co-infection with HCV and HIV viruses, diabetes mellitus, arterial hypertension, obesity, renal insufficiency, or even dyslipidemia did not constitute a contraindication for the use of EVER.

Cancer patients

Cancer patients deserve special mention, since the mTOR pathway is necessary for tumour cells to grow[25]. There are three potential profiles of cancer patients. Firstly, in patients transplanted for HCC outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver, EVER would be used as prophylaxis and would be introduced in the early post-transplantation period[10]. Secondly, in patients transplanted for HCC with recurrence of the original tumour during follow-up, EVER would be used as treatment[26]. Finally, in transplanted patients who develop a *de novo* tumour during follow-up, EVER would also be used as treatment[27,28].

In our study, in patients whose tumours were outside the Milan criteria in the explanted liver, either EVER or CNI was administered at low doses between six and twelve weeks post-transplant. We had difficulty finding appropriate historical matches for this subgroup of patients. Although macro- and microvascular invasion was greater in the EVER group, there was also a trend towards longer survival. This trend did not, however, reach statistical significance - probably due to the low number of patients. To date, no published randomized study has demonstrated the beneficial effect of the use of mTORi as prophylaxis, but we believe that EVER provides a benefit since it is the least pro-carcinogenic immunosuppressant and allows doses of known pro-carcinogenic immunosuppressants to be reduced. We await the results of a future randomized prospective study[29].

One of the main late indications in our study was the development of a *de novo* tumour or recurrence of the original HCC. Again, survival in the EVER group was longer but did not reach statistical significance compared to our historical cohort, probably due to the low number of patients included. Taking into account that the anti-tumour properties of mTORi are at doses much higher than those used in clinical practice[30], we agree with other authors[20,21] that EVER appears to be effective at reducing tumour recurrence.

Patients with acute rejection

The second most frequent indication for the introduction of EVER was to reinforce the immunosuppressive regimen in cases of severe or refractory acute rejection. In this situation, EVER could be safely administered together with CNI and steroids as triple therapy or with the addition of MMF as quadruple therapy as early as 10 d post-transplant, once healing was complete. The initial doses and trough levels reached were the highest. The phase II trial[31] comparing three doses of EVER showed

that freedom from rejection correlated with trough blood levels of 3 ng/mL or more. Six patients with chronic rejection did not benefit from the introduction of EVER and were finally retransplanted, suggesting that the drug has the greatest effect during the early post-transplantation period and that there is little or no benefit from EVER in the case of chronic rejection.

Neurotoxicity and other CNI-induced toxicities

Our experience with EVER without CNI was in patients with severe neurotoxicity or other severe adverse effects triggered by CNI, especially in the early post-transplantation period, although some cases were observed during the late post-transplantation period. Initial doses and trough levels were high, in the same range as for patients with refractory rejection. Our findings, consistent with other authors[32], indicate that EVER-based immunosuppression - either with or without other non-CNI drugs - is a feasible and effective option, at least for the time required for CNI-induced neurological complications to disappear. However, the risk of acute rejection during the first year post-transplantation indicates a need for caution. Therefore, we do not believe that regimens based on EVER without CNI should be the principal use of this drug, at least during the first year post-transplantation.

The improvement achieved in some patients with diabetes and arterial hypertension was probably due to the parallel decrease in CNI levels and/or steroids. None of these co-morbidities were indications for conversion and they were evaluated in a qualitative and global way that makes difficult to explain the real cause of improvement. However, we believe that regimens based on EVER and low levels of CNI could play a role in patients with metabolic syndrome[33], although further studies are required to ascertain their ability to modify the risk of cardiovascular disease[34].

Early and late renal dysfunction

In our study, an overall improvement in serum creatinine levels was observed in patients whose indication for receiving EVER was renal dysfunction. However, when we specifically analyzed the six patients converted for renal insufficiency, the maximum benefit was attained in those converted within the first year post-transplantation. Several liver studies and multicentre randomised trials[35,36] introducing EVER at one month post-transplantation have reported an amelioration in the glomerular filtration rate at 12 and 24 mo post-transplantation in patients receiving tacrolimus plus EVER and minimizing CNI compared to those receiving standard tacrolimus and steroids.

Adverse events and discontinuation at low doses

No life-threatening adverse events were observed. The main adverse event was dyslipidemia, which was easily controlled by reducing the EVER dose and adding a statin. None of our patients presented EVER-associated interstitial pneumonitis or severe sepsis, as had previously been reported in other studies[37], and drug-related deaths

Table 8 Future challenges in liver transplantation and the potential role of everolimus	
Future challenges	**Potential role of everolimus**
More marginal donors	Renal function protection
Recipients with more serious disease, selected by MELD	Renal function protection
Recipients with more serious disease, with metabolic syndrome	Prevention of cardiovascular events
Less HCV cirrhosis but more aggressive strains	Antifibrotic effect
More NASH	Prevention of cardiovascular events
More metabolic syndrome during follow-up	Prevention of cardiovascular events
More HCC recurrence	Antiproliferative effect
More *de novo* tumours	Antiproliferative effect
CNIe-related neurotoxicity	Good neurological profile

MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NASH: Non-alcoholic steato hepatitis; HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

did not occur. This was probably due to the low doses of EVER (Figure 1) and the lessons learned from our previous experience with sirolimus[38].

A good percentage of failure or discontinuation of the drug is probably related to the timing of the introduction of EVER in critical and irreversible situations where other immunosuppressants have failed. A real problem in the long-term management of mTORi is wound complications, which would render EVER inadvisable in stable patients with good liver function who must undergo some type of intercurrent surgery. In such cases, we would recommend withdrawal of the drug and its reintroduction, if necessary, four weeks after surgery.

Challenges in the 21st century and the potential role of EVER

According to our study, there are several potential indications for the use of EVER after liver transplantation. During the early post-transplantation period, EVER can be used as one component of a triple therapy for refractory rejection, as one component of a double therapy with CNI (both at half the normal dose) in cases of extended tumours in the explanted liver, and at low doses without CNI in cases of severe CNI-related adverse effects. During the late post-transplantation period, EVER can be used at low doses in patients with CNI-related adverse effects and in those with HCC recurrence or *de novo* tumours. In general, we recommend EVER at low doses and as a support immunosuppressant. In this scenario, the rate of adverse events, discontinuations and drug-related deaths will be acceptable.

The future of liver transplantation presents the following scenario (Table 8): (1) increasing acceptance of marginal donors to increase the pool of grafts; (2) recipients with more severe liver disease according to the MELD criteria[39]; (3) a higher frequency of recipients with metabolic syndrome as a comorbidity; (4) less HCV cirrhosis and more NASH as the reason for transplantation[40]; and (5) longer patient survival but with increased

HCV and HCC recurrence, *de novo* tumours and cardiovascular events. Looking at this scenario, we can imagine more renal dysfunction, more metabolic syndrome and cardiovascular events, and more cases of cancer. Marginal donors would increase the incidence of primary liver dysfunction and resultant renal dysfunction. The use of the MELD score to select patients for transplantation would increase the incidence of post-transplant renal dysfunction. The incidence of metabolic syndrome is increasing both in candidates for liver transplantation and in recipients during the post-transplantation period, as well as in the general non-transplanted population, which in turn would increase the risk of cardiovascular events in the long term. The new antiviral therapies for hepatitis C may affect the need for liver transplantation; however, the HCV in the small number of patients not responding to the new drugs will be more selected and perhaps more aggressive. The incidence of HCC secondary to HCV cirrhosis would decrease, but HCC secondary to NASH would increase. Improved post-transplantation management of patients would mean longer patient survival and thus a greater probability of tumour recurrence or a *de novo* tumour (Table 8). We urgently need an immunosuppressant that will meet all the requirements. EVER is a drug with a good profile for renal dysfunction, a certain antifibrotic effect, an ability to inhibit the mTOR pathway used by cancer cells, and a good degree of effectiveness in reducing cardiovascular risk events. Future trials will demonstrate if EVER is the immunosuppressant we need.

COMMENTS

Background
Calcineurine inhibitors (CNI) are the most powerful immunosuppressants used in liver transplantation, however the long-term survival and quality of life are partly overshadowed by the appearance of adverse effects of its chronic use, such as renal dysfunction, metabolic syndrome, cardiovascular complications, *de novo* tumor and recurrence of underlying disease. Previous attempts to overcome these complications with the use of mammalian target of rapamycin (mTOR) inhibitors (an immunosuppressant with a different way of action), did not succeed. However, everolimus seems to cope with them and to partially contribute to search their role.

Research frontiers
New emerging immunosuppressants must be powerful enough to avoid rejection in the same way as calcineurine inhibitors, but at the same time must avoid calcineurine inhibitors-related adverse events. The association of tacrolimus and everolimus could represent the best regimen to cope with the different profiles of patients after liver transplantation.

Innovations and breakthroughs
Recent multicentre trials have highlighted the important of everolimus introduction at one month post-transplant together with low dose tacrolimus to protect early and long term renal function after liver transplantation. In this single-centre study, the authors report other off-protocol indications for everolimus, that could fit into the various profiles of patients that most concern to medical teams, cancer patients and patients with co-morbidities derived from calcineurine inhibitors.

Applications
Due to the lack of new immunosuppressants, optimization of treatment regimens is of great value to increase patient and graft survival after liver transplantation. In the near future two facts will be relevant. First, survival will continue to increase over time, to the same extent that the need for calcineurine inhibitors sparing protocols. Second, the authors probably will see a change in the indications for liver transplantation, from hepatitis C liver cirrhosis toward cancer

patients. This article could serve as a starting point to be explored with further studies.

Terminology
Everolimus is an orally administered mTOR inhibitor, a proliferation signal employed by many mammalian cells, especially those with a high level of turnover (skin, intestinal and hematological cells), but also many cancer cells and T-cells implied in the second phase of the alloantigenic response. The same pathway used by different cells of the human body, explains the dual characteristic of this mTOR inhibitor as immunosuppressant and as antineoplastic.

Peer review
This an interesting review, it can be usefull for the readers.

REFERENCES

1   **Fortin MC**, Raymond MA, Madore F, Fugère JA, Pâquet M, St-Louis G, Hébert MJ. Increased risk of thrombotic microangiopathy in patients receiving a cyclosporin-sirolimus combination. *Am J Transplant* 2004; **4**: 946-952 [PMID: 15147429]

2   **Eisen HJ**, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sørensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med* 2003; **349**: 847-858 [PMID: 12944570]

3   **Tedesco-Silva H**, Vitko S, Pascual J, Eris J, Magee JC, Whelchel J, Civati G, Campbell S, Alves-Filho G, Bourbigot B, Garcia VD, Leone J, Esmeraldo R, Rigotti P, Cambi V, Haas T. 12-month safety and efficacy of everolimus with reduced exposure cyclosporine in de novo renal transplant recipients. *Transpl Int* 2007; **20**: 27-36 [PMID: 17181650]

4   **Saliba F**, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Jonas S, Sudan D, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Dong G, Lopez PM, Fung J, Junge G. Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study. *Am J Transplant* 2013; **13**: 1734-1745 [PMID: 23714399 DOI: 10.1111/ajt.12280]

5   **Motzer RJ**, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Kay A, Ravaud A. Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. *Cancer* 2010; **116**: 4256-4265 [PMID: 20549832 DOI: 10.1002/cncr.25219]

6   **Schuler W**, Sedrani R, Cottens S, Häberlin B, Schulz M, Schuurman HJ, Zenke G, Zerwes HG, Schreier MH. SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. *Transplantation* 1997; **64**: 36-42 [PMID: 9233698]

7   **Dumont FJ**. Everolimus. Novartis. *Curr Opin Investig Drugs* 2001; **2**: 1220-1234 [PMID: 11717808]

8   **Everson GT**. Everolimus and mTOR inhibitors in liver transplantation: opening the "box". *Liver Transpl* 2006; **12**: 1571-1573 [PMID: 17058246]

9   **Goralczyk AD**, Schnitzbauer A, Tsui TY, Ramadori G, Lorf T, Obed A. A therapeutic exploratory study to determine the efficacy and safety of calcineurin-inhibitor-free de-novo immunosuppression after liver transplantation: CILT. *BMC Surg* 2010; **10**: 15 [PMID: 20380712 DOI: 10.1186/1471-2482-10-15]

10 **Bilbao I**, Sapisochin G, Dopazo C, Lazaro JL, Pou L, Castells L, Caralt M, Blanco L, Gantxegi A, Margarit C, Charco R. Indications and management of everolimus after liver transplantation. *Transplant Proc* 2009; **41**: 2172-2176 [PMID: 19715864 DOI: 10.1016/j.transproceed.2009.06.087]

11 **Nielsen D**, Briem-Richter A, Sornsakrin M, Fischer L, Nashan B, Ganschow R. The use of everolimus in pediatric liver transplant recipients: first experience in a single center. *Pediatr Transplant* 2011; **15**: 510-514 [PMID: 21696525 DOI: 10.1111/j.1399-3046.2011.01515.x]

12 **Saliba F**, Dharancy S, Lorho R, Conti F, Radenne S, Neau-Cransac M, Hurtova M, Hardwigsen J, Calmus Y, Dumortier

J. Conversion to everolimus in maintenance liver transplant patients: a multicenter, retrospective analysis. *Liver Transpl* 2011; **17**: 905-913 [PMID: 21384525 DOI: 10.1002/lt.22292]

13 **Vallin M**, Guillaud O, Morard I, Gagnieu MC, Mentha G, Adham M, Morelon E, Boillot O, Giostra E, Dumortier J. Tolerability of everolimus-based immunosuppression in maintenance liver transplant recipients. *Clin Transplant* 2011; **25**: 660-669 [PMID: 21158921 DOI: 10.1111/j.1399-0012.2010.01370.x]

14 **De Simone P**, Metselaar HJ, Fischer L, Dumortier J, Boudjema K, Hardwigsen J, Rostaing L, De Carlis L, Saliba F, Nevens F. Conversion from a calcineurin inhibitor to everolimus therapy in maintenance liver transplant recipients: a prospective, randomized, multicenter trial. *Liver Transpl* 2009; **15**: 1262-1269 [PMID: 19790150 DOI: 10.1002/lt.21827]

15 **Levy G**, Schmidli H, Punch J, Tuttle-Newhall E, Mayer D, Neuhaus P, Samuel D, Nashan B, Klempnauer J, Langnas A, Calmus Y, Rogiers X, Abecassis M, Freeman R, Sloof M, Roberts J, Fischer L. Safety, tolerability, and efficacy of everolimus in de novo liver transplant recipients: 12- and 36-month results. *Liver Transpl* 2006; **12**: 1640-1648 [PMID: 16598777]

16 **Masetti M**, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, Begliomini B, Di Benedetto F, Gerunda GE. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in de novo liver transplant recipients preserves renal function. *Am J Transplant* 2010; **10**: 2252-2262 [PMID: 20486905 DOI: 10.1111/j.1600-6143.2010.03128.x]

17 **Castroagudín JF**, Molina E, Romero R, Otero E, Tomé S, Varo E. Improvement of renal function after the switch from a calcineurin inhibitor to everolimus in liver transplant recipients with chronic renal dysfunction. *Liver Transpl* 2009; **15**: 1792-1797 [PMID: 19938140 DOI: 10.1002/lt.21920]

18 **De Simone P**, Carrai P, Precisi A, Petruccelli S, Baldoni L, Balzano E, Ducci J, Caneschi F, Coletti L, Campani D, Filipponi F. Conversion to everolimus monotherapy in maintenance liver transplantation: feasibility, safety, and impact on renal function. *Transpl Int* 2009; **22**: 279-286 [PMID: 19054383 DOI: 10.1111/j.1432-2277.2008.00768.x]

19 **Bhoori S**, Toffanin S, Sposito C, Germini A, Pellegrinelli A, Lampis A, Mazzaferro V. Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. *J Hepatol* 2010; **52**: 771-775 [PMID: 20347502 DOI: 10.1016/j.jhep.2010.01.025]

20 **Gomez-Camarero J**, Salcedo M, Rincon D, Lo Iacono O, Ripoll C, Hernando A, Sanz C, Clemente G, Bañares R. Use of everolimus as a rescue immunosuppressive therapy in liver transplant patients with neoplasms. *Transplantation* 2007; **84**: 786-791 [PMID: 17893613]

21 **Zimmerman MA**, Trotter JF, Wachs M, Bak T, Campsen J, Skibba A, Kam I. Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 633-638 [PMID: 18324656 DOI: 10.1002/lt.21420]

22 **Schrader J**, Sterneck M, Klose H, Lohse AW, Nashan B, Fischer L. Everolimus-induced pneumonitis: report of the first case in a liver transplant recipient and review of treatment options. *Transpl Int* 2010; **23**: 110-113 [PMID: 19497063 DOI: 10.1111/j.1432-2277.2009.00900.x]

23 **Masetti M**, Rompianesi G, Montalti R, Romano A, Spaggiari M, Ballarin R, Guerrini GP, Gerunda GE. Effects of everolimus monotherapy on hematological parameters and iron homeostasis in de novo liver transplant recipients: preliminary results. *Transplant Proc* 2008; **40**: 1947-1949 [PMID: 18675097 DOI: 10.1016/j.transproceed.2008.05.068]

24 **De Simone P**, Petruccelli S, Precisi A, Carrai P, Doria R, Menichetti F, Filipponi F. Switch to everolimus for sirolimus-induced pneumonitis in a liver transplant recipient--not all proliferation signal inhibitors are the same: a case report. *Transplant Proc* 2007; **39**: 3500-3501 [PMID: 18089420]

25 **Finn RS**. Current and Future Treatment Strategies for

Patients with Advanced Hepatocellular Carcinoma: Role of mTOR Inhibition. *Liver Cancer* 2012; **1**: 247-256 [PMID: 24159589]

26 **De Simone P**, Crocetti L, Pezzati D, Bargellini I, Ghinolfi D, Carrai P, Leonardi G, Della Pina C, Cioni D, Pollina L, Campani D, Bartolozzi C, Lencioni R, Filipponi F. Efficacy and safety of combination therapy with everolimus and sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc* 2014; **46**: 241-244 [PMID: 24507059 DOI: 10.1016/j.transproceed.2013.10.035]

27 **Alegre C,** Jiménez C, Manrique A, Abradelo M, Calvo J, Loinaz C, García-Sesma A, Cambra F, Alvaro E, García M, Sanabria R, Justo I, Caso O, Moreno E. Everolimus monotherapy or combined therapy in liver transplantation: indications and results. *Transplant Proc* 2013; **45**: 1971-1974 [DOI: 10.1016/j.transproceed.2013.01.075]

28 **Klintmalm GB**, Saab S, Hong JC, Nashan B. The Role of Mammalian Target of Rapamycin Inhibitors in the Management of Posttransplant Malignancy. *Clin Transplant* 2014; Epub ahead of print [DOI: 10.1111/ctr.12357]

29 **Schinitzbauer AA**, Geissler EK (University of Regensburg. 2006-2011/2012 ClinicalTrials.gov: NCT00355862). Available from: URL: http://www.clinicaltrials.gov

30 **Yamanaka K**, Petrulionis M, Lin S, Gao C, Galli U, Richter S, Winkler S, Houben P, Schultze D, Hatano E, Schemmer P. Therapeutic potential and adverse events of everolimus for treatment of hepatocellular carcinoma - systematic review and meta-analysis. *Cancer Med* 2013; **2**: 862-871 [PMID: 24403259 DOI: 10.1002/cam4.150]

31 **Calmus Y**, Durrbach A. Everolimus de novo in liver transplantation. *Gastroenterol Clin Biol* 2009; **33** Suppl 4: S247-S252 [PMID: 20004330 DOI: 10.1016/S0399-8320(09)73161-6]

32 **Catalano G,** De Simone P, Mazzoni A, Ghinolfi D, Coletti L, Filipponi F. Everolimus-based immunosuppression in a case of ABO-incompatible liver transplantation with calcineurin inhibitor-related posterior occipital syndrome. *Transpl Int* 2014 Mar 7; Epub ahead of print [PMID: 24606320 DOI: 10.1111/tri.12304]

33 **McKenna GJ**, Trotter JF, Klintmalm E, Ruiz R, Onaca N, Testa G, Saracino G, Levy MF, Goldstein RM, Klintmalm GB. Sirolimus and cardiovascular disease risk in liver transplantation. *Transplantation* 2013; **95**: 215-221 [PMID: 23232369 DOI: 10.1097/TP.0b013e318279090c]

34 **Peddi VR**, Wiseman A, Chavin K, Slakey D. Review of combination therapy with mTOR inhibitors and tacrolimus minimization after transplantation. *Transplant Rev* (Orlando) 2013; **27**: 97-107 [PMID: 23932018 DOI: 10.1016/j.trre.2013.06.001]

35 **Keating GM**, Lyseng-Williamson KA. Everolimus: a guide to its use in liver transplantation. *BioDrugs* 2013; **27**: 407-411 [PMID: 23696253 DOI: 10.1007/s40259-013-0041-6]

36 **Fischer L**, Klempnauer J, Beckebaum S, Metselaar HJ, Neuhaus P, Schemmer P, Settmacher U, Heyne N, Clavien PA, Muehlbacher F, Morard I, Wolters H, Vogel W, Becker T, Sterneck M, Lehner F, Klein C, Kazemier G, Pascher A, Schmidt J, Rauchfuss F, Schnitzbauer A, Nadalin S, Hack M, Ladenburger S, Schlitt HJ. A randomized, controlled study to assess the conversion from calcineurin-inhibitors to everolimus after liver transplantation--PROTECT. *Am J Transplant* 2012; **12**: 1855-1865 [PMID: 22494671 DOI: 10.1111/j.1600-6143.2012.04049.x]

37 **Kaplan B**, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. *Transplant Rev* (Orlando) 2014; Epub ahead of print [PMID: 24685370 DOI: 10.1016/j.trre.2014.03.002]

38 **Bilbao I**, Sapisochin G, Dopazo C, Lazaro JL, Pou L, Catells L. Indications and Management of m-TOR Inhibitors after Liver Transplantation. In: Editor: Charles B Taylor. Immunosuppression: New Research Nova Science Publishers, 2009: 117-132

39 **Sharma P**, Schaubel DE, Guidinger MK, Goodrich NP, Ojo AO, Merion RM. Impact of MELD-based allocation on end-stage renal disease after liver transplantation. *Am J Transplant* 2011; **11**: 2372-2378 [PMID: 21883908 DOI: 10.1111/j.1600-6143.2011.03703.x]

40 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

RESEARCH REPORT

Everolimus immunosuppression reduces the serum expression of fibrosis markers in liver transplant recipients

Ainhoa Fernández-Yunquera, Cristina Ripoll, Rafael Bañares, Marta Puerto, Diego Rincón, Ismael Yepes, Vega Catalina, Magdalena Salcedo

Ainhoa Fernández-Yunquera, Cristina Ripoll, Rafael Bañares, Diego Rincón, Ismael Yepes, Vega Catalina, Magdalena Salcedo, Department of Liver Transplantation, Hospital General Gregorio Marañón, 28028 Madrid, Spain
Rafael Bañares, Marta Puerto, Diego Rincón, On-Line Biomedical Investigation Center for Hepatic and Gastrointestinal Diseases, 28028 Madrid, Spain
Author contributions: Fernández-Yunquera A performed the study and wrote the paper; Ripoll C analyzed the data, wrote the paper and gave final approval of the version to be published; Bañares R analyzed and interpreted the data; Puerto M contributed important reagents; Rincón D, Yepes I and Catalina C collected the data; and Salcedo M designed the study.
Supported by Novartis Pharma Schweiz AG
Correspondence to: Magdalena Salcedo, Chief Doctor, Department of Liver Transplantation, Hospital General Gregorio Marañón, C/ Doctor Esquerdo nº 46, 28028 Madrid, Spain. magdalena.salcedo@salud.madrid.org
Telephone: +34-630-991228 Fax: +34-91-5868308
Received: October 30, 2013 Revised: January 29, 2014
Accepted: February 18, 2014
Published online: June 24, 2014

Abstract

AIM: To evaluate the expression of serum fibrosis markers in liver transplantation (LT) recipients on everolimus monotherapy compared to patients on an anti-calcineurin regimen.

METHODS: This cross-sectional case-control study included LT patients on everolimus monotherapy (cases) (E) (n = 30) and matched controls on an anti-calcineurin regimen (calcineurin inhibitors, CNI), paired by etiology of liver disease and time since LT (n = 30). Clinical characteristics, blood tests and elastography were collected. Serum levels of transforming growth factor-β (TGF-β), angiopoietin-1, tumor necrosis factor (TNF), platelet derived growth factor, amino-terminal propeptide of type Ⅲ procollagen (PⅢNP), hyaluronic acid (HA), VCM-1 (ng/mL), interleukin (IL)-10, interferon-inducible protein 10 (IP-10), vascular endothelial growth factor and hepatocyte growth factor (HGF) (pg/mL) were determined by enzyme-linked immunosorbent assay. Expression of these markers between E and CNI was compared. Stratified analysis was done according to factors that may influence liver fibrosis. Variables are described with medians (interquartillic range) or percentages.

RESULTS: A total of 60 patients [age: 59 (49-64), hepatitis C virus (HCV): n = 21 (35%), time from LT: 73 mo (16-105)] were included. Patients had been on everolimus for a median of 15 mo. No differences in inflammatory activity, APRI test or liver elastography were found between the groups. No significant differences were observed between the groups in serum levels of PⅢNP, metalloproteinase type = 1, angiopoietin, HGF, IP-10, TNF-α, IL-10 and *vascular* cell adhesion molecule. Patients on E had a lower expression of TGF-β [E: 12.7 (3.7-133.6), CNI: 152.5 (14.4-333.2), P = 0.009] and HA [E: 702.89 (329.4-838.2), CNI: 1513.6 (691.9-1951.4), P = 0.001] than those on CNI. This difference was maintained in the stratified analysis when recipient age is more than 50 years (TFG-β1: P = 0.06; HA: P = 0.005), in patients without active neoplasia (TFG-β1, P = 0.009; HA: P = 0.01), according to time since LT (> than 5 years, TFG-β1: P = 0.001; HA: P = 0.002), related to previous history of biliary complications (HA: P = 0.01) and HCV recurrence (HA: P = 0.004). Liver transplant recipients with everolimus monotherapy had less serum expression of TGF-β y HA than matched patients with anti-calcineurins. This difference remains when classifying patients according to donor age and time since LT. Due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference was non-significant but trends towards the lower expression of TFG-β1 in the everolimus group. Mammalian target of rapamycin (mTOR) plays a role in the transformation of quiescent hepatocellular stellate cell to their active

profibrotic state, and experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrogenesis.

CONCLUSION: This study supports a possible role of everolimus in liver fibrosis modulation after LT in a clinical setting and suggests that tailoring immunosuppression could avoid fibrosis progression in the allograft.

Core tip: This study tries to approach the possible antifibrotic effect of everolimus, a mammalian target of a rapamycin inhibitor, in the clinical setting. Some studies in animal models suggest that it could also have an antifibrotic effect. The main conclusion of this study is that liver transplantation recipients with everolimus monotherapy had less serum expression of transforming growth factor-β and hyaluronic acid than matched patients with anti-calcineurins that play an important role in liver fibrosis. The study offers the rationale for much needed future randomized controlled trials that evaluate the modulation of post-transplant fibrosis.

INTRODUCTION

Liver transplantation (LT) is the definitive treatment for end-stage liver disease. However, the outcome of a liver transplant can be compromised by allograft dysfunction due to fibrosis, which can even lead to cirrhosis. Approximately 75% of liver biopsies conducted in long-term LT survivors in whom liver tests are anomalous show significant histopathological abnormalities[1,2]. Fibrosis in the graft may be due to the recurrence of native disease [especially recurrent hepatitis C virus (HCV)], hepatotoxicity, *de novo* disease, non-alcoholic steatohepatitis, chronic rejection and/or vascular and biliary complications.

Strategies designed to prevent the progression of fibrosis in the allograft include the specific treatment of native disease[3,4] and/or stricter control of factors that can accelerate this fibrosis[5]. In addition, tailoring the immunosuppressive regime has been proposed as a strategy to regulate fibrogenesis in the post-transplant period. In HCV patients, measures such as avoiding the use of adjuvant pulse steroids for acute rejection and slow withdrawal of low-dose steroids beyond 12 mo have been proposed to avoid any immune-mediated graft injury that could induce an inflammatory and fibrogenic response[6-8].

However, the results of a meta-analysis indicate no differences in mortality, graft survival, rejection, fibrosing cholestatic hepatitis or severe fibrosis related to the use of the calcineurin inhibitors, cyclosporine and tacrolimus at 1 year of follow-up[9].

For prophylaxis against rejection in kidney transplant patients, new immunosuppressors known as mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been recently introduced[10]. Small observational studies have described their use[11], particularly in patients with renal failure[12-14] and in those who develop post-transplant neoplasia[15,16]. mTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis [synthesis of interleukins interleukin (ILs) and transforming growth factor-β (TGF-β)][17,18], angiogenesis[19] and cell metabolism (hypoxia inducible factor)[20].

Due to the role played by mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell (HSC) and portal fibroblasts[21], it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In effect, a recent study conducted on bile duct-ligated (BDL) cirrhotic rats showed that the mTOR inhibitors, sirolimus and everolimus, reduced liver fibrosis compared to the effects of calcineurin inhibitors (CNI) after 5 wk of treatment[22].

The aim of this study was to compare serum levels of mediators of liver fibrosis in liver transplant patients under immunosuppressive regimes based on everolimus (E) with those based on calcineurin inhibitors.

MATERIALS AND METHODS

This cross-sectional study was conducted over the period April to October 2010. All consecutive patients who underwent liver transplantation between 1995 and 2010 under everolimus immunosuppression alive at the time of the study were enrolled. Patients were matched with control LT patients undergoing calcineurin inhibitor treatment according to liver disease etiology and time since LT. Exclusion criteria for cases and control patients were acute rejection in the previous 6 mo, uncontrolled infection or antiviral treatment, or unresolved biliary complications.

Everolimus (Certican®, Novartis Pharma Schweiz AG, Bern, Switzerland) is approved for prophylaxis against rejection in *de novo* renal transplant recipients[10], for management of malignancy (chemotherapy resistant kidney cancer, subependymal giant cell astrocytoma and neuroendocrine neoplasm)[20] and for use in drug-eluting coronary stents[23]. However, this drug has also been used off-label in liver and lung transplantation patients[24-27]. At our center, the use of everolimus in LT recipients is approved in situations such as renal dysfunction or adverse events like neurotoxicity due to CNI, development of *de novo* malignancies, recurrence of hepatocellular carcinoma, and the presence of predictors of a high risk of hepatocellular carcinoma recurrence in the explanted liver (satellitosis, vascular infiltration and multinodularity disease)[28,29]. Con-

traindications for the use of everolimus are a prior history of hepatic artery thrombosis, proteinuria greater than 800 mg/d and/or surgery in the previous 4 wk[29,30].

Everolimus dosing and switching

An initial dose of 0.5-0.75 mg bid E was administered and then increased 0.5 mg weekly to obtain a trough level of 3-8 ng/mL. Tacrolimus and cyclosporine were tapered by 15%-25% of the usual dose every 2 wk until complete withdrawal. The overlap period between both drugs in the E group treatment was a median of 1 or 2 mo[31-33] before monotherapy was achieved. In patients who were started on everolimus, steroids were given according to the usual schedule, and then progressively tapered and withdrawn by month 12 after liver transplantation. Trough levels of everolimus, hematological and lipid profiles, renal and liver function tests and proteinuria were monitored weekly until stable levels of the drug were achieved[34].

Clinical and laboratory variables

Information was compiled on patient demographics, etiology of cirrhosis, LT surgical variables, postoperative period and laboratory data. The immunosuppression regime data recorded were present dose and blood levels, time of administration, and combined treatment with corticosteroids and/or mycophenolate.

Laboratory tests were performed to determine transaminases, cholestasis enzymes and simple validated fibrosis scores (APRI)[35-37]. Additionally, 20 mL of blood were obtained to determine serum biomarkers of fibrosis that had been identified in previous studies[38-42]. We determined serum markers that could be correlated with late liver fibrosis by enzyme-linked immunosorbent assay, including those linked to matrix deposition such as hyaluronic acid (Echelos, Bioscience Inc.), amino-terminal propeptide of type Ⅲ procollagen (PⅢNP; Cusabio, Bionova), those linked to matrix degradation such as tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1; Ray-Biotech, Bionova), growth factors like angiopoietin (Ray-Biotech, Bionova), hepatocyte growth factor (HGF; Ray-Biotech, Bionova), platelet derived growth factor (PDGF; RayBiotech, Bionova) and finally, inflammatory markers that participate in the fibrogenesis-like TGF-β1 (Diaclone, Bionova), adiponectin and leptin, IP-10 (interferon-inducible protein 10 calcineurin inhibitor; Diaclone, Bionova), tumor necrosis factor alpha (TNF-α; Diaclone, Bionova), interleukin 10 (IL-10; Diaclone, Bionova) and *vascular* cell adhesion molecule (VCAM; Cusabio, Bionova).

Liver stiffness was measured by a trained nurse or physician by transient elastography using a Fibroscan instrument (Echosens, Paris, France). Measurements in which 10 acquisitions were achieved, with a success rate of at least 60% and an interquartile range lower than 30 were considered valid[43,44].

Definitions

The following definitions were made: (1) early allograft dysfunction[45]: one or more of the following postop-

erative findings: bilirubin > 10 mg/dL, INR > 1.6 on postoperative day or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2000 IU/mL within the first 7 postoperative days; (2) previous acute rejection: a histological picture compatible with rejection or in cases of an abnormal liver test, conversion to a normal test result after reaching optimal serum levels of immunosuppressants[1]; (3) chronic rejection: a compatible histological picture[1]; (4) biliary tract disease: anastomotic and non-anastomotic biliary strictures detected on imaging showing biochemical expression. Resolution of strictures was defined as a non-requirement for endoscopic, radiological or surgical treatment of the stricture in the 6 mobefore inclusion; (5) uncontrolled neoplasia: a remission time under 2 years; (6) HCV recurrence: histological indicators of inflammation or fibrosis in patients with HCV viremia detected in protocol biopsies at 6 and 12 mo; and (7) arterial hypertension, diabetes mellitus or dyslipidemia: defined according to the criteria established by the European Society of Hypertension and the International Diabetes Federation[46,47].

RESULTS

Sixty LT patients were recruited for the study (30 on everolimus and 30 on CNI). The demographic characteristics of the participants are provided in Table 1. Patients were predominantly men of median age 60 (49-64) years in the everolimus group and 54 (46-60) years in the control CNI group. The most common cause of liver disease that led to transplantation was alcoholic liver cirrhosis. Median time since LT was approximately 6 years (IQR 16.7-106.4 mo) for both groups. No difference in donor age or in the proportion of patients with early allograft dysfunction was observed.

The median time of everolimus treatment was 15 (5-29) mo and the median time of the initial dose of everolimus given from the time of LT was 2.7 years (0.7-8.3). This is because the main indication to use everolimus in our center was developing neoplasia *de novo* (85.71%). Monotherapy with everolimus was achieved in 25 patients (83.3%). Of the 5 patients on combination therapy (everolimus plus CNI), 1 patient was under cyclosporine treatment and 4 patients received tacrolimus. These patients did not tolerate monotherapy with everolimus immunosuppression. Most patients in the CNI group were receiving tacrolimus (24 patients) and had been under CNI treatment for a median time of 72 (17-108) mo. Approximately 25% of patients in the CNI group were given concomitant mycophenolate mofetil to minimize adverse effects linked to CNI treatment, while only one patient in the everolimus group received this drug. No differences in the proportions of patients under concomitant steroid treatment were observed between the two groups (Table 2).

No differences between treatment groups were detected in: HCV recurrence, previous episodes of acute, chronic rejection and biliary complications, proportion of patients with diabetes mellitus, arterial hypertension,

Table 1 Demographic and clinical characteristics of patients before liver transplantation			
	Everolimus patients (*n* = 30)	CNI patients (*n* = 30)	*P* value
Male	24 (80)	24 (80)	1.000
Age (yr)	46 (44-60)	51 (44-59)	0.760
Etiology of liver disease			
EtOH	16 (53.3)	13 (43.3)	0.541
HCV	11 (36.7)	10 (33.3)	
HBV	0	2 (6.7)	
Autoimmune	1 (3.3)	2 (6.7)	
Hemochromatosis	1 (3.3)	2 (6.7)	
Cholestatic disorders	1 (3.3)	0	
Cryptogenic	0	1 (3.3)	
Time from LT (mo)	75 (16-113)	72 (17-108)	0.859
Indication for LT			
HCC	2 (6.7)	4 (13. 3)	0.041
Decompensated cirrhosis	16 (53.3)	21 (70)	
Decompensated cirrhosis and HCC	12 (40)	4 (13.3)	
Acute liver Failure	0	1 (3.3)	
Donor age (yr)	55 (34-72)	49 (31-63)	0.521
Early allograft dysfunction	5 (16.7)	2 (6.7)	0.212

Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartillic range. CNI: Calcineurin inhibitors; HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma.

Table 2 Clinical characteristics of patients after liver transplantation			
	Everolimus patients (*n* = 30)	CNI patients (*n* = 30)	*P* value
Age	60 (49-64.5)	54 (46.5- 60.5)	0.756
BMI (kg/m^2)	26.2 (23.3-28.2)	27.9 (25.2-31.3)	0.211
Dyslipemia	10 (33.3)	12 (40)	0.789
Diabetes mellitus	9 (30)	13 (43.3)	0.284
HTA	18 (60)	18 (60)	1.000
Neoplasia			
HCC	8 (26.7)	1 (3.3)	0.026
Solid non-hepatic neoplasia	2 (6.7)	0	
Skin neoplasia	4 (13.3)	1 (3.3)	
Hematological neoplasia	2 (6.7)	0	
Recurrent HCV	11 (36.7)	10 (33.3)	1.000
AST (IU/L)	23 (18-53)	35 (25-66)	0.081
ALT (IU/L)	43 (18- 65)	24 (18-62)	0.260
Bilirubin (mg/dL)	0.5 (0.37-0.7)	0.85 (0.5–1.1)	0.002
Cholesterol (mg/dL)	188.5 (167-220.75)	158 (141.25-178.25)	0.002
Acute rejection	7 (23.3)	9 (30)	0.771
Chronic rejection	2 (6.7)	1 (3.3)	1.000
Biliary complications	10 (33.3)	12 (40)	0.789
APRI	0.74 (0.48-2)	0.47 (0.34-1.4)	0.135
Liver stiffness (kPa)	7.6 (5.1-8.6)	8.4 (5.6-10.7)	0.134
Concomitant steroids	6 (20)	6 (20)	1.000
Concomitant Mycophenolate mofetil	1 (3.3)	8 (26.7)	0.026

Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartillic range. HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma; HTA: Arterial hypertension; APRI: APRI score.

obesity or dyslipidemia. Patients in the everolimus group had higher serum levels of cholesterol, a well-known side effect of the drug. As expected, given the accepted local indications for everolimus treatment, patients in this group had a greater proportion of neoplasms and hepatocellular carcinoma outside the Milan criteria (data not shown) at the time of the study.

Although bilirubin levels were higher in the CNI group ($P = 0.002$), no differences were observed in transaminase levels (AST and ALT), GGT or in the proportion of patients with hyperbilirubinemia. Similarly, no differences in APRI or elastography were detected between the groups.

No significant differences were observed between the groups in serum levels of PⅢNP, TIMP-1, angiopoietin, HGF, IP-10, TNF-α, IL-10 and VCAM (Table 3). Interestingly, patients on everolimus showed a markedly lower expression of TGF-β1 [12.7 (3.7-133.6) ng/mL *vs* 152.5 (IQR 14.4-333.2) ng/mL; $P = 0.009$] (Figure 1A). TGF-β1 is the most potent stimulus for hepatic fibrogenesis through activation of hepatic stellate cells[48]. Furthermore, patients on everolimus showed the lower expression of hyaluronic acid [702.89 (329.4-838.2) ng/mL *vs* 1513.6 (691.9-1951.4) ng/mL; $P = 0.001$] (Figure 1B), an essential component of the extracellular matrix (ECM) mostly synthesized by hepatic stellate cells[49].

To determine whether the results could be influenced by other factors, markers were compared in different patient subgroups by univariate analysis (Table 4). First of all, we examined the expression of fibrosis markers in patients without active neoplasia given the uneven distribution of neoplasia between groups. Other patient

subsets were established according to time since LT (> than 5 years), recipient age (> 50 years), previous history of biliary complications and HCV recurrence. The differences observed between TGF-β1 and hyaluronic acid expression in the main everolimus and CNI groups persisted in our analysis by subgroups. This difference was statistically significant when classifying patients according to donor age and time since LT. However, due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference emerged as a non-significant trend towards the lower expression of TFGβ-1 in the everolimus group. Although there were differences in the use of mycophenolate mofetil among both groups, the results described before remained when we compared both groups excluding patients who were receiving mycophenolate mofetil.

DISCUSSION

In this study we show that LT patients on everolimus therapy have lower serum levels of TGF-β1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving CNI. TGF-β1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as
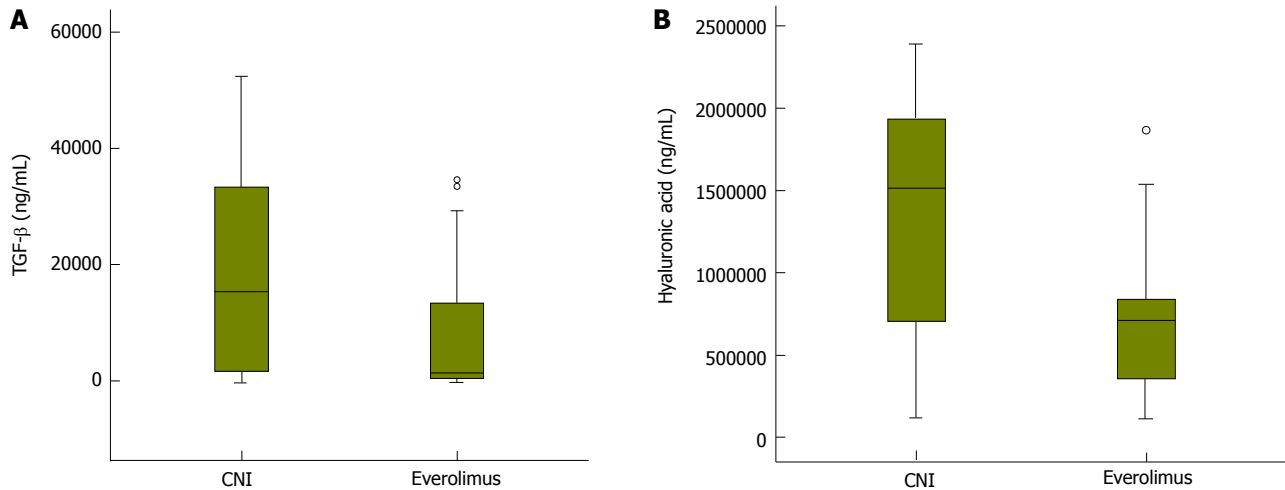
Figure 1 Box plots of serum transforming growth factor-β (A) and serum Hyaluronic acid (B) in patients under calcineurin inhibitors or everolimus regime. TGF-β: Transforming growth factor-β; CNI: Calcineurin inhibitors.

Table 3 Serum levels of liver fibrosis mediators

	Everolimus patients (n = 30)	CNI patients (n = 30)	P value
VCAM (ng/mL)	68.25 (25.98-135.17)	58.88 (35.30-115.52)	0.668
PⅢNP (ng/mL)	172.4 (119.75-1195.90)	879.40 (140.10-1555.15)	0.193
IP-10 (pg/mL)	86.01 (51.10-210.91)	79.61 (59.2-172.64)	0.669
HGF (pg/mL)	225.17 (163.30-320.17)	205.53 (152.59-297.86)	0.363
Angiopoietin (ng/mL)	26.97 (19.58-32.25)	30.108 (24.60-38.8)	0.122
TNF-α (ng/mL)	41.12 (38.35-44.8)	42.70 (40.20-45.07)	0.435
IL-10 (pg/mL)	8.52 (6.07-9.23)	8.66 (6.95-9.40)	0.856
TGF-β (ng/mL)	12.7 (3.7-133.6)	152.5 (14.4-333.2)	0.009
HA (ng/mL)	702.89 (329.4-838.2)	1513.6 (691.9-1951.4)	0.001
PDGF (ng/mL)	1.5630 (1.4663-1.6369)	1.5630 (1.4616-1.6369)	0.720

Variables are expressed as medians and interquartillic range. VCAM: Vascular cell adhesion molecule; PⅢNP: Amino-terminal propeptide of type Ⅲ procollagen; IP10: Interferon-inducible protein 10 calcineurin inhibitor; TNF-α: Transforming necrosis factor alpha; TGF: Tissue growth factor; HGF: Hepatocyte growth factor; HA: Hyaluronic acid; PDGF: Platelet derived growth factor.

Table 4 Stratified analysis according to factors that could influence liver fibrosis

	TGF-β (ng/mL)	P value	HA (ng/mL)	P value
All patients				
E (n = 30)	12.7 (3.7-133.6)	0.009	702.89 (329.4-838.2)	0.001
CNI (n = 30)	152.5 (14.4-333.2)		1513.6 (691.9-1951.4)	
Free of neoplasia				
E (n = 29)	11.1 (3.2-22.4)	0.005	754.8 (351.3-837)	0.030
CNI (n = 22)	137.5 (14.4-333.2)		1296.7 (703.7-1936.1)	
Time from LT > 5 yr				
E (n = 17)	16.5 (7.6-264.6)	0.010	462.0 (351.3-770.3)	0.002
CNI (n = 16)	296.8 (125.4-337.1)		1084.7 (523.7-1665.8)	
Donor age > 50 yr				
E (n = 12)	14.0 (6.0-67.1)	0.06	910.0 (589.2-1510.8)	0.005
CNI (n = 15)	96.0 (14.5-297.1)		1897.0 (1519-2136.5)	
Biliary complications				
E (n = 12)	20.6 (7.6-265)	0.110	516.75 (235.6-1079.4)	0.010
CNI (n = 10)	272.3 (16.5-403.4)		1545.37 (1085.8-1888.7)	
Recurrent HCV				
E (n = 10)	6.5 (1.6-15.3)	0.260	914.55 (768.8-1513.6)	0.410
CNI (n = 11)	14.5 (6.1-225)		1991.17 (1532.4-2168.9)	

Variables are expressed as medians and interquartillic range. TGF-β: Transforming growth factor-β; HA: Hyaluronic acid; E: Everolimus; CNI: Calcineurin inhibitors.

hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including matrix metalloproteinases (MMP) and their inhibitors (TIMP)[48,50-52].

MTOR signaling includes several steps in the transformation of quiescent HSC to their active profibrotic state[53]. Although some studies have addressed the modulation of liver fibrosis in patients on CNI, no study has assessed the role of mTOR inhibitors in fibrogenesis in a clinical setting.

The potential role of mTOR inhibition in attenuating fibrogenic pathways has been examined in experimental models of cirrhosis. After bile duct ligation- and thioacetamide induced cirrhosis, low dose rapamycin led to the reduced accumulation of ECM-producing cells, ECM components, reduced interstitial MMP-2 activity and a reduced spleen weight as an indicator of portal hypertension than in vehicle-treated cirrhotic rats[54]. Higher doses of rapamycin in the BDL rats gave rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts[21]. Lastly, mTOR inhibitors have been noted to reduce liver fibrosis up to 70% and portal pressure up to 50% in BDL rats compared to CNI-treated rats. Furthermore, in mTOR inhibitor-treated rats, the clinical manifestation of portal hypertension was lessened, as indicated by factors such as the development of ascites.

In the context of LT, one of the main causes of fibrosis is recurrent hepatitis C. The activation of HSC has been correlated not only with the fibrosis stage, but

also with the rate of liver fibrosis progression[55]. In a retrospective clinical study, the use of sirolimus compared to CNI was associated with a trend towards diminished disease activity and fibrosis in serial biopsies, although no differences were observed in incidence and time to recurrence of HCV[56].

No differences in the extent of fibrosis as measured by transient elastography and APRI score were detected, although our study was not designed to assess this factor. Elastography has not been validated in long-term liver grafts and its sensitivity to determine fibrosis is probably not comparable to the use of direct molecular markers of fibrogenesis. The limitations of our study include those inherent to its cross-sectional design, which precludes establishing a temporal relationship between drug initiation and serum levels of fibrosis markers. In addition, it has been well established that different etiologies of liver disease produce different fibrosis patterns. Unfortunately, our sample size was insufficient to determine the effect of everolimus according to the etiology of liver disease. Also, serum biomarker expression could be influenced by factors secondary to the inflammatory response or to other forms of chronic visceral damage. To avoid this bias, patients with acute conditions were not included and the influence of other chronic conditions was assessed by examining different patient subgroups.

In conclusion, patients under everolimus therapy show reduced serum expression of fibrosis markers such as TGF-β1 and hyaluronic acid compared to patients matched for LT etiology and time since LT under a CNI immunosuppressive regimen. The results of this study provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies.

COMMENTS

Background
The outcome of liver transplant may be conditioned by allograft dysfunction associated with the development of fibrosis which can even lead to cirrhosis. Tailoring immunosuppression has been postulated to have a role in fibrosis progression. Mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been introduced for prophylaxis against rejection in transplant patients and, because of their antiangiogenic, antiproliferative and antifibrotic properties, it has been postulated that they could modulate liver fibrosis in liver transplant (LT) grafts.

Research frontiers
Low dose rapamycin can reduce accumulation of extracellular matrix (ECM)-producing cells (extracellular matrix), ECM components, interstitial matrix metalloproteinases (MMP)-2 activity (metalloproteinases) and a reduced spleen weight as an indicator of portal hypertension in cirrhotic rats. Experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrosis but there is no evidence in a clinical setting. The hotspot of this article is the study about the impact of everolimus immunosuppression in serum levels of liver mediator fibrosis expression in a clinical practice.

Innovations and breakthroughs
MTOR signaling includes several steps in the transformation of quiescent hepatic stellate cell (HSC) to their active profibrotic state. Higher doses of rapamycin in rats give rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts. These rats had reduced liver fibrosis up to 70% and portal pressure up to 50% compared to calcineurin inhibitors-treated rats. Clinical manifestation of portal hypertension

like ascites development was lessened in mTOR inhibitor-treated. Due to the potential role mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell and portal fibroblasts, it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In this study we show that liver transplant patients on everolimus therapy have lower serum levels of transforming growth factor-β (TGF-β) 1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving calcineurin inhibitors. TGF-β1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including MMP and their inhibitors (TIMP).

Applications
The study results suggest that mTOR inhibitors could modulate fibrosis progression in liver grafts. Although this is not a prospective study, the results support the need to investigate the role of an immunosuppression regime in fibrosis development after liver transplantation.

Terminology
MTOR: MTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis (synthesis of interleukins and transforming growth factor beta), angiogenesis and cell metabolism (hypoxia inducible factor).

Peer review
This is a good descriptive study in which the authors compare serum liver fibrosis expression between both immunosuppression regimens (anti-calcineurin *vs* everolimus). The results of this study are interesting and provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies in clinical practice.

REFERENCES

1   **Demetris AJ**, Adeyi O, Bellamy CO, Clouston A, Charlotte F, Czaja A, Daskal I, El-Monayeri MS, Fontes P, Fung J, Gridelli B, Guido M, Haga H, Hart J, Honsova E, Hubscher S, Itoh T, Jhala N, Jungmann P, Khettry U, Lassman C, Ligato S, Lunz JG, Marcos A, Minervini MI, Mölne J, Nalesnik M, Nasser I, Neil D, Ochoa E, Pappo O, Randhawa P, Reinholt FP, Ruiz P, Sebagh M, Spada M, Sonzogni A, Tsamandas AC, Wernerson A, Wu T, Yilmaz F. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 2006; **44**: 489-501 [PMID: 16871565 DOI: 10.1002/hep.21280]

2   **Benlloch S**, Berenguer M, Prieto M, Rayón JM, Aguilera V, Berenguer J. Prediction of fibrosis in HCV-infected liver transplant recipients with a simple noninvasive index. *Liver Transpl* 2005; **11**: 456-462 [PMID: 15776403 DOI: 10.1002/lt.20381]

3   **O'Grady JG**. Phenotypic expression of recurrent disease after liver transplantation. *Am J Transplant* 2010; **10**: 1149-1154 [PMID: 20353464 DOI: 10.1111/j.1600-6143.2010.03080.x]

4   **Berenguer M**, Palau A, Aguilera V, Rayón JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant* 2008; **8**: 679-687 [PMID: 18294165 DOI: 10.1111/j.1600-6143.2007.02126.x]

5   **Hanouneh IA**, Feldstein AE, McCullough AJ, Miller C, Aucejo F, Yerian L, Lopez R, Zein NN. The significance of metabolic syndrome in the setting of recurrent hepatitis C after liver transplantation. *Liver Transpl* 2008; **14**: 1287-1293 [PMID: 18756451 DOI: 10.1002/lt.21524]

6   **Gane EJ**. The natural history of recurrent hepatitis C and what influences this. *Liver Transpl* 2008; **14** Suppl 2: S36-S44 [PMID: 18825724 DOI: 10.1002/lt.21646]

7   **Berenguer M**. What determines the natural history of recurrent hepatitis C after liver transplantation? *J Hepatol* 2005; **42**: 448-456 [PMID: 15763325 DOI: 10.1016/j.jhep.2005.01.011]

8   **Samuel D**, Forns X, Berenguer M, Trautwein C, Burroughs A, Rizzetto M, Trepo C. Report of the monothematic EASL conference on liver transplantation for viral hepatitis (Paris, France, January 12-14, 2006). *J Hepatol* 2006; **45**: 127-143 [PMID: 16723165 DOI: 10.1016/j.jhep.2006.05.001]

9   **Berenguer M**, Royuela A, Zamora J. Immunosuppression

with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. *Liver Transpl* 2007; **13**: 21-29 [PMID: 17192906 DOI: 10.1002/lt.21035]

10   **Rostaing L**, Kamar N. mTOR inhibitor/proliferation signal inhibitors: entering or leaving the field? *J Nephrol* 2010; **23**: 133-142 [PMID: 20155724]

11   **Calmus Y**, Dharancy S. Indications of mTOR inhibitors in liver transplantation. *Gastroenterol Clin Biol* 2009; **33** Suppl 4: S245-S246 [PMID: 20004329 DOI: 10.1016/S0399-8320(09)73160-4]

12   **Asrani SK**, Leise MD, West CP, Murad MH, Pedersen RA, Erwin PJ, Tian J, Wiesner RH, Kim WR. Use of sirolimus in liver transplant recipients with renal insufficiency: a systematic review and meta-analysis. *Hepatology* 2010; **52**: 1360-1370 [PMID: 20815021 DOI: 10.1002/hep.23835]

13   **De Simone P**, Precisi A, Petruccelli S, Balzano E, Carrai P, Catalano G, Campani D, Filipponi F. The impact of everolimus on renal function in maintenance liver transplantation. *Transplant Proc* 2009; **41**: 1300-1302 [PMID: 19460545 DOI: 10.1016/j.transproceed.2009.03.051]

14   **Masetti M**, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, Begliomini B, Di Benedetto F, Gerunda GE. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in de novo liver transplant recipients preserves renal function. *Am J Transplant* 2010; **10**: 2252-2262 [PMID: 20486905 DOI: 10.1111/j.1600-6143.2010.03128.x]

15   **Toso C**, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. *Hepatology* 2010; **51**: 1237-1243 [PMID: 20187107 DOI: 10.1002/hep.23437]

16   **Gomez-Camarero J**, Salcedo M, Rincon D, Lo Iacono O, Ripoll C, Hernando A, Sanz C, Clemente G, Bañares R. Use of everolimus as a rescue immunosuppressive therapy in liver transplant patients with neoplasms. *Transplantation* 2007; **84**: 786-791 [PMID: 17893613 DOI: 10.1097/01.tp.0000280549.93403.dd]

17   **Le Pabic H**, L'Helgoualc'h A, Coutant A, Wewer UM, Baffet G, Clément B, Théret N. Involvement of the serine/threonine p70S6 kinase in TGF-beta1-induced ADAM12 expression in cultured human hepatic stellate cells. *J Hepatol* 2005; **43**: 1038-1044 [PMID: 16139919 DOI: 10.1016/j.jhep.2005.05.025]

18   **Gäbele E**, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schrum LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. *J Biol Chem* 2005; **280**: 13374-13382 [PMID: 15677443 DOI: 10.1074/jbc.M409444200]

19   **Tepperman E**, Ramzy D, Prodger J, Sheshgiri R, Badiwala M, Ross H, Raoa V. Surgical biology for the clinician: vascular effects of immunosuppression. *Can J Surg* 2010; **53**: 57-63 [PMID: 20100415]

20   **Gabardi S**, Baroletti SA. Everolimus: a proliferation signal inhibitor with clinical applications in organ transplantation, oncology, and cardiology. *Pharmacotherapy* 2010; **30**: 1044-1056 [PMID: 20874042 DOI: 10.1592/phco.30.10.1044]

21   **Bridle KR**, Popa C, Morgan ML, Sobbe AL, Clouston AD, Fletcher LM, Crawford DH. Rapamycin inhibits hepatic fibrosis in rats by attenuating multiple profibrogenic pathways. *Liver Transpl* 2009; **15**: 1315-1324 [PMID: 19790156 DOI: 10.1002/lt.21804]

22   **Patsenker E**, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. *J Hepatol* 2011; **55**: 388-398 [PMID: 21168455 DOI: 10.1016/j.jhep.2010.10.044]

23   **Almalla M**, Schröder JW, Pross V, Stegemann E, Marx N, Hoffmann R. Everolimus-eluting versus paclitaxel-eluting stents for treatment of bare metal stent restenosis. *Am J Cardiol* 2011; **108**: 518-522 [PMID: 21624553 DOI: 10.1016/j.amjcard.2011.03.080]

24   **Molinari M**, Berman K, Meeberg G, Shapiro JA, Bigam D, Trotter JF, Kneteman N. Multicentric outcome analysis of sirolimus-based immunosuppression in 252 liver transplant recipients. *Transpl Int* 2010; **23**: 155-168 [PMID: 19765266 DOI: 10.1111/j.1432-2277.2009.00969.x]

25   **Mártinez JM**, Pulido LB, Bellido CB, Usero DD, Aguilar LT, Moreno JL, Artacho GS, Díez-Canedo JS, Gómez LM, Bravo MA. Rescue immunosuppression with mammalian target of rapamycin inhibitor drugs in liver transplantation. *Transplant Proc* 2010; **42**: 641-643 [PMID: 20304212 DOI: 10.1016/j.transproceed.2010.02.011]

26   **Bilbao I**, Sapisochin G, Dopazo C, Lazaro JL, Pou L, Castells L, Caralt M, Blanco L, Gantxegi A, Margarit C, Charco R. Indications and management of everolimus after liver transplantation. *Transplant Proc* 2009; **41**: 2172-2176 [PMID: 19715864 DOI: 10.1016/j.transproceed.2009.06.087]

27   **Zaghla H**, Selby RR, Chan LS, Kahn JA, Donovan JA, Jabbour N, Genyk Y, Mateo R, Gagandeep S, Sher LS, Ramicone E, Fong TL. A comparison of sirolimus vs. calcineurin inhibitor-based immunosuppressive therapies in liver transplantation. *Aliment Pharmacol Ther* 2006; **23**: 513-520 [PMID: 16441472 DOI: 10.1111/j.1365-2036.2006.02770.x]

28   **Zimmerman MA**, Trotter JF, Wachs M, Bak T, Campsen J, Skibba A, Kam I. Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 633-638 [PMID: 18324656 DOI: 10.1002/lt.21420]

29   **Dunkelberg JC**, Trotter JF, Wachs M, Bak T, Kugelmas M, Steinberg T, Everson GT, Kam I. Sirolimus as primary immunosuppression in liver transplantation is not associated with hepatic artery or wound complications. *Liver Transpl* 2003; **9**: 463-468 [PMID: 12740787 DOI: 10.1053/jlts.2003.50079]

30   **Sayin B**, Karakayali H, Colak T, Sevmis S, Pehlivan S, Demirhan B, Haberal M. Conversion to sirolimus for chronic allograft nephropathy and calcineurin inhibitor toxicity and the adverse effects of sirolimus after conversion. *Transplant Proc* 2009; **41**: 2789-2793 [PMID: 19765436 DOI: 10.1016/j.transproceed.2009.07.094]

31   **De Simone P**, Carrai P, Precisi A, Petruccelli S, Baldoni L, Balzano E, Ducci J, Caneschi F, Coletti L, Campani D, Filipponi F. Conversion to everolimus monotherapy in maintenance liver transplantation: feasibility, safety, and impact on renal function. *Transpl Int* 2009; **22**: 279-286 [PMID: 19054383 DOI: 10.1111/j.1432-2277.2008.00768.x]

32   **Alamo JM**, Barrera L, Casado MD, Bernal C, Marin LM, Suarez G, Sanchez-Moreno L, Jimenez R, Suarez-Grau JM, Sousa JM, Cordero E, Gomez-Bravo MA. Efficacy, tolerance, and safety of mammalian target of rapamycin inhibitors as rescue immunosuppressants in liver transplantation. *Transplant Proc* 2009; **41**: 2181-2183 [PMID: 19715866 DOI: 10.1016/j.transproceed.2009.06.083]

33   **Levy G**, Schmidli H, Punch J, Tuttle-Newhall E, Mayer D, Neuhaus P, Samuel D, Nashan B, Klempnauer J, Langnas A, Calmus Y, Rogiers X, Abecassis M, Freeman R, Sloof M, Roberts J, Fischer L. Safety, tolerability, and efficacy of everolimus in de novo liver transplant recipients: 12- and 36-month results. *Liver Transpl* 2006; **12**: 1640-1648 [PMID: 16598777 DOI: 10.1002/lt.20707]

34   **Masetti M**, Rompianesi G, Montalti R, Romano A, Spaggiari M, Ballarin R, Guerrini GP, Gerunda GE. Effects of everolimus monotherapy on hematological parameters and iron homeostasis in de novo liver transplant recipients: preliminary results. *Transplant Proc* 2008; **40**: 1947-1949 [PMID: 18675097 DOI: 10.1016/j.transproceed.2008.05.068]

35   **Beckebaum S**, Iacob S, Klein CG, Dechêne A, Varghese J, Baba HA, Sotiropoulos GC, Paul A, Gerken G, Cicinnati VR. Assessment of allograft fibrosis by transient elastography and noninvasive biomarker scoring systems in liver transplant patients. *Transplantation* 2010; **89**: 983-993 [PMID: 20335832 DOI: 10.1097/TP.0b013e3181cc66ca]

36 **Pissaia A**, Borderie D, Bernard D, Scatton O, Calmus Y, Conti F. APRI and FIB-4 Scores Are Useful After Liver Transplantation Independently of Etiology. *Transplant Proc* 2009; **41**: 679-681 [PMID: 19328955 DOI: 10.1016/j.transproceed.2008.12.014]

37 **Cholongitas E**, Tsochatzis E, Goulis J, Burroughs AK. Noninvasive tests for evaluation of fibrosis in HCV recurrence after liver transplantation: a systematic review. *Transpl Int* 2010; **23**: 861-870 [PMID: 20704691 DOI: 10.1111/j.1432-2277.2010.01142.x]

38 **Carrión JA**, Fernández-Varo G, Bruguera M, García-Pagán JC, García-Valdecasas JC, Pérez-Del-Pulgar S, Forns X, Jiménez W, Navasa M. Serum fibrosis markers identify patients with mild and progressive hepatitis C recurrence after liver transplantation. *Gastroenterology* 2010; **138**: 147-158.e1 [PMID: 19786026 DOI: 10.1053/j.gastro.2009.09.047]

39 **Micheloud D**, Salcedo M, Bañares R, Rincón D, Lorente R, Muñoz-Fernández MA, Resino S. Serum levels of fibrosis biomarkers measured early after liver transplantation are associated with severe hepatitis C virus recurrence. *Transpl Infect Dis* 2009; **11**: 183-188 [PMID: 19254326 DOI: 10.1111/j.1399-3062.2009.00370.x]

40 **Jarcuska P**, Janicko M, Veselíny E, Jarcuska P, Skladaný L. Circulating markers of liver fibrosis progression. *Clin Chim Acta* 2010; **411**: 1009-1017 [PMID: 20399764 DOI: 10.1016/j.cca.2010.04.009]

41 **Fontana RJ**, Dienstag JL, Bonkovsky HL, Sterling RK, Naishadham D, Goodman ZD, Lok AS, Wright EC, Su GL. Serum fibrosis markers are associated with liver disease progression in non-responder patients with chronic hepatitis C. *Gut* 2010; **59**: 1401-1409 [PMID: 20675691 DOI: 10.1136/gut.2010.207423]

42 **Leroy V**. Other non-invasive markers of liver fibrosis. *Gastroenterol Clin Biol* 2008; **32**: 52-57 [PMID: 18973846 DOI: 10.1016/S0399-8320(08)73993-9]

43 **Vergniol J**, de Lédinghen V. [Transient elastography (FibroScan): a new tool in hepatology]. *Presse Med* 2009; **38**: 1516-1525 [PMID: 19195821 DOI: 10.1016/j.lpm.2008.08.018]

44 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798 [PMID: 16823833 DOI: 10.1002/lt.20857]

45 **Olthoff KM**, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, Shaked A, Christie JD. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010; **16**: 943-949 [PMID: 20677285 DOI: 10.1002/lt.22091]

46 Summary of the 2007 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines for the management of arterial hypertension. *Vasc Health Risk Manag* 2007; **3**: 783-795 [PMID: 18200799]

47 **Lorenzo C**, Haffner SM. Performance characteristics of the new definition of diabetes: the insulin resistance atherosclerosis study. *Diabetes Care* 2010; **33**: 335-337 [PMID: 19880585 DOI: 10.2337/dc09-1357]

48 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

49 **Parsian H**, Rahimipour A, Nouri M, Somi MH, Qujeq D, Fard MK, Agcheli K. Serum hyaluronic acid and laminin as biomarkers in liver fibrosis. *J Gastrointestin Liver Dis* 2010; **19**: 169-174 [PMID: 20593050]

50 **Rahimi RA**, Andrianifahanana M, Wilkes MC, Edens M, Kottom TJ, Blenis J, Leof EB. Distinct roles for mammalian target of rapamycin complexes in the fibroblast response to transforming growth factor-beta. *Cancer Res* 2009; **69**: 84-93 [PMID: 19117990 DOI: 10.1158/0008-5472.CAN-08-2146]

51 **Liu Y**, Wen XM, Lui EL, Friedman SL, Cui W, Ho NP, Li L, Ye T, Fan ST, Zhang H. Therapeutic targeting of the PDGF and TGF-beta-signaling pathways in hepatic stellate cells by PTK787/ZK22258. *Lab Invest* 2009; **89**: 1152-1160 [PMID: 19668241 DOI: 10.1038/labinvest.2009.77]

52 **Moustakas A**, Heldin CH. Non-Smad TGF-beta signals. *J Cell Sci* 2005; **118**: 3573-3584 [PMID: 16105881 DOI: 10.1242/jcs.02554]

53 **Zhu J**, Wu J, Frizell E, Liu SL, Bashey R, Rubin R, Norton P, Zern MA. Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. *Gastroenterology* 1999; **117**: 1198-1204 [PMID: 10535884 DOI: 10.1016/S0016-5085(99)70406-3]

54 **Neef M**, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. *J Hepatol* 2006; **45**: 786-796 [PMID: 17050028 DOI: 10.1016/j.jhep.2006.07.030]

55 **Russo MW**, Firpi RJ, Nelson DR, Schoonhoven R, Shrestha R, Fried MW. Early hepatic stellate cell activation is associated with advanced fibrosis after liver transplantation in recipients with hepatitis C. *Liver Transpl* 2005; **11**: 1235-1241 [PMID: 16184580 DOI: 10.1002/lt.20432]

56 **Asthana S**, Toso C, Meeberg G, Bigam DL, Mason A, Shapiro J, Kneteman NM. The impact of sirolimus on hepatitis C recurrence after liver transplantation. *Can J Gastroenterol* 2011; **25**: 28-34 [PMID: 21258665]

RETROSPECTIVE STUDY

Impact of transplant nephrectomy on peak PRA levels and outcome after kidney re-transplantation

Dietlind Tittelbach-Helmrich, Przemyslaw Pisarski, Gerd Offermann, Marcel Geyer, Oliver Thomusch, Ulrich Theodor Hopt, Oliver Drognitz

Dietlind Tittelbach-Helmrich, Przemyslaw Pisarski, Oliver Thomusch, Ulrich Theodor Hopt, Oliver Drognitz, Department of General Surgery, University of Freiburg, 79106 Freiburg i. Brsg., Germany
Dietlind Tittelbach-Helmrich, Department of General Surgery, University of Luebeck, 23538 Luebeck, Germany
Gerd Offermann, Transplant Center, Medical Department IV, Campus Benjamin Franklin, University Charité of Berlin, 12200 Berlin, Germany
Marcel Geyer, Department of Nephrology, University of Freiburg, 79106 Freiburg i. Brsg., Germany
Author contributions: Drognitz O designed the study; Gerd Offermann G and Tittelbach-Helmrich D collected and analyzed the data and wrote the paper; Geyer M and Thomusch O were involved in interpretation of data and editing the manuscript; Pisarski P and Hopt UT were involved in the design of the study and revised the paper critically.
Correspondence to: Dr. Dietlind Tittelbach-Helmrich, Department of General Surgery, University of Luebeck, Ratzeburger Allee 160, 23538 Luebeck,
Germany. dietlind.tittelbach-helmrich@uksh.de
Telephone: +49-451-5002001 Fax: +49-451-5002069
Received: November 27, 2013 Revised: April 16, 2014
Accepted: May 16, 2014
Published online: June 24, 2014

Abstract

AIM: To determine the impact of transplant nephrectomy on peak panel reactive antibody (PRA) levels, patient and graft survival in kidney re-transplants.

METHODS: From 1969 to 2006, a total of 609 kidney re-transplantations were performed at the University of Freiburg and the Campus Benjamin Franklin of the University of Berlin. Patients with PRA levels above (5%) before first kidney transplantation were excluded from further analysis ($n = 304$). Patients with graft nephrectomy ($n = 245$, NE+) were retrospectively compared to 60 kidney re-transplants without prior graft nephrectomy (NE-).

RESULTS: Peak PRA levels between the first and the second transplantation were higher in patients undergoing graft nephrectomy ($P = 0.098$), whereas the last PRA levels before the second kidney transplantation did not differ between the groups. Age adjusted survival for the second kidney graft, censored for death with functioning graft, were comparable in both groups. Waiting time between first and second transplantation did not influence the graft survival significantly in the group that underwent nephrectomy. In contrast, patients without nephrectomy experienced better graft survival rates when re-transplantation was performed within one year after graft loss ($P = 0.033$). Age adjusted patient survival rates at 1 and 5 years were 94.1% and 86.3% *vs* 83.1% and 75.4% group NE+ and NE-, respectively ($P < 0.01$).

CONCLUSION: Transplant nephrectomy leads to a temporary increase in PRA levels that normalize before kidney re-transplantation. In patients without nephrectomy of a non-viable kidney graft timing of re-transplantation significantly influences graft survival after a second transplantation. Most importantly, transplant nephrectomy is associated with a significantly longer patient survival.

Core tip: In our paper, presented as "poster of distinction" at the ATC, we show that graft nephrectomy of a first non-functioning kidney graft leads to an increase in peak panel reactive antibody that normalizes before re-transplantation. In 305 low-risk patients who underwent re-transplantation, graft survival did not differ between those with or without prior nephrectomy. Interestingly, patient survival was significantly better in patients with nephrectomy. This supports the find-

ings of Ayus *et al*, who investigated patients staying on maintenance dialysis after graft failure. Therefore graft nephrectomy should be considered in patients returning to dialysis after failure of a kidney transplant.

INTRODUCTION

Kidney transplantation is the therapy of choice for patients suffering from end-stage renal failure. Due to improvements in immunosuppressive therapy and operative technique, contemporary graft survival rates in first deceased donor transplants have reached 90% after one year and 68% after five years, respectively[1]. Patients returning to dialysis after failure of the primary graft have a significantly higher mortality rate compared to patients awaiting their first kidney graft[2]. Repeat kidney transplantation has been shown to offer a significant survival benefit in these cases[3,4]. However, the outcome of repeat kidney transplantation is known to be inferior to primary transplantation[1]. In 2005 18.7% of patients on the waiting list in the United States had been transplanted previously (OPTN/SRTR Annual report 1995-2004) and recent research indicates that the number of patients undergoing kidney retransplantation is growing rapidly[1].

The indication and timing of primary allograft nephrectomy in patients awaiting a secondary renal transplant are still a matter of debate[5]. Graft nephrectomy is a safe procedure in experienced centers. It is associated with perioperative morbidity that depends on the surgical technique used (*e.g.*, extra- *vs* intracapsular) and the indication for nephrectomy. Morbidity ranges from 4% to 48% and encompasses bleeding, infection or, less frequently, injury of iliac vessels[6,7]. Due to perioperative complications some authors recommend not to remove the non-functional kidney until graft associated complications occur[8-11]. However, others advise the routine removal of the failed graft to avoid infection, bleeding, hypertension or erythropoietin resistance due to chronic inflammation[10,11]. The most common practice seems to be nephrectomy after early graft loss, while in patients with graft failure after more than one year, nephrectomy is often exclusively reserved for cases experiencing complications[12-15].

The impact of a non-functioning kidney graft left in situ or graft nephrectomy on antibody production and outcome after secondary renal transplantation remains unclear, although PRA levels in patients undergoing nephrectomy seem to be higher than in patients in which the graft is not removed[16,17].

The aim of this study was to determine the influence

Table 1 Pretransplant demographic data of all patients

Characteristics	NE+	NE	*P*
n	245	60	
Sex (M/F)	158/87	41/19	0.650
Age at 1. Tx (yr; mean)	35.5 ± 13.9	39.2 ± 12.9	0.056
Age at 2. Tx (yr; mean)	41.6 ± 13.3	47.2 ± 13.3	0.004
Date of 1. Tx	09/1969-03/2005	10/1979-09/2002	
Date of 2. Tx	09/1981-12/2005	04/1991-09/2006	

M: Male; F: Female.

of nephrectomy on PRA levels and the outcome after secondary renal transplantations.

MATERIALS AND METHODS

Patients

The records of all retransplant renal allograft recipients at the University of Freiburg and the University of Berlin, Campus Benjamin Franklin, between 1969 and 2006 were reviewed.

In total 609 re-transplantations were performed, of which 305 (50.1%) were included in our study. Inclusion criteria were as follows: second renal transplantation (third or fourth transplantations were excluded from analysis), PRA prior to first kidney transplantation ⩽ 5%, available data on nephrectomy and a minimum of three documented PRA values (before first, between first and second and immediately before second transplantation). Of 305 patients meeting these criteria, 245 patients underwent nephrectomy (NE+) and 60 patients retained their failed first graft (NE-).

The mean age at the time of the first kidney transplantation was 35.5 ± 13.9 years and 39.3 ± 12.8 years for NE+ and NE- patients, respectively (*P* = 0.056). At the time of second transplantation patients were 41.6 ± 13.3 years old in group NE+ and 47.2 ± 13.3 years in the group NE- (*P* = 0.004). Demographic data of patients are shown in Table 1.

The immunosuppressive regimen included steroids plus cyclosporin A (CsA; *n* = 175), CsA plus azathioprine or mycophenolate mofetil (*n* = 106) or other regimens containing tacrolimus or an induction therapy with antibodies (*n* = 22). All patients in the group NE- received CsA for maintenance therapy.

Graft failure was defined as the irreversible loss of graft function with the need to resume dialysis. Immunosuppression (prednisone 5 mg per day) was continued as long as diuresis exceeded 500 mL/d. If urine production fell below 500 mL/d, immunosuppression was discontinued. In group NE-, the non-functioning kidney graft remained in situ, unless patients developed complications (*e.g.*, infections, bleeding or hypertension). Patients in the group NE+ underwent nephrectomy soon after resuming dialysis. Transplant nephrectomy was performed according to the technique described by Rosenthal *et al*[6].

Statistical analysis

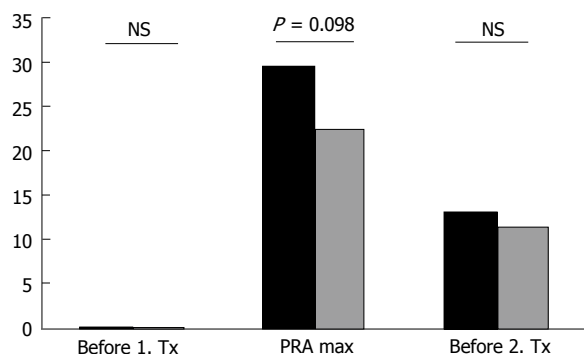Perioperative and follow-up data of patients were gained

Figure 1 Levels of panel reactive antibodies before the first transplantation, peak panel reactive antibodies between first and second transplantation and before second transplantation in the groups NE+ (black) and NE- (grey).
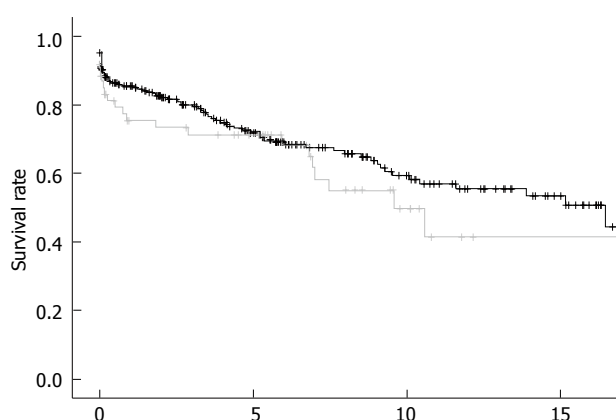

Figure 2 Kidney graft survival of a second renal allograft in patients with (black) or without (grey) prior nephrectomy of a first non-functioning kidney graft, censored for "death with functioning graft".

Table 2 Multivariate Cox regression analysis for graft survival after second renal transplantation

	OR	95%CI	*P*
NE+/-	1.06	0.71-1.56	0.79
PRA before 1. Tx	1.59	1.11-2.30	0.01
PRA max	0.99	0.98-1.00	0.18
PRA before 2. Tx	1.02	1.00-1.04	0.01
Time from 1. Tx to graft loss	0.96	0.88-1.05	0.37
Time from graft loss to nephrectomy	0.89	0.76-1.07	0.22
Time from nephrectomy to 2. Tx	0.89	0.79-1.02	0.09
Time from graft loss to 2. Tx	0.98	0.91-1.06	0.65
Age at 1. Tx	1.01	0.99-1.03	0.45
Age at 2. Tx	1.01	0.99-1.03	0.13

PRA: Peak panel reactive antibody.

retrospectively from electronic health care records or from Eurotransplant Network Information System. Statistical analysis was performed using SPSS. Values are expressed as mean ± SD unless otherwise stated. Patient and graft survival rates were calculated according to the Kaplan-Meier method. Survival rates among both groups were compared using the univariate log-rank analysis. Group comparisons were calculated by independent Students *t* tests. *P* values of < 0.05 were considered significant. Non-significant differences are indicated as ns.

RESULTS

Follow-up data were available for all patients. Mean follow-up was 7.9 years (range 0.3-22.8 years) in the group NE+ and 6.2 years (range 0.4-19.3 years) in the group NE-. Mean waiting time from graft loss to re-transplantation was 3.44 ± 2.68 years in the group NE+ and 2.55 ± 2.55 years in the group NE- (*P* = 0.021). In the group NE+, nephrectomy was performed 0.53 ± 1.47 years after graft loss and 3.05 ± 2.57 years before second transplantation.

The last recorded PRA levels before second transplantation did not differ between groups (Figure 1). In contrast, the mean maximum PRA levels were higher in the group NE+ than in the group NE- (29.7% *vs* 22.5%), although this difference did not reach statistical significance (*P* = 0.09). When comparing the median, the difference in maximum PRA levels reached statistical significance (18.5 in NE+ *vs* 9 in NE-; *P* = 0.038). The maximum PRA level was detected 1.6 ± 1.9 years (NE+) and 0.5 ± 2.9 years (NE-) after graft loss and 2.2 ± 2.3 years (NE+) *vs* 2.1 ± 3.4 years (NE-) before re-transplantation. Maximum PRA levels in the group NE+ were observed at an average of one year after nephrectomy (1.0 ± 2.2 years).

The uni- and multivariate analysis of potential risk factors show that PRA levels measured directly before transplantations were the only factor being associated with a significantly higher risk of graft loss (Table 2).

Graft survival for the entire cohort differed significantly with 1, 5 and 10-year graft survival rates of 81.4%, 62.4% and 46.3% *vs* 66.8%, 59.0% and 30.2% for patients of the groups NE+ and NE- (*P* = 0.01), respectively. However, this advantage disappeared when the analysis was censored for death with a functioning graft (Figure 2).

Graft survival rates after the second kidney transplantation did not differ between patients with early failure of the first graft (within 6 mo) and patients with graft loss occurring later than 6 mo.

To further exclude potential confounding variables, any failure of the second graft within one year after re-transplantation, which is mainly related to technical or early immunological complications, was censored (Figure 3). Graft survival rates at 5 and 10 years did not differ and were 77.4% and 56.9% in the group NE+ and 88.8% and 45.4% in group NE- (*P* = 0.214).

In addition, we evaluated the influence of center-specific factors on graft survival rates due to different immunosuppressive regimens. According to our data, patients on triple immunosuppressive regimens using calcineurin inhibitors (mainly CsA) and azathioprine or MMF and steroids experienced significantly better graft survival rates if compared to patients using only CsA and steroids. The graft survival rates of patients in the groups NE+ and NE-, respectively, receiving the same immunosuppressive regimen did not differ between the two centers.
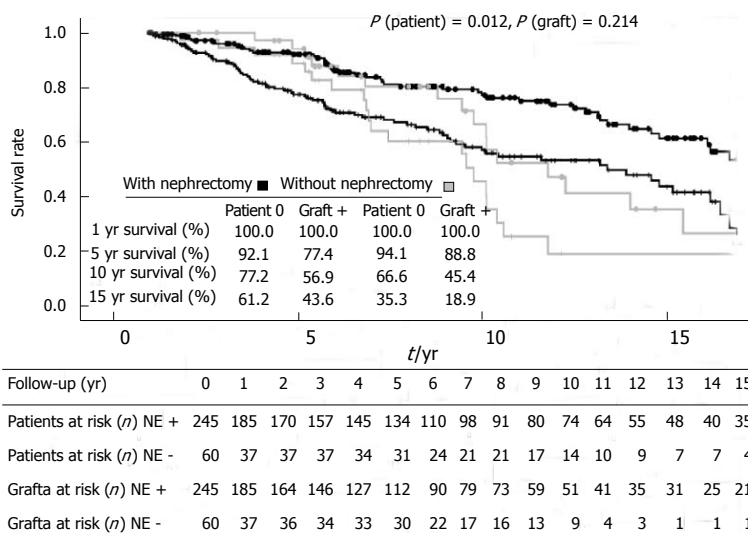
Figure 3 Patient and graft survival, censored for graft loss within 1 year, in second kidney transplants with prior nephrectomy of a non-functioning first kidney graft (black: group NE+) compared to controls without nephrectomy (grey: NE-).

P (patient) = 0.012, P (graft) = 0.214

	With nephrectomy ■		Without nephrectomy ▢	
	Patient 0	Graft +	Patient 0	Graft +
1 yr survival (%)	100.0	100.0	100.0	100.0
5 yr survival (%)	92.1	77.4	94.1	88.8
10 yr survival (%)	77.2	56.9	66.6	45.4
15 yr survival (%)	61.2	43.6	35.3	18.9

Follow-up (yr)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Patients at risk (n) NE +	245	185	170	157	145	134	110	98	91	80	74	64	55	48	40	35
Patients at risk (n) NE -	60	37	37	37	34	31	24	21	21	17	14	10	9	7	7	4
Grafta at risk (n) NE +	245	185	164	146	127	112	90	79	73	59	51	41	35	31	25	21
Grafta at risk (n) NE -	60	37	36	34	33	30	22	17	16	13	9	4	3	1	1	1

Interestingly, in patients undergoing nephrectomy prior to re-transplantation (NE+) the timing of second kidney transplantation (within one year after graft loss *vs* later than one year) did not significantly influence the outcome. In contrast, patients without nephrectomy experienced better graft survival rates when re-transplantation was performed within one year after graft loss ($P = 0.033$) (Figure 4).

Patient survival rates according to the Kaplan-Meier method at 1, 5 and 10 years were 94.1%, 86.3%, 72.2% and 79.2%, 73.1%, 44.1% in the group NE+ and in the group NE-, respectively ($P < 0.01$). Since patients of the group NE- were significantly older than patients in the group NE+, patient survival may have been influenced by differences in age at the time of second transplantation. However, Log-rank analysis of age-adjusted patient survival rates after the exclusion of all patients older than 65 years at time of second transplantation, still revealed a significant survival benefit for patients in the nephrectomy group, compared to patients without nephrectomy (94.1% and 86.3% *vs* 83.1% and 75.4% at 1 and 5 years; $P = 0, < 0.01$)

DISCUSSION

Therapeutic strategies for patients having lost a primary kidney graft and awaiting re-transplantation differ from center to center. Until now, there is no consensus regarding the indication and timing of the removal of a nonviable graft.

It is known that graft and patient survival is worse after second kidney transplantation compared to the first transplantation[1]. Several factors may contribute to this finding: Kidney re-transplants acquire additional waiting time on dialysis after failure of the first transplant which in itself is well known to increase morbidity and mortality after re-transplantation[2,18]. Moreover, patients who undergo repeat renal transplantation are older than at the time of first transplantation and often receive grafts from extended-criteria donors[19-22].

The main finding of our study was a significantly in-

creased patient survival in those second graft recipients who had undergone nephrectomy of their first nonviable graft before receiving a repeat transplantation. This striking effect was observed despite a lack of difference in second kidney graft survival rates between patients who had their first transplant removed before re-transplantation and those who retained their failed graft.

The reasons for the improved survival of repeat transplant candidates who had undergone prior nephrectomy are unclear. However, patients staying on maintenance dialysis after failure of a first kidney graft also show improved survival after graft nephrectomy[23]. The residual non-functioning graft in patients not undergoing nephrectomy may thus be a source of complications in itself or through the need for continued immunosuppressive therapy (*e.g.*, infections or a chronic inflammatory condition).

By analyzing graft survival rates censored for death with functioning graft or graft loss within one year, our results revealed no differences for patients with or without nephrectomy, which is in accordance with recent literature[24]. Therefore, nephrectomy of the failed first kidney graft does not influence survival of the second graft.

Patients considered for re-transplantation are often immunized or even highly immunized due to the development of HLA-specific antibodies to previous transplant antigens. Yong Won Cho showed that panel reactive antibodies are observed more often after graft loss than after blood transfusions or prior pregnancies[25]. Therefore, even with negative complement dependent cytotoxicity crossmatch, these patients are more likely to develop acute humoral rejection episodes[25-27]. This correlates with our findings that higher PRA values before first and second transplantation are associated with an increased risk of graft loss. The impact of the elevated PRA levels in the group NE+ remains unclear but was also observed in other studies[5,28,29]. Our study design precluded information on presensitized patients. Schleicher *et al*[29] could show that in their collective patients undergoing nephrectomy had significantly higher PRA levels at the time of
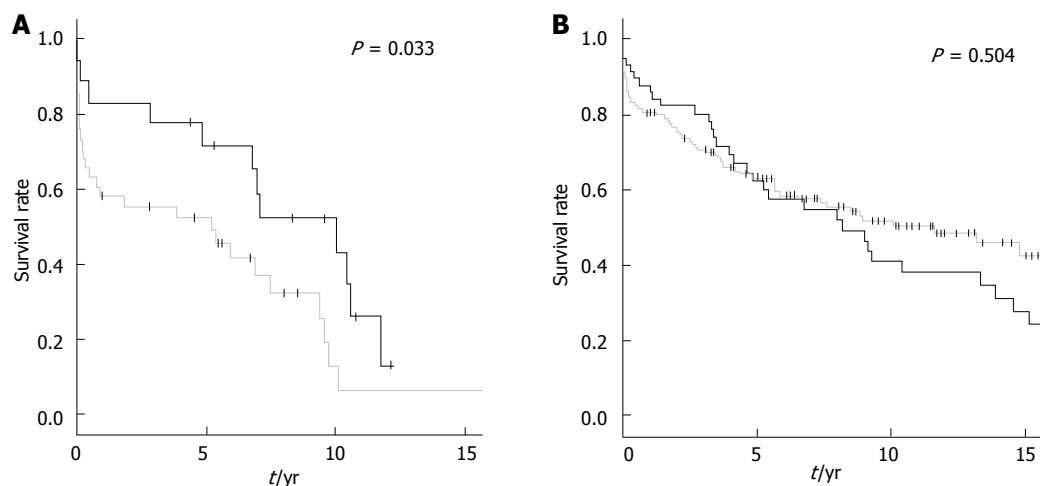
Figure 4 Graft survival of a second renal allograft in patients without (A) or with (B) prior nephrectomy of a first non-functioning kidney graft and retransplantation within (black curve) or later than one year after nephrectomy (grey curve).

retransplantation, which led to a worse graft survival in that group. Especially a PRA level > 70% was an independent risk factor for graft loss. In our study, graft survival did not differ between the groups. This may be due to similar PRA levels before retransplantation. Lucarelli *et al*[30] also did not find a difference in second graft survival in patients with or without prior nephrectomy. They also observed comparable PRA levels in both groups at the time of retransplantation[30].

Intensified immunosuppression may therefore improve graft and patient survival in patients with elevated PRA after a first graft nephrectomy and can also be found in our data considering the different immunosuppressive regimens.

Other authors state that the rise in HLA antibodies after nephrectomy is an expression of the capacity of even a nonfunctional graft to absorb donor specific antibodies or mount an immune response to the donor's MHC antigens. This may protect a second renal graft[31,32]. The graft intolerance syndrome, which leads to chronic inflammatory disease that can be treated by embolization of nonfunctioning renal allografts[33-35], favors the aforementioned hypothesis. However, neither murine and nor human studies could proof these findings[36].

By analyzing graft survival rates censored for death with functioning graft or graft loss within one year, we observed no difference for patients with or without nephrectomy. Therefore, nephrectomy of the failed first kidney graft does not influence survival of the second graft.

Although we observed no influence of prior graft survival, we could confirm the importance of waiting time to retransplantation. In patients undergoing nephrectomy prior to re-transplantation, no difference was evident. In contrast, in patients without nephrectomy, a survival benefit was evident when re-transplantation was performed within one year after graft loss. In our patient group waiting time to retransplantation was about two to three years; in the United States waiting times of more than five years are common[35]. This also needs to be taken into account when considering a graft nephrectomy

with its associated perioperative risk.

This study is limited by its retrospective design and the long timeframe in which patients have undergone transplantation. It still offers novel insights into the advantages of graft nephrectomy on the outcome of secondary kidney transplantation.

In a conclusion, Nephrectomy of a nonfunctioning kidney graft prior to re-transplantation is a save procedure in experienced centers that, despite a temporary increase in PRA levels, results in significantly better patient survival. Therefore transplant nephrectomy should be considered in all patients awaiting a kidney re-transplantation.

COMMENTS

Background
Kidney transplantation is the treatment of choice for patients with end-stage renal disease. Despite excellent results, the half-live after deceased or living donor transplantation was 8.8 and 11.9 years after transplantation in 2005 in the United States, the management of patients with graft failure is still under debate. Some authors favor removal of the non viable kidney to prevent complications such as infection or chronic inflammatory response, others recommend to leave the nonfunctioning kidney in order to prevent surgery associated complications and a rise in panel reactive antibodies.

Research frontiers
There are many studies showing that panel reactive antibodies rise after the removal of a non viable kidney transplant. The long-term outcome concerning morbidity and mortality of patients as well as the outcome of a second kidney transplant after graft nephrectomy remains unclear. Prior studies demonstrated controversial results regarding complication rates and mortality with or without nephrectomy in patients staying on dialysis after graft failure or undergoing secondary renal transplantation.

Innovations and breakthroughs
Nephrectomy of a non-viable kidney graft leads to a temporary increase in panel reactive antibodies (PRA) level which equalizes before the time of retransplantation. Graft survival after a second kidney transplantation is not influenced by nephrectomy of the first graft. If nephrectomy is not performed, re-transplantation should be undertaken within one year after graft loss due to significantly better graft survival rates. Most importantly, patient survival one or five years after a second kidney transplantation is significantly better in patients undergoing nephrectomy of the first failed graft.

Applications
The study results suggest that in patients with graft failure nephrectomy should be

considered due to a better patient survival after a second renal transplantation.

Terminology
Kidney or renal transplantation is the process of transferring a kidney of a deceased or living donor to a patient with end-stage renal disease, including not only the surgical procedure but also the immunological management. Graft survival is the rate of kidney transplants that remains with good function after a certain time period. PRA are pre-existing antibodies against cell proteins, which present, if elevated, a risk factor for rejection after organ transplantation.

Peer review
The article aims to determine the impact of transplant nephrectomy on peak panel reactive antibody levels and patient and graft survival in kidney re-transplants. It is conducted as a retrospective study in a large patient cohort and with a long follow-up. The article is very interesting for anybody involved in the care of renal transplant patients since it offers new insights into in the dilemma of management of patients with graft loss and the usefulness of transplant nephrectomy prior to re-transplantation.

REFERENCES

1   **Magee JC**, Barr ML, Basadonna GP, Johnson MR, Mahade-van S, McBride MA, Schaubel DE, Leichtman AB. Repeat organ transplantation in the United States, 1996-2005. *Am J Transplant* 2007; **7**: 1424-1433 [PMID: 17428290 DOI: 10.1111/j.1600-6143.2007.01786.x]

2   **Rao PS**, Schaubel DE, Jia X, Li S, Port FK, Saran R. Survival on dialysis post-kidney transplant failure: results from the Scientific Registry of Transplant Recipients. *Am J Kidney Dis* 2007; **49**: 294-300 [PMID: 17261432 DOI: 10.1053/j.ajkd.2006.11.022]

3   **Rao PS**, Schaubel DE, Wei G, Fenton SS. Evaluating the survival benefit of kidney retransplantation. *Transplantation* 2006; **82**: 669-674 [PMID: 16969291 DOI: 10.1097/01.tp.0000235434.13327.11]

4   **Ojo A**, Wolfe RA, Agodoa LY, Held PJ, Port FK, Leavey SF, Callard SE, Dickinson DM, Schmouder RL, Leichtman AB. Prognosis after primary renal transplant failure and the beneficial effects of repeat transplantation: multivariate analyses from the United States Renal Data System. *Transplantation* 1998; **66**: 1651-1659 [PMID: 9884254 DOI: 10.1097/00007890-199812270-00014]

5   **Johnston O**, Rose C, Landsberg D, Gourlay WA, Gill JS. Nephrectomy after transplant failure: current practice and outcomes. *Am J Transplant* 2007; **7**: 1961-1967 [PMID: 17617860 DOI: 10.1111/j.1600-6143.2007.01884.x]

6   **Rosenthal JT**, Peaster ML, Laub D. The challenge of kidney transplant nephrectomy. *J Urol* 1993; **149**: 1395-1397 [PMID: 8501774]

7   **Secin FP**, Rovegno AR, del Rosario Brunet M, Marrugat RE, Dávalos Michel M, Fernandez H. Cumulative incidence, indications, morbidity and mortality of transplant nephrectomy and the most appropriate time for graft removal: only nonfunctioning transplants that cause intractable complications should be excised. *J Urol* 2003; **169**: 1242-1246 [PMID: 12629335 DOI: 10.1097/01.ju.0000050658.94353.24]

8   **Martínez Urrutia MJ**, López Pereira P, Perdiguero M, Gutiérrez E, Gar Jaureguizar Monereo E. [Problems associated with nephrectomy of transplanted kidneys]. *Cir Pediatr* 1995; **8**: 151-154 [PMID: 8679390]

9   **O'Sullivan DC**, Murphy DM, McLean P, Donovan MG. Transplant nephrectomy over 20 years: factors involved in associated morbidity and mortality. *J Urol* 1994; **151**: 855-858 [PMID: 8126809]

10 **Gregoor PJ**, Kramer P, Weimar W, van Saase JL. Infections after renal allograft failure in patients with or without low-dose maintenance immunosuppression. *Transplantation* 1997; **63**: 1528-1530 [PMID: 9175823 DOI: 10.1097/00007890-199705270-00028]

11 **López-Gómez JM**, Pérez-Flores I, Jofré R, Carretero D, Rodríguez-Benitez P, Villaverde M, Pérez-García R, Nassar GM, Niembro E, Ayus JC. Presence of a failed kidney transplant in patients who are on hemodialysis is associated with chronic inflammatory state and erythropoietin resistance. *J Am Soc Nephrol* 2004; **15**: 2494-2501 [PMID: 15340000 DOI: 10.1097/01.ASN.0000137879.97445.6E]

12 **Casali R**, Simmons RL, Ferguson RM, Mauer MM, Kjellstrand CM, Buselmeier TJ, Najarian JS. Factors related to success or failure of second renal transplants. *Ann Surg* 1976; **184**: 145-154 [PMID: 782386 DOI: 10.1097/00000658-197608000-00002]

13 **Cacciarelli T**, Sumrani N, Delaney V, Hong JH, DiBenedetto A, Sommer BG. The influence of delayed renal allograft function on long-term outcome in the cyclosporine era. *Clin Nephrol* 1993; **39**: 335-339 [PMID: 8334761 DOI: 10.1097/00007890-199201000-00009]

14 **Chiverton SG**, Murie JA, Allen RD, Morris PJ. Renal transplant nephrectomy. *Surg Gynecol Obstet* 1987; **164**: 324-328 [PMID: 3551155]

15 **Zargar MA**, Kamali K. Reasons for transplant nephrectomy: a retrospective study of 60 cases. *Transplant Proc* 2001; **33**: 2655-2656 [PMID: 11498109 DOI: 10.1016/S0041-1345(01)02134-0]

16 **Douzdjian V**, Rice JC, Carson RW, Gugliuzza KK, Fish JC. Renal retransplants: effect of primary allograft nephrectomy on early function, acute rejection and outcome. *Clin Transplant* 1996; **10**: 203-208 [PMID: 8664520]

17 **Abouljoud MS**, Deierhoi MH, Hudson SL, Diethelm AG. Risk factors affecting second renal transplant outcome, with special reference to primary allograft nephrectomy. *Transplantation* 1995; **60**: 138-144 [PMID: 7624955 DOI: 10.1097/00007890-199507270-00005]

18 **Gill JS**, Abichandani R, Kausz AT, Pereira BJ. Mortality after kidney transplant failure: the impact of non-immunologic factors. *Kidney Int* 2002; **62**: 1875-1883 [PMID: 12371992 DOI: 10.1046/j.1523-1755.2002.00640.x]

19 **Gjertson DW**. A multi-factor analysis of kidney regraft outcomes. *Clin Transpl* 2002; 335-349 [PMID: 12971460]

20 **Miles CD**, Schaubel DE, Jia X, Ojo AO, Port FK, Rao PS. Mortality experience in recipients undergoing repeat transplantation with expanded criteria donor and non-ECD deceased-donor kidneys. *Am J Transplant* 2007; **7**: 1140-1147 [PMID: 17331109 DOI: 10.1111/j.1600-6143.2007.01742.x]

21 **Howard RJ**, Reed AI, Van Der Werf WJ, Hemming AW, Patton PR, Scornik JC. What happens to renal transplant recipients who lose their grafts? *Am J Kidney Dis* 2001; **38**: 31-35 [PMID: 11431178 DOI: 10.1053/ajkd.2001.25178]

22 **Kaplan B**, Meier-Kriesche HU. Death after graft loss: an important late study endpoint in kidney transplantation. *Am J Transplant* 2002; **2**: 970-974 [PMID: 12482151 DOI: 10.1034/j.1600-6143.2002.21015.x]

23 **Ayus JC**, Achinger SG, Lee S, Sayegh MH, Go AS. Transplant nephrectomy improves survival following a failed renal allograft. *J Am Soc Nephrol* 2010; **21**: 374-380 [PMID: 19875809 DOI: 10.1681/ASN.2009050480]

24 **Surga N**, Viart L, Wetzstein M, Mazouz H, Collon S, Tillou X. Impact of renal graft nephrectomy on second kidney transplant survival. *Int Urol Nephrol* 2013; **45**: 87-92 [PMID: 23328966 DOI: 10.1007/s11255-012-0369-8]

25 **Bryan CF**, Baier KA, Nelson PW, Luger AM, Martinez J, Pierce GE, Ross G, Shield CF, Warady BA, Aeder MI, Helling TS, Muruve N. Long-term graft survival is improved in cadaveric renal retransplantation by flow cytometric cross-matching. *Transplantation* 1998; **66**: 1827-1832 [PMID: 9884283 DOI: 10.1097/00007890-199812270-00043]

26 **Mahoney RJ**, Norman DJ, Colombe BW, Garovoy MR, Leeber DA. Identification of high- and low-risk second kidney grafts. *Transplantation* 1996; **61**: 1349-1355 [PMID: 8629295 DOI: 10.1097/00007890-199605150-00011]

27 **Scornik JC**. The flow cytometry crossmatch in second kidney grafts. *Transplantation* 1996; **62**: 1698-1700 [PMID: 8970637 DOI: 10.1097/00007890-199612150-00035]

28 **Khakhar AK**, Shahinian VB, House AA, Muirhead N, Hol-

lomby DJ, Leckie SH, McAlister VC, Chin JL, Jevnikar AM, Luke PP. The impact of allograft nephrectomy on percent panel reactive antibody and clinical outcome. *Transplant Proc* 2003; **35**: 862-863 [PMID: 12644168 DOI: 10.1016/S0041-1345(02)04031-9]

29   **Schleicher C**, Wolters H, Kebschull L, Anthoni C, Suwelack B, Senninger N, Mersfeld B, Palmes D. Impact of failed allograft nephrectomy on initial function and graft survival after kidney retransplantation. *Transpl Int* 2011; **24**: 284-291 [PMID: 21155899 DOI: 10.1111/j.1432-2277.2010.01197.x]

30   **Lucarelli G**, Vavallo A, Bettocchi C, Losappio V, Gesualdo L, Grandaliano G, Selvaggi FP, Battaglia M, Ditonno P. Impact of transplant nephrectomy on retransplantation: a single-center retrospective study. *World J Urol* 2013; **31**: 959-963 [PMID: 23152125 DOI: 10.1007/s00345-012-0986-y]

31   **Lucchiari N**, Panajotopoulos N, Xu C, Rodrigues H, Ianhez LE, Kalil J, Glotz D. Antibodies eluted from acutely rejected renal allografts bind to and activate human endothelial cells. *Hum Immunol* 2000; **61**: 518-527 [PMID: 10773356 DOI: 10.1016/S0198-8859(00)00109-9]

32   **Martin L**, Guignier F, Bocrie O, D'Athis P, Rageot D, Rifle G, Justrabo E, Mousson C. Detection of anti-HLA antibodies with flow cytometry in needle core biopsies of renal transplants recipients with chronic allograft nephropathy. *Transplantation* 2005; **79**: 1459-1461 [PMID: 15912120 DOI: 10.1097/01.TP.0000156164.54216.DE]

33   **Pérez Martínez J**, Gallego E, Juliá E, Llamas F, López A, Palao F, Lorenzo I, López E, Illescas ML, Gómez Roldán C. Embolization of non-functioning renal allograft: efficacy and control of systemic inflammation. *Nefrologia* 2005; **25**: 422-427 [PMID: 16231510]

34   **Solinas A**, De Giorgi F, Frongia M. Ablation of non functioning renal allograft by embolization: a valid alternative to graft nephrectomy? *Arch Ital Urol Androl* 2005; **77**: 99-102 [PMID: 16146270]

35   **Morales A**, Gavela E, Kanter J, Beltrán S, Sancho A, Escudero V, Crespo J, Pallardó LM. Treatment of renal transplant failure. *Transplant Proc* 2008; **40**: 2909-2911 [PMID: 19010144 DOI: 10.1016/j.transproceed.2008.09.047]

36   **Lair D**, Coupel S, Giral M, Hourmant M, Karam G, Usal C, Bignon JD, Brouard S, Soulillou JP. The effect of a first kidney transplant on a subsequent transplant outcome: an experimental. *Kidney International* 2005; **67**: 2368-2375 [DOI: 10.1111/j.1523-1755.2005.00343.x]

CASE REPORT

Intra-abdominal desmoid tumor after liver transplantation: A case report

Vidya A Fleetwood, Shannon Zielsdorf, Sheila Eswaran, Shriram Jakate, Edie Y Chan

Vidya A Fleetwood, Shannon Zielsdorf, Edie Y Chan, Department of General Surgery, Division of Transplantation Surgery, Rush University Medical Center, Chicago, IL 60612, United States

Sheila Eswaran, Department of Medicine, Section of Hepatology, Rush University Medical Center, Chicago, IL 60612, United States

Shriram Jakate, Department of Pathology, Rush University Medical Center, Chicago, IL 60612, United States

Author contributions: Fleetwood VA contributed to primary content, manuscript creation and revisions, literature review; Zielsdorf S contributed to literature review, manuscript revisions; Eswaran S contributed to manuscript revisions, follow-up information, chart review; Jakate S contributed to manuscript revisions, pathology review, immunostaining; Chan EY contributed to manuscript revisions, language editing, preceptorship.

Correspondence to: Vidya Fleetwood, MD, Department of General Surgery, Division of Transplantation Surgery, Rush University Medical Center, Jelke Bldg Suite 785, 1750 West Harrison St, Chicago, IL 60612,
United States. vidyaratna_a_fleetwood@rush.edu
Telephone: +1-843-6873783   Fax: +1-312-9422867
Received: March 11, 2014   Revised: May 6, 2014
Accepted: June 10, 2014
Published online: June 24, 2014

Abstract

We are reporting the first documented case of an abdominal desmoid tumor presenting primarily after liver transplantation. This tumor, well described in the literature as occurring both in conjunction with familial adenomatous polyposis as well as in the post-surgical patient, has never been noted after solid organ transplantation and was therefore not included in our differential upon presentation. Definitive diagnosis required the patient to undergo surgical excision and immunochemical staining of the mass for confirmation. A review of the literature showed no primary tumors after transplantation. In a population of patients who received a small bowel transplant after they developed short gut post radical resection of aggressive fibromatosis, only rare recurrences were seen. No connection of tumor development with immunosuppression or need to decrease immunosuppressant treatment has been demonstrated in these patients. Our case and the literature show the risk of this tumor presenting in the post-transplantation patient and the need for a high index of suspicion in patients who present with a complex mass after transplantation to prevent progression of the disease beyond a resectable lesion. Results of a thorough search of the literature are detailed and the medical and surgical management of both resectable and unresectable lesions is reviewed.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Desmoid; Intra-abdominal fibromatosis; Immunosuppression; Liver transplantation; Solid organ transplantation; Recurrence

Core tip: Desmoid tumor is a soft tissue tumor seen primarily after surgical resection. A high index of suspicion is necessary as delayed diagnosis can cause significant morbidity with resection. This case presents the first observed desmoid after liver transplantation as well as a literature search detailing the observed desmoid presentations in the context of immunosuppression.

INTRODUCTION

We present the first documented case of desmoid tumor appearing after solid organ transplantation. Desmoid

Figure 1 Desmoid tumor at initial presentation.

Figure 2 Immunostained slide of tumor histology, demonstrating low cellularity tumor in a myxoid matrix with positive beta-catenin staining.

tumors are a rare malignancy characterized by benign histology and aggressive local recurrence. Incidence and recurrence of desmoids in patients who have undergone transplantation and effects of immunosuppression on desmoid development have not yet been studied.

CASE REPORT

A 60-year-old male presented to our clinic with a three day history of right upper quadrant pain. He noted two months of fatigue and a recent history of diarrhea, resolved at admission. He denied nausea, vomiting, fevers, chills, or weight loss. Medical history was notable Hepatitis C cirrhosis status post orthotopic liver transplant approximately six months prior, as well as type Ⅱ diabetes mellitus. Postoperatively, he received basiliximab on the day of transplant and on postoperative day 4 for induction along with a methylprednisolone taper in the days immediately post transplantation. He was maintained on cyclosporine and had previously shown no signs of graft rejection with historically appropriate levels of immunosuppressive therapy. He denied alcohol or drug abuse.

Physical exam was remarkable for tenderness to palpation over the right upper quadrant with a new marked right upper quadrant mass. Laboratory measurements revealed a white blood cell count of 4.32×10^9/L (reference range 1.5-10.5 $\times 10^9$/L) with a normal differential, hemoglobin of 11.1 g/dL (reference range 13.5-17.5 g/dL), and creatinine of 1.29 mg/dL (reference range 0.75-1.2 mg/dL). Alkaline phosphatase was elevated at 281 mmol/L (reference range 30-125 mmol/L) and the transaminases and total bilirubin were within normal limits. Cyclosporine levels were within therapeutic ranges (100-150 ng/mL). He had received a colonoscopy at an outside institution two years prior significant only for benign polyps. Alfa-fetoprotein measurement was within normal limits (< 10 ng/mL). Computed tomography imaging showed a 13 cm multi-loculated heterogeneous fluid collection with hyperdense and hypodense components inferior to right hepatic tip with multiple cystic locules (Figure 1). The final radiologic report was read as "large complex multiloculated subhepatic peritoneal 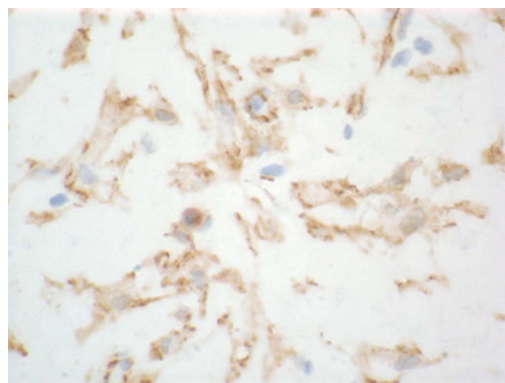collection(s), likely multiloculated hematoma(s) as well as adjacent loculated hemoperitoneum. The adjacent mesentery now demonstrates amorphous enhancement and therefore superimposed infection/phlegmon cannot be excluded. These collection(s) are essentially new since the prior study (previously mild hemoperitoneum was present in the subhepatic region and both paracolic gutters).

Based on the concern for infected hematoma, the patient was sent for placement of an interventional radiology drain into loculated fluid collection. Fluid pathology and cultures were nondiagnostic. The drain was placed under imaging guidance but was clearly unable to access loculated areas due to solid components interfering with catheter passage. As his symptoms were persistent and mass was well visualized with computed tomography, no further imaging appeared warranted; he was taken to the operating room, where exploratory laparotomy was performed. Intra-operatively the infrahepatic mass was noted to be white, inflamed, and fibrotic. Ten centimeters of small bowel and the mesentery were noted to be inseparably adhered to mass and inflamed, and they were resected en bloc with the mass. Grossly negative margins at abdominal wall and the involved small bowel were achieved but microscopic positivity was confirmed by frozen section in the posterior portion of the mass adjacent to the retroperitoneum. Final pathology returned with a low cellularity tumor with myofibroblasts in disarray in a myxoid matrix, beta-catenin stain positive (Figure 2), consistent with desmoid fibromatosis.

Our patient tolerated the procedure well and was discharged home at prior functional status within ten days. Repeat imaging has been negative for signs of recurrence (Figure 3) in the 23 mo in which he has followed up since resection. He has been started on treatment for chronic hepatitis C with sobosfuvir and simeprevir therapy.

DISCUSSION

Desmoid tumors are rare tumors which fall into two types: sporadic and those associated with familial adenomatous polyposis. The incidence of desmoid is less than three percent of soft tissue sarcomas and about 0.03% of all malignancies[1]. They appear between 15 and 60 years

Figure 3 Imaging 18 mo after excision.

with a peak age of appearance at 30 years[2]. Both types are characterized by monoclonal, fibroblastic proliferation with 80% rate of positive nuclear and cytoplasmic staining for beta-catenin[3]. They are histologically benign and do not metastasize but are frequently locally invasive and highly recurrent. Only 5% of sporadic desmoid tumors, in contrast with 80% of FAP-associated desmoids, occur intra-abdominally; other locations include extremities and trunk[1]. Both sporadic and FAP-associated desmoids have been well described in the surgical literature as recurring both within the surgical field and intra-compartmentally outside the surgical field. Differential diagnosis encompasses both fibroblastic sarcomas and other reactive fibroblastic process, but these can be distinguished from desmoids as the latter tend to occur with a diagnosis of FAP, nuclear staining for beta-catenin - present in roughly 80% of cases - and screening for mutations of beta-catenin gene, found in approximately 85% of sporadic cases[3]. Factors which prognosticate recurrence have been suggested to be sex[2] and mutations of the beta-catenin gene, the latter of which were found to be associated with significantly higher rates of local recurrence[4].

Treatment for these tumors includes a wide variety of approaches. As these tumors have no metastatic potential, treatment is usually dictated by rapidity of growth and functional considerations such as pain or local obstruction. Surgical management has historically been the first-line treatment of desmoids[5]. Negative margins are generally the goal of surgical intervention; however, for intra-abdominal type especially, morbidity associated with surgery may prevent definitive excision with negative margins. Results have been mixed on whether negative margins were predictive of lower recurrence rate[6]. In a case series including 56 patients with either intra- or extra-abdominal primary disease, microscopically positive margins were associated with an almost fourfold increase in local recurrence compared to microscopically negative margins, but no difference in overall survival was observed[7]. A review of multiple studies addressing margin status concluded that no definitive conclusion could be reached based on available evidence and that negative margins should be strove for if they did not compromise functional status[8].

Adjunctive radiotherapy in patients with positive margins has been explored in depth and shown to result in decreased relapse rates but significant complications, including tissue fibrosis[9]. Radiotherapy has not been strongly evaluated in patients with negative surgical margins, as many patients with negative margins elect not to undergo radiation therapy. In a comparative review of 22 cases, radiation therapy alone demonstrated a local control rate of 78% as opposed to 61% with surgery alone[10]. However, multiple complications were noted and, given the accompanying tissue damage and peak occurrence of desmoids in young patients, it is generally recommended to use radiation therapy only as an adjunct to surgery or for unresectable disease[11].

For unresectable disease, a wide variety of medical treatments have been used, although few have been systematically evaluated. A systematic review of the literature addressed the different strategies noted below[12]. As most desmoid tumors express nuclear estrogen receptor-B, tamoxifen and other anti-estrogens have been used with some anecdotal reports of response; however, this has not been evaluated in a larger series. Non-steroidal anti-inflammatory drugs have also been tested and have demonstrated activity against tumors with partial or complete response. In cases of rapidly growing or symptomatic tumor, cytotoxic agents such as methotrexate and vinblastine have been studied; however, these were evaluated largely in the pediatric population and are associated with high, although tolerable, levels of toxicity[6]. Imatinib is another agent which is currently under study and has shown promise in multiple low-powered studies[13,14] but has not yet been licensed for this indication[6].

No incidences of primary desmoid tumor development have yet been documented in patients who have undergone transplantation of liver or other solid organs. Recurrence of pre-transplant desmoids in patients known to be on immunosuppression, in this case for intestinal transplant, is only addressed in one series. Fourteen patients with desmoid tumors underwent intestinal transplantation, of which three recurred; time interval to recurrence was 15, 17 and 69 mo. Of these patients, eleven were maintained on immunosuppression[15]. In a European study of both intra- and extra-abdominal fibromatosis, recurrence was seen at between 0 and 204 mo in 37 patients with a mean time of 14 to 17 mo[7]. Although these studies show similar time to recurrence, the power of the study addressing the immunosuppressed patient is so low that it is difficult to draw conclusions on the impact of immunosuppressive therapy on recurrence.

Our patient presented with a sporadic primary tumor. We were able to achieve a grossly negative resection but pathology revealed microscopic disease at the margins; he received no adjunctive therapy but has not recurred at 23 mo, suggesting that his immunosuppression has not caused rapid growth or recurrence.

In conclusion, desmoid tumors are a rare disease for which the primary standard of care differs between surgical excision or watchful waiting, depending on extent of involvement of surrounding structures and postoperative morbidity. We presented a hitherto undocumented case of sporadic desmoid tumor after liver transplantation. The patient has no personal or family history of familial adenomatous polyposis. The primary manifestation was treated with surgical excision. No incidences of primary desmoid tumor development have yet been documented in patients who have undergone transplantation of liver or other solid organs. Influence of immunosuppression on the development of desmoids is unknown; on recurrence, poorly studied. Further study would be helpful to elaborate the effect of immunosuppression on development of desmoids and the rates of recurrence after solid organ transplant.

COMMENTS

Case characteristics
A 60-year-old male with a history of liver transplantation presented with right upper quadrant pain.

Clinical diagnosis
Clinical findings were a palpable tender mass in right upper quadrant.

Differential diagnosis
Differential diagnosis includes abscess, malignancy, and recurrent hepatitis.

Laboratory diagnosis
Alkaline phosphatase 281 mmol/L, hemoglobin 11.1 g/dL, and creatinine of 1.29 mg/dL with otherwise normal complete metabolic panel and blood counts.

Imaging diagnosis
CT showed multiloculated, heterogeneous fluid collection with varying densities and cystic components inferior to and distinct from right hepatic tip; scattered periaortic lymph nodes were seen.

Pathological diagnosis
Pathology showed a low cellularity tumor with myofibroblasts in disarray in a myxoid matrix with positive beta-catenin staining.

Treatment
The patient was treated with surgical resection; no adjunctive radiotherapy or chemotherapy was used.

Related reports
This is the first case of desmoid tumor occurring primarily after organ transplantation; one previous case series addressing viability of small bowel transplantation after resection of desmoid tumor addressed recurrence of fibromatosis on immunosuppression but was insufficiently powered to reach a conclusion.

Term explanation
Nuclear beta catenin is a protein which regulates cell-cell adhesion and gene transcription encoded by the *CTNNB1* gene; positive staining has been noted with desmoid tumors as well as with colorectal cancer, hepatocellular carcinoma, and lung cancer.

Experiences and lessons
This case reports the first noted desmoid tumor occurring after solid organ transplantation and presents methods of treatment for both resectable and unresectable lesions as well as describing the literature available on desmoid tumors in immunosuppressed patients.

Peer review
The manuscript entitled, "Intra-abdominal desmoid tumor after liver transplantation: a case report" by Fleetwood *et al*, reported a case of intra-abdominal desmoid tumor developing in a post liver transplant recipient. This is the first case presentation of intraabdominal desmoid tumor after liver transplantation, and worth for publication.

REFERENCES

1   **Shields CJ**, Winter DC, Kirwan WO, Redmond HP. Desmoid tumours. *Eur J Surg Oncol* 2001; **27**: 701-706 [PMID: 11735163 DOI: 10.1053/ejso.2001.1169]

2   **Mankin H**, Hornicek F, Springfield, D. Extra-abdominal desmoid tumors: A report of 234 cases. *J Surg Oncol* 2010; **102**: 380-384 [PMID: 19877160 DOI: 10.1002/jso.21433]

3   **Carlson JW**, Fletcher CD. Immunohistochemistry for beta-catenin in the differential diagnosis of spindle cell lesions: analysis of a series and review of the literature. *Histopathology* 2007; **51**: 509-514 [PMID: 17711447 DOI: 10.1111/j.1365-2559.2007.02794.x]

4   **Dômont J**, Salas S, Lacroix L, Brouste V, Saulnier P, Terrier P, Ranchère D, Neuville A, Leroux A, Guillou L, Sciot R, Collin F, Dufresne A, Blay JY, Le Cesne A, Coindre JM, Bonvalot S, Bénard J. High frequency of beta-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management. *Br J Cancer* 2010; **102**: 1032-1036 [PMID: 20197769 DOI: 10.1038/sj.bjc.6605557]

5   **Shinagare AB**, Ramaiya NH, Jagannathan JP, Krajewski KM, Giardino AA, Butrynski JE, Raut CP. A to Z of desmoid tumors. *AJR Am J Roentgenol* 2011; **197**: W1008-W1014 [PMID: 22109314 DOI: 10.2214/AJR.11.6657]

6   **Kasper B**, Ströbel P, Hohenberger P. Desmoid tumors: clinical features and treatment options for advanced disease. *Oncologist* 2011; **16**: 682-693 [PMID: 21478276 DOI: 10.1634/theoncologist.2010-0281]

7   **Stoeckle E**, Coindre JM, Longy M, Binh MB, Kantor G, Kind M, de Lara CT, Avril A, Bonichon F, Bui BN. A critical analysis of treatment strategies in desmoid tumours: a review of a series of 106 cases. *Eur J Surg Oncol* 2009; **35**: 129-134 [PMID: 18760561 DOI: 10.1016/j.ejso.2008.06.1495]

8   **Melis M**, Zager JS, Sondak VK. Multimodality management of desmoid tumors: how important is a negative surgical margin? *J Surg Oncol* 2008; **98**: 594-602 [PMID: 19072851 DOI: 10.1002/jso.21033]

9   **Jelinek JA**, Stelzer KJ, Conrad E, Bruckner J, Kliot M, Koh W, Laramore GE. The efficacy of radiotherapy as postoperative treatment for desmoid tumors. *Int J Radiat Oncol Biol Phys* 2001; **50**: 121-125 [PMID: 11316554 DOI: 10.1016/S0360-3016(00)01570-4]

10 **Nuyttens JJ**, Rust PF, Thomas CR, Turrisi AT. Surgery versus radiation therapy for patients with aggressive fibromatosis or desmoid tumors: A comparative review of 22 articles. *Cancer* 2000; **88**: 1517-1523 [PMID: 10738207 DOI: 10.1002/(SICI)1097-0142(20000401)88]

11 **National Comprehensive Cancer Network**. NCCN Practice Guidelines in Oncology – Guidelines for Treatment of Sarcoma. Available from: URL: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#sarcoma. Web. 09/13/2013

12 **Janinis J**, Patriki M, Vini L, Aravantinos G, Whelan JS. The pharmacological treatment of aggressive fibromatosis: a systematic review. *Ann Oncol* 2003; **14**: 181-190 [PMID: 12562642 DOI: 10.1093/annonc/mdg064]

13 **Heinrich MC**, McArthur GA, Demetri GD, Joensuu H, Bono P, Herrmann R, Hirte H, Cresta S, Koslin DB, Corless CL, Dirnhofer S, van Oosterom AT, Nikolova Z, Dimitrijevic S, Fletcher JA. Clinical and molecular studies of the effect of imatinib on advanced aggressive fibromatosis (desmoid tumor). *J Clin Oncol* 2006; **24**: 1195-1203 [PMID: 16505440 DOI: 10.1200/JCO.2005.04.0717]

14 **Penel N**, Le Cesne A, Bui BN, Perol D, Brain EG, Ray-Coquard I, Guillemet C, Chevreau C, Cupissol D, Chabaud S, Jimenez M, Duffaud F, Piperno-Neumann S, Mignot L, Blay JY. Imatinib for progressive and recurrent aggressive fibromatosis (desmoid tumors): an FNCLCC/French Sarcoma Group phase II trial with a long-term follow-up. *Ann Oncol*

2011; **22**: 452-457 [PMID: 20622000 DOI: 10.1093/annonc/mdq341]

15   **Moon JI**, Selvaggi G, Nishida S, Levi DM, Kato T, Ruiz P, Bejarano P, Madariaga JR, Tzakis AG. Intestinal transplantation for the treatment of neoplastic disease. *J Surg Oncol* 2005; **92**: 284-291 [PMID: 16299803 DOI: 10.1002/jso.20416]

World Journal of

Transplantation

W J T World Journal of Transplantation

Contents

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Andrew Leon Lobashevsky, MD, PhD, D(ABHI), Associate professor, IUSM, Department of Medicine, Histocompatibility Laboratory Director, Riley Hospital, 702 Barnhill Dr, R#0615, Indianapolis, IN 46033, United States

AIM AND SCOPE

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

    WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

AIM AND SCOPE

World Journal of Transplantation is now indexed in PubMed Central, PubMed, Digital Object Identifier.

FLYLEAF

REVIEW

Methodological aspects of anti-human leukocyte antigen antibody analysis in solid organ transplantation

Andrew L Lobashevsky

Andrew L Lobashevsky, Histocompatibility Laboratory, Department of Medicine, School of Medicine, Indiana University, Indianapolis, IN 46202, United States
Andrew L Lobashevsky, Histocompatibility Laboratory, Methodist Hospital, Indiana University Health Inc., Indianapolis, IN 46202, United States
Author contributions: Lobashevsky AL solely contributed to this paper.
Correspondence to: Andrew L Lobashevsky, MD, PhD, Associate Professor, Director, Histocompatibility Laboratory, Department of Medicine, School of Medicine, Indiana University, 702 Barnhill Dr., Room #0615, Indianapolis, IN 46202, United States. alobashe@iuhealth.org
Telephone: +1-317-9488717   Fax: +1-317-9481108

Abstract

Donor human leukocyte antigen (HLA)-specific antibodies (DSA) play an important role in solid organ transplantation. Preexisting IgG isotype DSA are considered a risk factor for antibody mediated rejection, graft failure or graft loss. The post-transplant development of DSA depends on multiple factors including immunogenicity of mismatched antigens, HLA class Ⅱ typing of the recipient, cytokine gene polymorphisms, and cellular immunoregulatory mechanisms. *De novo* developed antibodies require special attention because not all DSA have equal clinical significance. Therefore, it is important for transplant clinicians and transplant immunologists to accurately characterize DSA. In this review, the contemporary immunological techniques for detection and characterization of anti-HLA antibodies and their pitfalls are described.

Core tip: In solid organ transplantations the graft outcomes critically depend on the degree of human leukocyte antigen (HLA) matching between the donor and recipient. Although the cellular component of the allogeneic immune response to the transplanted tissue plays a key role, the contribution of antibodies should not be underestimated. The detection of anti-HLA class Ⅰ and class Ⅱ antibodies is an important component of the initial work-up of a potential transplant candidate. The introduction of new highly sensitive technologies such as solid-phase based technologies has had a tremendous effect on organ allocation and immunomodulation strategies.

INTRODUCTION

In most cases the development of alloantibodies against human leukocyte antigens (HLAs) is related to immunization *via* blood and/or blood product transfusions, pregnancy, and transplants. There are scattered reports in the literature indicating the production of HLA antibodies may be elicited by vaccinations and infections due to cross reactivity between viral/bacterial antigens and HLAs[1-4] or through the bystander effect[5-8]. Humoral or antibody-mediated immunity requires noncovalent contact between antigens and antibodies. The hyper variable regions of the light and heavy immunoglobulin chains are termed complementarity-determining regions and they are primarily involved in the interaction with antigens. Antibody effector functions are specified by the constant domains of the heavy chains. The most

important function of these domains is the activation of the complement cascade, which is triggered by conformational changes in the hinge area after antigen binding. Complement activation results in the destruction of the cell membrane.

In solid organ transplantations of the kidney, heart, lung, and pancreas graft outcomes critically depend on the degree of HLA matching between the donor and recipient[9-17]. The cellular components of the allogeneic immune response to the transplanted tissue play a key role in this matching and the contribution of antibodies should not be underestimated[18-22]. The detection of anti-HLA class I and class II antibodies is an important component of the initial work-up of a potential transplant candidate (TC). The rationale for obtaining this information is related to clinical studies, which have universally demonstrated that pre-existing donor specific antibodies (DSAs) represent a significant risk factor for graft outcome[23-34]. The importance of the post-transplant monitoring of DSAs in kidney and cardiac transplants has been widely described[35-45]. The *de novo* development of DSAs strictly depends on the antigenicity and immunogenicity of mismatched HLAs. The substantial influence on antibody production involves other factors such as the HLA class II type of the responder, immunosuppressive medications, cytokine and chemokine genomic polymorphisms, and the hormonal background of the recipient[11,45-50].

It is generally accepted that *de novo* developed DSAs represent a risk factor for graft failure even at low concentrations. The early detection of DSAs considerably reduces the incidence of antibody-mediated rejection (AMR) and transplant glomerulopathy[23,51-58]. A post-transplant antibody analysis is a part of the routine monitoring of recipients. The introduction of new highly sensitive technologies such as solid-phase based technologies has had a tremendous effect on the clinical approach to anti-HLA antibody analysis[56-58]. The purpose of this review is to familiarize the reader with the methodological aspects and pitfalls of anti-HLA antibody analysis in solid-organ TC. Solid phase (SP) techniques will be specifically addressed.

METHODS OF ANTIBODY IDENTIFICATION

Cell based assays

Complement-dependent cytotoxicity: The long-established NIH complement-dependent cytotoxicity (CDC) method and its modifications are still widely used[59-64]. This assay allows the identification of high concentrations of antibodies to HLAs. There are two main purposes for applying the CDC method. This method can be used to estimate the percent of reactive antibodies (PRA) when the recipient serum is incubated with a panel of HLA typed T- or B-lymphocytes. If the serum contains antibodies against a particular HLA then the addition of rabbit complement causes cell death that is

visualized by staining and microscopic examination. This test is also used for the cross-matching (CM) or detection of complement binding antibodies against the HLAs of a particular donor. Various modifications of the NIH CDC method including extended incubation, additional washings, and the addition of secondary antihuman light kappa chain specific antibodies have been used to increase the sensitivity of the assay. Notably, the NIH CDC assay detects anti-HLA antibodies of the IgG and IgM isotypes. However, the IgM isotype has considerably less clinical significance[20,64-68]. Donors with HLA recipient antibodies detected by CDC should be avoided due to the high risk of hyperacute or delayed hyperacute rejection. Currently, this assay is primarily used to determine the efficacies of the desensitization or immunomodulation of recipients with high concentrations of anti-HLAs and identify recipients that are CDC-CM positive for their donors. Numerous reports have demonstrated that changing CDC-CM from positive to negative *via* durable DSA removal significantly reduces the risk of graft loss. The short-term graft survival in such recipients is not significantly different from recipients without CDC-positive DSA[69-75].

In the early 1970s, Patel *et al*[76] reported a considerable rate of graft failure in recipients who were CDC-CM negative with their donors. These observations indicated that the sensitivities of the CDC assay and its modifications were insufficient to detect low concentrations of DSA and was deleterious for the transplanted organ. Methods for increasing the sensitivity of antibody detection by flow cytometry (FC) based techniques were introduced more than 30 years ago[77].

Flow cytometry methods

Although FC methods of analysis are more sensitive than the CDC method they are subject to the effects of non-HLA and autologous antibodies that complicate the interpretation of the results. This complication is particularly important in the case of B-lymphocytes because they express Fc receptors and various adhesion molecules on their surfaces that facilitate non-specific binding[67,78]. B-cell false positive FC CM results may erroneously preclude transplants that should have favorable outcomes. The non-specific antibody binding may be reduced by the incubation of the lymphocytes with pronase, enzyme destroying Fc receptors, and other members of the Ig superfamily[79-82]. FC cell-based assays can also be used for PRA analysis. In this case, pooled HLA-typed lymphocytes are incubated with the recipient serum, and the percentage of positively reactive cells is determined based on the median channel shift[83]. This method has limited applicability because further testing is required to determine the antibody specificity. Additionally, the absorption of anti-HLA class I is required when class II PRA is analyzed.

SOLID PHASE ASSAYS

SP technologies use purified HLA class I and/or class

II proteins that are attached to an artificial substrate or matrix. These assays offer significantly higher sensitivities and specificities than cellular methods[84-86].

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was the first solid-phase analysis developed for antibody screening and specificity determination[87]. In this assay, HLA molecules are bound to the wells of plastic plates and positive reactions are measured by the color signal intensity produced by enzymes conjugated to anti-human antibodies following the addition of substrate to the wells. Purified pooled HLA- I and -II molecules bound to the wells are used for antibody screening/detection and PRA analysis. To define the antibody specificity, HLA proteins isolated from one individual are used to coat each well of the plate.

FC

FC SP assays use microspheres that have been coated with soluble HLA proteins that are extracted from a single cell line for specificity analysis or mixed for PRA analysis (FlowPRA Specific, FlowPRA, One Lambda)[88]. After the addition of the fluorescence-conjugated anti-human secondary antibodies a light signal is generated that indicates a positive binding of the antibody to HLA molecule(s). As with ELISA, this method can be designed to detect antibodies of IgG and IgM isotypes depending on the specificity of the secondary antibodies[84,89,90].

Luminex

Luminex-based technology has revolutionized the approach to anti-HLA antibody analysis and resolved ambiguities associated with the interpretation of CDC and FC results. This highly sensitive methodology has become an integral component of clinical decision-making and pathological diagnosis of transplanted organ injury. Luminex technology also incorporates microparticles (beads) that have been conjugated to varying amounts of two dyes, which enable the identification of 100 sets of beads. HLA-specific alloantibodies are detected *via* the addition to a reaction mixture of secondary phycoerythrin (PE)-conjugated anti-human antibodies. Each group of beads can be identified by the amount of conjugated fluorochromes and it is possible to identify which HLAs have bound antibodies. The light signal produced by bound antibody is proportional to its concentration and is expressed as the mean fluorescence intensity (MFI). The original assay was introduced as a combination of beads that were coated with HLA proteins extracted from individual cells. More recently, Luminex technology introduced a modification of the assay that included beads coated with a single class I or class II HLA. This methodological approach significantly improved antibody specificity analyses, particularly in highly sensitized patients[88,91-95]. The considerably higher surface density of HLAs on the microbeads compared to that on lymphocytes makes the Luminex single-antigen (SA) methodology extremely specific and highly sensitive. As a result, investigators can detect very low concentrations of HLA-directed antibodies. In the last decade, numerous reports have addressed the methodological aspects, clinical relevance, and standardization of the Luminex SP SA assay for the detection of antibodies[53,54,95-100]. The results of these studies have significantly expanded our understanding of anti-HLA antibody biology and the mechanism of the interaction between antibodies with antigens. This increased understanding includes improved information on isotypes and subtypes of antibodies, their abilities to bind complement, and fine epitope specificity. Fine epitope specificity is particularly important for the prediction of graft rejection[93,101-103]. There are also many new questions such as how does the signal produced by the DSAs detected in the SA Luminex assay correlate with positive FC CM, what is the clinical relevance of minimally reactive DSA, and how can the bead saturation effect be identified and overcome. Additionally, there are many other questions to address.

In this review, I describe the pitfalls, caveats, and limitations of the Luminex SP SA assay based on seven years of experience and clinical outcomes/observations at our transplant center.

Luminex SA SP assay: Technical challenges

Correlation of DSA MFI values with the results of FC CM tests: It is generally accepted that preexisting DSA directed against HLA can cause allograft injury or loss. FC CM is the most sensitive immunological method and it allows for the detection of DSA in TC serum. Positive FC CM is associated with an elevated risk of AMR[12,16,17,21,32,104-107]. The accurate detection of the spectrum of anti-HLA antibodies is critical for organ allocation and the prediction of FC CM results. Comparisons of the antibody profiles in the sera of TCs with the HLA typing of the potential donors are called virtual cross matches (VCMs). VCMs are particularly important in cases of heart and lung allocation in which the cold ischemia time is limited. VCM has been widely used at our transplant center for several years. Although SP Luminex technology allows for the very specific detection of DSAs at relatively low concentrations, issues regarding how strong the DSAs must be to cause positive FC CM results have to be addressed. The results of a multicenter study performed by Reed *et al*[89] suggested optimal cutoffs from 1000 to 1500 MFIs for antibodies to the HLA-A, -B, -DRB1, and -DQB1 loci. DSAs with MFIs within the indicated range are considered weak, and those below this range are considered negative. The correlation analyses between MFI values of DSAs and the results of FC CM assays performed in our laboratory have demonstrated that MFIs $\geq$ 2600 produced by anti-HLA I antibodies (HLA-A and -B loci) most likely result in T cell-positive FC CM results (positive predictive value 97%). Furthermore, MFIs $\geq$ 3100 produced by anti-HLA II antibodies (HLA-DRB1 and -DQB1 loci) most likely generate B cell-positive FC CM results (positive predictive value
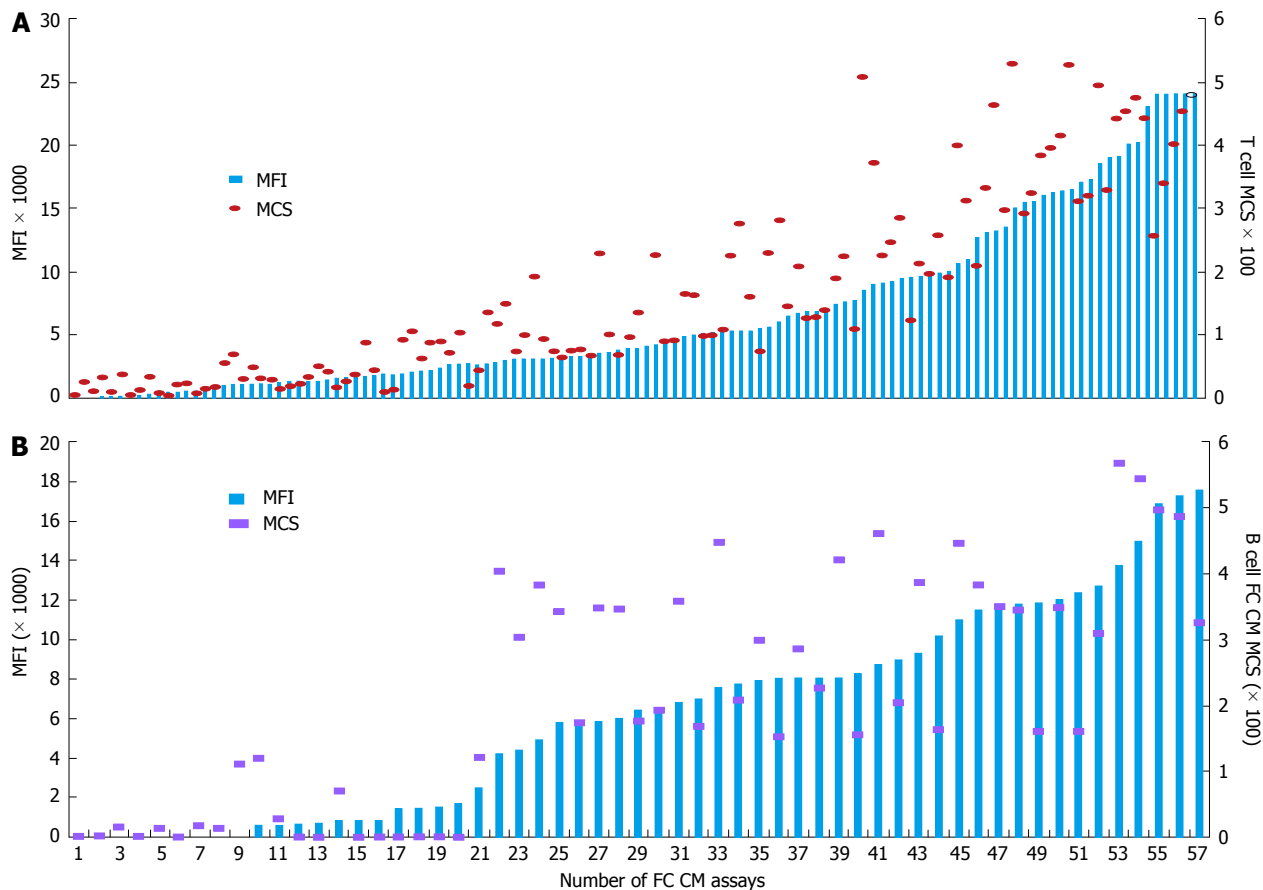
Figure 1 Concordance of mean fluorescence intensity values of monospecific anti-human leukocyte antigen class I (A) and class II (B) antibodies and results of flow cytometry cross match assay. Left Y axis indicates MFI values of antibodies, right Y axis indicates MCS for T- and B-cells FC CM assay, respectively. Blue bars indicate class I and class II MFI values, respectively; red circles and purple squares indicate MCS values of each FC CM test for T and B lymphocytes, respectively. MCS: Median channel shift; MFI: Mean fluorescence intensity; FC: Flow cytometry; CM: Cross match.
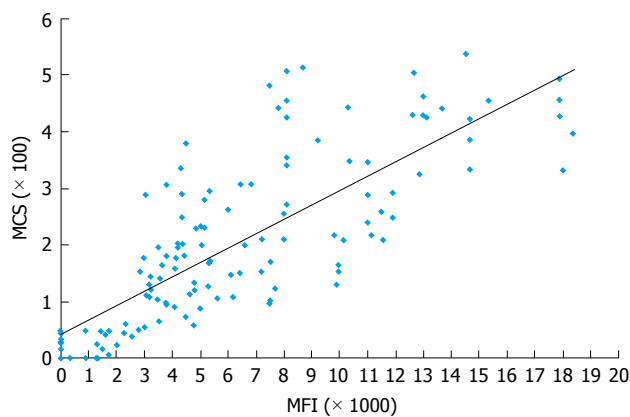
Figure 2 Correlation analysis between mean fluorescence intensity of anti-human leukocyte antigen class I antibodies and T cell flow cytometry cross match median channel shift. Each blue diamond represents a single test. MCS: Median channel shift; MFI: Mean fluorescence intensity.

95%, R = 0.78)[93] (Figures 1 and 2). The positive cutoffs determined in this assay also predict positive FC CM results when the recipient has multi-specific DSAs.

Quantities of HLA proteins on the beads

The density of the HLA molecules on the beads is significantly higher than that on lymphocytes (5×10^4-10^5

Table 1 Donor specific antibodies solid phase single-antigen Luminex analysis in unmodified and Dithiothreitol treated serum

Serum treatment	HLA specificities		
	B8	**B18**	**DR53**
DDT treated	24777[1]	23500	23100
Untreated	550	1764	4852

[1]The numbers in the table indicate MFI values. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity; DDT: Dithiothreitol.

molecules per cell) or endothelial cells. Therefore, even a minor admixture of anti-HLA antibodies or anti-idiotypic antibodies of the IgM isotype may cause false negative results. To overcome this obstacle, serum Dithiothreitol (DTT)-treatment is recommended[108-110].

We have observed strong positive T cell FC CM results and weakly reactive DSAs to HLA-B8 by SA Luminex. Subsequent DTT treatment of the serum resolved this discrepancy and revealed DSAs to the antigen with MFI values of 24000 (Table 1).

Denatured HLA and cryptic epitopes

Discrepancies between negative FC CM results and strongly reactive DSAs are observed when biochemical
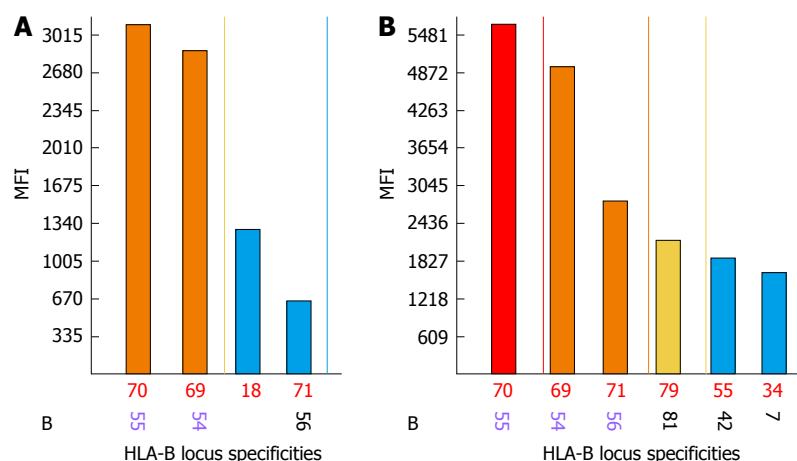
Figure 3 High affinity antibodies against denatured human leukocyte antigen-B55 and human leukocyte antigen-B54 proteins. A: Acid untreated sample; B: Acid treated sample; Y axis indicates MFI values of the antibodies; X axis indicates HLA-B locus specific antibodies. Colored bars represent MFI values of different intensity: Blue 500-2000, yellow 2001-3000, brown 3001-5000, and red > 5000. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity.
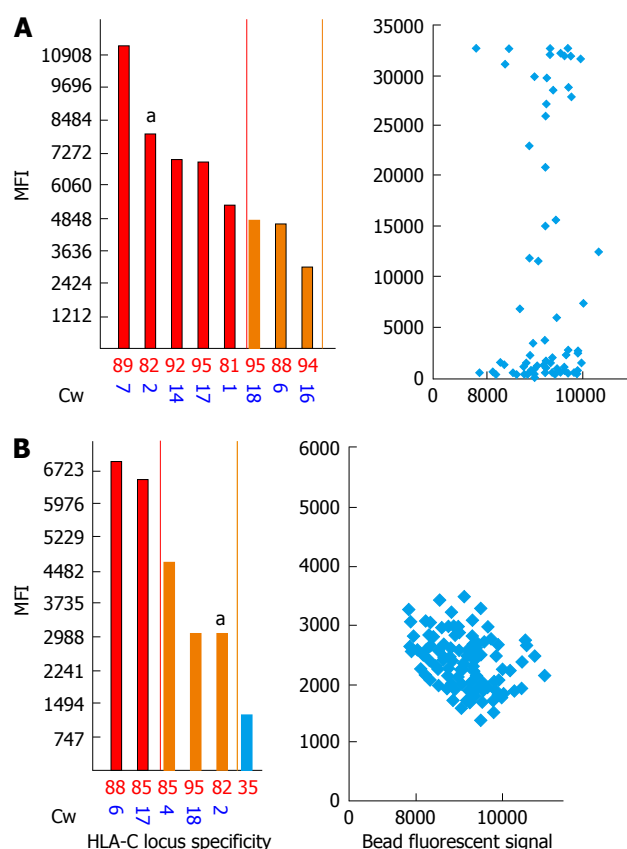
Figure 4 Uneven bead (bead #82) distribution results in falsely positive reactivity of antibodies to human leukocyte antigen-C2. [a]Anti-HLA-C2 antibodies MFI values in original (A) and repeated (B) samples, respectively. Left panels in figure A and B represent HLA-C locus specific antibody analysis, whereas right panels represent beads distribution on the basis of their fluorescence and MFI values produced by bound antibodies. Colored bars represent MFI values of different intensity: Blue 500-2000, yellow 2001-3000, brown 3001-5000, and red > 5000. Blue diamonds represent beads coated with HLA-C2 proteins. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity.

modification (denaturing) of HLA proteins during the bead conjugation process occurs. Denatured HLAs have

higher affinities for DSAs and when present in large amounts they produce strong false positive signals. An acid treatment is used to exclude antibodies against denatured HLAs. Antibodies against denatured HLA-B55 and HLA-B54 proteins are likely present when no differences or increased MFI values are observed between treated and untreated beads[110-113] (Figure 3). The acid treatment procedure is ineffective when analyzing anti-class II antibodies. In these types of situations, Luminex screening tests or Luminex class II phenotypic bead assays are performed.

Uneven bead distributions on SA Luminex dot blot histograms

Strong DSAs to HLA-C2 in the recipient serum have been shown to yield negative FC CM results[114] (Figure 4). To investigate this discrepancy, we analyzed the bead counts and bead distributions on the SA dot blot histograms of the aforementioned bead sets. Figure 4A shows the MFI values of 13100 for the anti-HLA-C2 antibodies and an uneven bead distribution. As shown in the figure, a majority of the beads are located within the negative MFI range and only a few have MFI values of approximately 30000. These findings indicate that the MFIs observed on SA antibody panels represent an average number between the lowest and highest values. A repeat of the assay confirmed the absence of DSAs, and the SA dot blot C2 histogram formed single clusters of beads located in the negative area (Figure 4B).

Non-specific antibody reactivity

One of the limitations of SA SP Luminex technology detailed in the literature is non-specific reactivity[90,106]. The main reasons for high background levels may be related to antibodies reacting to latex, autoimmune disease(s), and some medications (IVIG)[75,115-117]. Non-specific antibody binding complicates antibody analysis due to the appearance of multiple false positive antibodies and may affect organ allocation and the interpretation of VCM
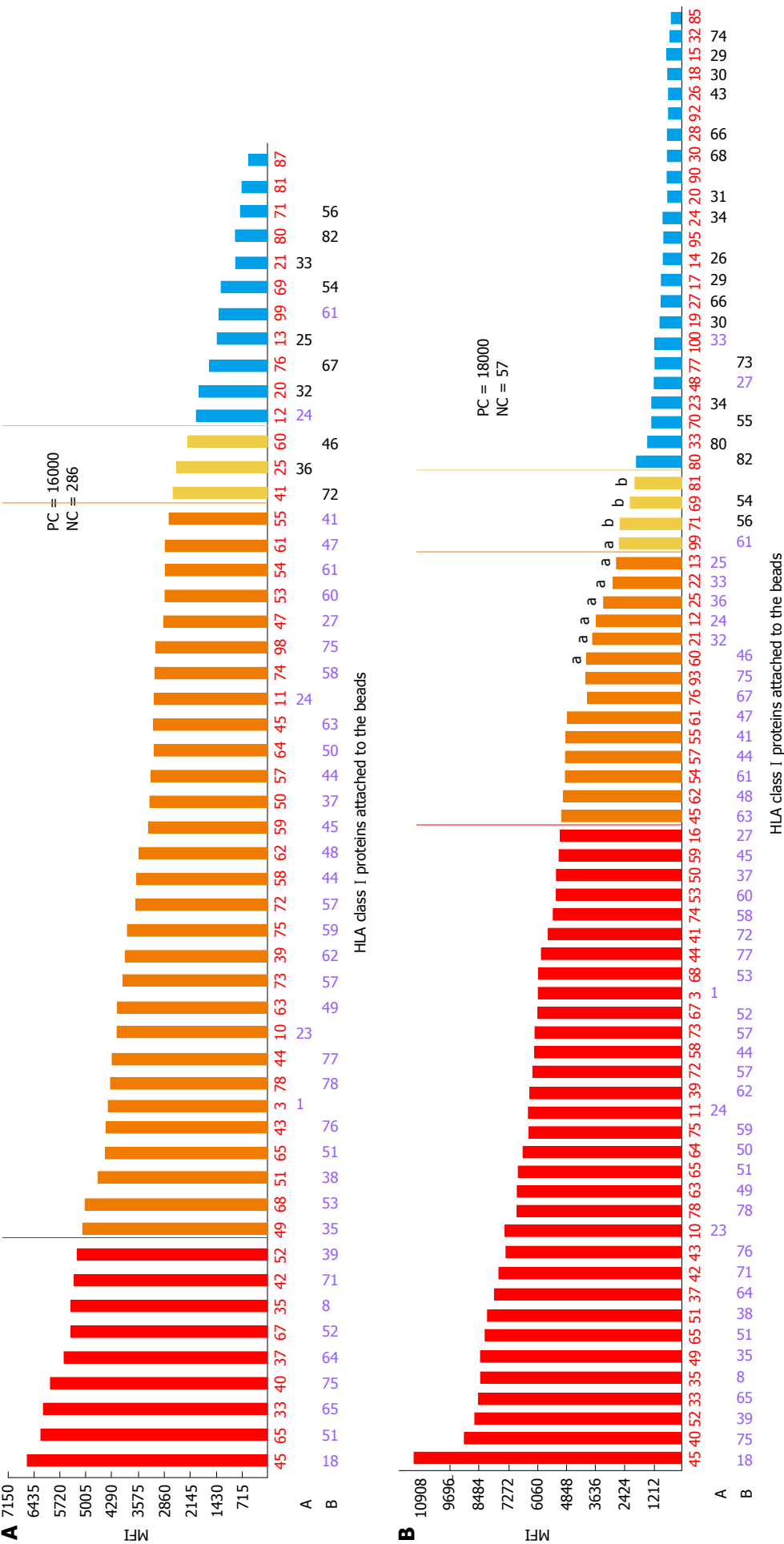
Figure 5 Anti-Class I antibodies detected in unabsorbed (A) and absorbed (B) serum samples. The numbers on the right indicate control values before and after absorption; [a]Additional antibody specificities with MFI ≥ 2600; [b]Additional antibody specificities with MFI ≥ 2600; HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity; NC: Negative control intensity; PC: Positive control MFI values; Y axis indicates MFI values; X axis indicates bead number and HLA-A and -B loci specificities. Colored bars represent MFI values of different intensity: Blue 500-2000, yellow 2001-3000, brown 3001-5000, and red > 5000.

results. The reagent Adsorb Out™ is manufactured by One Lambda and consists of microparticles designed for serum pre-incubation. This product reduces or removes strong background signals due to non-specific binding. Figures 5 and 6 demonstrate the additional seven anti-Class I and two anti-Class II antibody specificities that are associated with increasing the MFI (≥ 2600 for anti-Class I, and ≥ 3100 for Class II) and decreasing the negative control MFI values from 286 to 57 and from 230 to 60 for class I and class II antibodies, respectively. Additionally, the number of weakly reactive anti-Class I (MFI = 1500-2600) and anti-Class II (MFI = 1500-3100) antibodies also increased from nine to eleven and from two to three, respectively. Accounting for the MFI values of the antibodies to self-HLA also improves the TC antibody profiles. The high MFI values for the
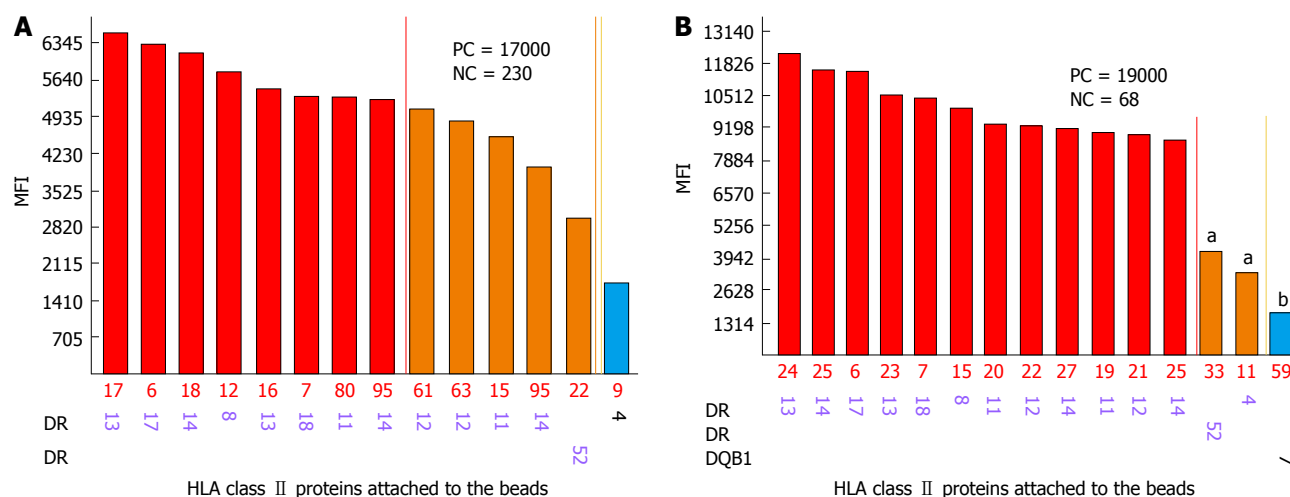
Figure 6 Anti-Class II antibodies detected in unabsorbed (A) and absorbed (B) serum samples. The numbers on the right indicate control values before and after absorption; [a]Additional antibody specificities with MFI $\geq$ 3100; [b]Additional antibody specificities with MFI = 1500-3100; MFI: Mean fluorescence intensity; NC: Negative control MFI values; PC: Positive control MFI values; Y axis indicates MFI value, X axis indicates bead number and HLA-A and -B loci specificities. Colored bars represent MFI values of different intensity: Blue 2001-3000, brown 3001-5000, and red > 5000.

negative control (MFI > 100) usually indicate high background and require the test to be repeated. However, if serum samples taken at different time points consistently exhibit elevated non-specific reactivity after adsorption then antibody analysis can be performed appropriately[118]. An unusual anti-class II antibody profile was observed in one kidney TC. The serum of this patient contained pan-reactive anti-DRB1 antibodies including self-specificities (Figure 7; the self-antigens are circled in red). A subsequent autologous FC CM assay was B cell positive and Luminex SP screening PRA analysis did not detect any antibodies. The results of these tests led to the conclusion that the anti-DRB1 antibodies in the serum of this TC serum were clinically irrelevant.

Bead oversaturation

The sera from patients may contain strong high-titer antibodies that can cause a prozoning or oversaturation effect. Figure 8 shows the oversaturation of SA beads with antibodies to HLA-I. In our experience this phenomenon is suspected when the MFI values exceed 20000. As shown in Figure 8, the antibodies against A2, A69, B51, and B52 exhibited oversaturation that disappeared upon the dilution of the serum. This prozoning effect has been reported by others with the SA Luminex bead assay[119-123]. Dilution of the serum to exclude the oversaturation of antibodies is critical when the antibody analysis is performed on the sera of highly sensitized TCs who have been subjected to immunomodulation/desensitization.

component 1q SA SP binding assay

The classical pathway of complement activation following antibody/HLA interactions is usually associated with graft cell damage and poor outcomes. Over the last decade it has been demonstrated that some of the DSAs detected with SA SP analysis but not with CDC (FC pos/CDC neg) can activate the complement system[124-128].

Numerous studies have demonstrated that inferior graft outcomes, graft loss, and C4d deposition are observed more frequently among recipients with DSAs that bind complement component 1 (C1q)[129-135]. The recently developed C1q SA SP assay (C1qScreen™) represents a reliable tool for distinguishing the binding IgG antibody from the complement-fixing antibodies of the IgG and IgM isotypes. The complement-fixing antibodies (C1q+) are detected using external C1q and anti-C1q antibodies conjugated to PE. The fluorescence intensity of the signal is proportional to the amount of bound C1q and is measured by MFI. The presence of C1q+ DSA is more strongly correlated with graft failure than the presence of antibodies that do not bind complement[126,136-139]. However, the absence of complement fixing antibodies has recently been reported in recipients with documented AMR. This result may indicate a low sensitivity of the C1q assay. The elegant studies of R. Liwski have demonstrated a good relationship between the anti-human globulin (AHG)-C1qScreen™ assay and CDC-AHG reactivity[128,129]. This highly sensitive modification of the original C1qScreen™ protocol would certainly be useful for risk assessment.

SA SP Luminex-based methodology and structural analysis of HLA epitopes

Each HLA protein represents a linear sequence of amino acid residues (AAR) or triplets, and the degree of mismatch is assessed as the number of triplets that are not shared between the donor and the recipient. There are two important points regarding this approach. First, only the AARs accessible to antibodies that reside in α-helical coils and β-loops are considered. In contrast, the triplets that are located in the β-pleated floor and beneath the α-chains are not available for antibody binding and are often not critical for antibody production because they are not immunogenic[140-143]. Second, alloantibodies can be

Figure 7 Pan-reactive anti-human leukocyte antigen-DRB1 antibodies detected in kidney transplant candidate serum. Y axis indicates MFI values, X axis indicate beads number and HLA-DR specificities; HLA-DR specificities in the blue circles are self. Colored bars represent MFI values of different intensity: Green < 500; blue 501-2000; yellow 2001-3000; brown 3001-3500; red > 3500. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity.

Table 2 Epitope analysis of human leukocyte antigen recipient developed antibodies to "antigens"

HLA	MFI	Reactive eplets/pair of eplets	Number of mismatched eplets	Mismatched eplets	Comments
B*07:02[a]	1689	44re	5	44re[b], 65qia, 70iaq, 113hd, 177dk	44re + self 69at
B*45:01[a]	1433	167es	1	167es[b]	44re + self 69at
B*44:03	1398	167es	5	76ent, 79rt, 94ii, 167es, 199v	44re + self 69at
B*82:01	1077	44re, 167es	4	44re, 65qia, 70iaq, 167es	44re + self 69at
B*44:02	841	167es	5	76ent, 79rt, 94ii, 167es, 199v	44re + self 69at
C*07:02	657	44re, 167es	5	44re, 65qia, 70iaq, 94ii, 167es	44re + self 69at
B*81:01	1222	44re	4	44re, 65qia, 70iaq, 254ta	44re + self 69at
B*27:08	1130	44re	3	44re, 65qia, 71ka	44re + self 69at
B*56:01	1059	44re	3	44re, 65qia, 71ka	44re + self 69at
B*55:01	837	44re	3	44re, 65qia, 71ka	44re + self 69at
B*42:01	761	44re	3	44re, 65qia, 71ka	44re + self 69at
B*67:01	681	44re	4	44re, 65qia, 70iaq, 158t	44re + self 69at
B*27:05	607	44re	5	44re, 65qia, 71ka, 76 edt, 79rt	44re + self 69at
B*73:01	327	44re	6	44re, 65qia, 71ka, 103 m, 267qe, 275kp	44re + self 69at
C*06:02[a]	379	44re, 167es	5	44re, 65qia, 70iaq, 94ii, 167es	
B*08:01	2046	44re	1	44r	44re + 69m
B*15:12 (B76)	1294	ND[c]	3	45rma, 113hd, 163lg	Unexplained

[a] Alleles in bold font are mismatches with the 1st donor; [b] Reactive eplets; [c] Not determined. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity.
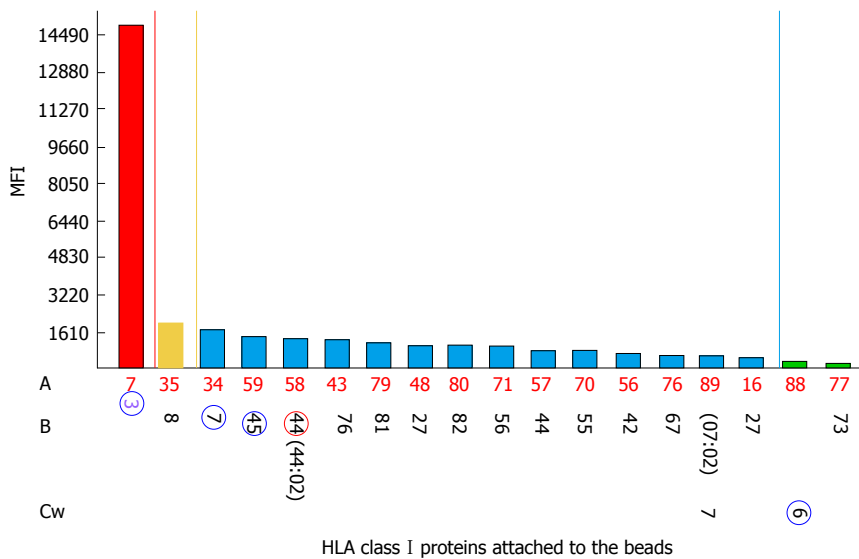
Figure 9 Anti-Class I antibodies detected in the serum of kidney transplant candidate C. The numbers in blue circles indicate HLA specificities mismatched with the 1st donor. The number in red circle indicates HLA specificity mismatched with the current donor; the numbers in parenthesis indicate allelic assignment of HLA. Y axis indicates MFI values, X axis indicates bead number and HLA-A and -B loci specificities. Colored bars represent MFI values of different intensity: Green < 500; blue 501-1600; brown 1601-2500; red > 2500. HLA: Human leukocyte antigen; MFI: Mean fluorescence intensity.

Table 3 Human leukocyte antigen Class I typing results of the recipient and previous and current donors						
	A locus		**B locus**		**C locus**	
Recipient	23:01	66:01	41:01	49:01	07:01	17:01
1st donor (immunizer)	**03:01**[a]	26:01	**07:02**	**45:01**	**06:02**	07:01
Current donor	23:01	**24:01**	**44:02**	49:01	**03:03**	07:01

[a]Mismatched alleles are given in bold font.

produced only against non-self-mismatched triplets.

Furthermore, AAR triplet analysis (HLA Matchmaker computer algorithm) can explain or predict the development of post-transplant antibodies in kidney allograft recipients[46,50]. Subsequent analyses of patients' antibodies and HLA-specific monoclonal antibodies have revealed that each HLA consists of structurally defined "eplets" that represent epitopes comprised of the AARs within a 3 Å-5 Å radius of the surface of the molecule[48,10,122]. An example of such analysis is presented in Figure 9, Figure 10 and Table 2. The HLA typing results of the recipient, previous donors, and current donors are given in Table 3. The pre-transplant evaluation of the second kidney TC revealed multiple weakly reactive (MFI cutoff ≥ 2600) antibodies including DSA B*44:02 (Figure 9) and strongly positive T cell FC CM results (ΔMCS = 129, positive cutoff = 50) (the autologous T cell FC CM results appeared to be negative). A HLAMatchmaker analysis of the HLA-B locus-specific antibodies determined that the antibody reactivity was restricted to two epitopes/eplets 44re and 167es on the immunizing HLA-B*45:01antigen. FC CM and SA bead assays revealed that the antigens targeted by the recipient antibodies shared the eplet pair 44re69at. Eplets that were mismatched with the immunizer were 44re and 167es, while 69at was shared by both
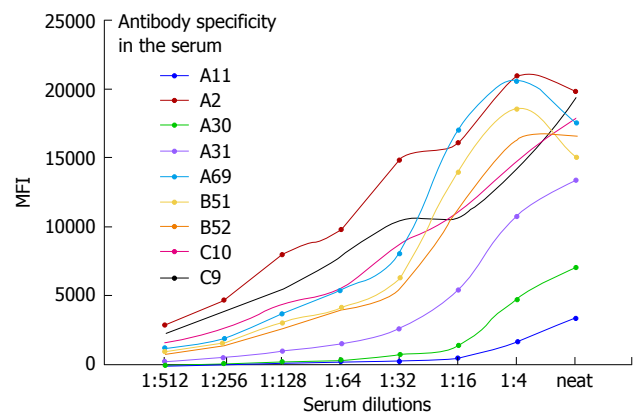
Figure 8 Phenomenon of class I bead oversaturation with antibodies. Y axis indicates MFI values, X axis indicates serum dilution. Ten percent fetal bovine serum in roswell park memorial institute medium was used as diluents. MFI: Mean fluorescence intensity.
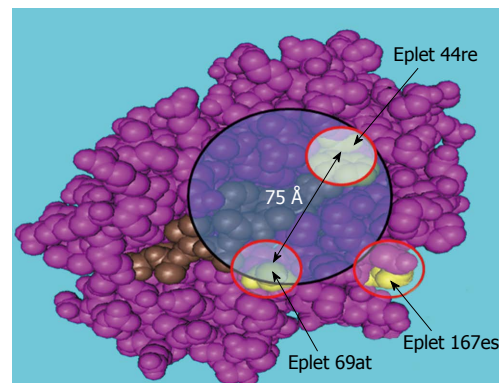
Figure 10 Three dimensional map of mismatched and shared eplets. Alpha chain domains are in purple; peptide in the antigen presenting groove is in brown color; eplets are in yellow; black transparent circle specifies the patch comprising two eplets.

the donor's B7 and the patient's A23 and A66 (Figure 10).

The identification of immunogenic epitopes significantly affects the prediction of post-transplant alloantibody specificities, donor selection, graft outcome, and organ allocation. The recent studies of Zeevi *et al*[142] and Duquesnoy *et al*[143] used monoclonal antibodies to demonstrate the anti-HLA antibody complement-fixing abilities strictly depend on the configuration of the critical contact eplet(s)[140,141]. The results of their studies indicated that complete complement cascade activation is determined by the energy produced from the antibody-HLA interaction. The amount of this energy should be sufficient to induce conformational changes of the constant region of the antibody to elicit C1q binding and subsequent component activation. The authors hypothesized that the binding energies of the SA C1q-negative antibodies are insufficient to induce conformational changes in the constant region. However, in the cases of the C1q+ and CDC+ antibodies this energy is sufficient to trigger complete complement activation and cell membrane damage[141,143].

CONCLUSION

The identification of anti-HLA antibodies in TC serum is a major task of HLA laboratories and transplant physicians and is important for graft failure risk assessment and donor selection. Furthermore, antibody detection is critical in highly sensitized TCs who have been subjected to desensitization (immunomodulation). SA SP analysis is a highly sensitive and highly specific method of antibody characterization that enables the detection of low concentrations of antibodies and their fine HLA specificities. However, this assay is not free from limitations including large variation in the numbers of HLA molecules per bead and the effects of manual (*i.e.*, technologist-to-technologist) factors on assay variance. In this review, I attempted to share multiple years of experience performing SA SP assays for pre- and post-transplant antibody analyses in my laboratory and address some pitfalls and caveats of this assay. Evaluations of the clinical significance of anti-HLA antibodies should undeniably include their concentrations, isotypes, ability to fix the complement, and fine epitope specificity.

REFERENCES

1   **Candon S**, Thervet E, Lebon P, Suberbielle C, Zuber J, Lima C, Charron D, Legendre C, Chatenoud L. Humoral and cellular immune responses after influenza vaccination in kidney transplant recipients. *Am J Transplant* 2009; **9**: 2346-2354 [PMID: 19656126 DOI: 10.1111/j.1600-6143.2009.02787.x]

2   **Danziger-Isakov L**, Cherkassky L, Siegel H, McManamon M, Kramer K, Budev M, Sawinski D, Augustine JJ, Hricik DE, Fairchild R, Heeger PS, Poggio ED. Effects of influenza immunization on humoral and cellular alloreactivity in humans. *Transplantation* 2010; **89**: 838-844 [PMID: 20179666 DOI: 10.1097/TP.0b013e3181ca56f8]

3   **Di Genova G**, Roddick J, McNicholl F, Stevenson FK. Vaccination of human subjects expands both specific and bystander memory T cells but antibody production remains

4   **Kennedy RB**, Ovsyannikova IG, Vierkant RA, Jacobson RM, Poland GA. Effect of human leukocyte antigen homozygosity on rubella vaccine-induced humoral and cell-mediated immune responses. *Hum Immunol* 2010; **71**: 128-135 [PMID: 19896518 DOI: 10.1016/j.humimm.2009.11.002]

5   **Benson MJ**, Elgueta R, Schpero W, Molloy M, Zhang W, Usherwood E, Noelle RJ. Distinction of the memory B cell response to cognate antigen versus bystander inflammatory signals. *J Exp Med* 2009; **206**: 2013-2025 [PMID: 19703988 DOI: 10.1084/jem.20090667]

6   **Dougan SK**, Ashour J, Karssemeijer RA, Popp MW, Avalos AM, Barisa M, Altenburg AF, Ingram JR, Cragnolini JJ, Guo C, Alt FW, Jaenisch R, Ploegh HL. Antigen-specific B-cell receptor sensitizes B cells to infection by influenza virus. *Nature* 2013; **503**: 406-409 [PMID: 24141948 DOI: 10.1038/nature12637]

7   **Kumar D**, Campbell P, Humar A. Donor-specific alloantibody upregulation after influenza vaccination in transplant recipients. *Am J Transplant* 2011; **11**: 2538; author's reply 2539-2540 [PMID: 21906260 DOI: 10.1111/j.1600-6143.2011.03745.x]

8   **Kumar D**, Danziger-Isakov L. Immunization against influenza: a balancing act. *Am J Transplant* 2011; **11**: 1561-1562 [PMID: 21672158 DOI: 10.1111/j.1600-6143.2011.03605.x]

9   **Abe M**, Kawai T, Futatsuyama K, Tanabe K, Fuchinoue S, Teraoka S, Toma H, Ota K. Postoperative production of anti-donor antibody and chronic rejection in renal transplantation. *Transplantation* 1997; **63**: 1616-1619 [PMID: 9197356]

10 **Bas J**, Mestre M, Grinyó JM, Massip E, Alsina J, Castelao AM, Corominas M, Buendia E. Peripheral blood lymphoid subsets and long-term clinical course of kidney recipients: a longitudinal study. *Cytometry* 1998; **34**: 103-112 [PMID: 9579608 DOI: 10.1002/(SICI)1097-0320(19980415)34:2<103::AID-CYTO7>3.0.CO;2-J]

11 **Cippà PE**, Gaspert A, Etter C, Guenduez Z, Ferrari-Lacraz S, Rüsi B, Fehr T. Late antibody-mediated rejection by de novo donor HLA-DP-specific antibody after renal transplantation: a case report. *Hum Immunol* 2014; **75**: 462-465 [PMID: 24530822 DOI: 10.1016/j.humimm.2014.02.008]

12 **Claas FH**, Dankers MK, Oudshoorn M, van Rood JJ, Mulder A, Roelen DL, Duquesnoy RJ, Doxiadis II. Differential immunogenicity of HLA mismatches in clinical transplantation. *Transpl Immunol* 2005; **14**: 187-191 [PMID: 15982562 DOI: 10.1016/j.trim.2005.03.007]

13 **Deng CT**, El-Awar N, Ozawa M, Cai J, Lachmann N, Terasaki PI. Human leukocyte antigen class II DQ alpha and beta epitopes identified from sera of kidney allograft recipients. *Transplantation* 2008; **86**: 452-459 [PMID: 18698250 DOI: 10.1097/TP.0b013e3181804cd2]

14 **Scornik JC**, LeFor WM, Cicciarelli JC, Brunson ME, Bogaard T, Howard RJ, Ackermann JR, Mendez R, Shires DL, Pfaff WW. Hyperacute and acute kidney graft rejection due to antibodies against B cells. *Transplantation* 1992; **54**: 61-64 [PMID: 1631946 DOI: 10.1097/00007890-199207000-00010]

15 **Takemoto S**, Port FK, Claas FH, Duquesnoy RJ. HLA matching for kidney transplantation. *Hum Immunol* 2004; **65**: 1489-1505 [PMID: 15603878 DOI: 10.1016/j.humimm.2004.06.008]

16 **Terasaki PI**. Humoral theory of transplantation. *Am J Transplant* 2003; **3**: 665-673 [PMID: 12780557 DOI: 10.1034/j.1600-6143.2003.00135.x]

17 **Terasaki PI**, Cai J. Humoral theory of transplantation: further evidence. *Curr Opin Immunol* 2005; **17**: 541-545 [PMID: 16098722 DOI: 10.1016/j.coi.2005.07.018]

18 **Arnold ML**, Kloecker S, Roppelt D, Kalden JR. HLA DQ antibodies in patients awaiting kidney re-transplantation. *Genes and Immunity* 2005; **6**: S68

19 **Bartel G**, Wahrmann M, Exner M, Regele H, Schillinger M, Hörl WH, Böhmig GA. Determinants of the complement-fixing ability of recipient presensitization against HLA anti-

gens. *Transplantation* 2007; **83**: 727-733 [PMID: 17414705 DOI: 10.1097/01.tp.0000256337.18347.aa]

20   **Sumitran-Holgersson S**. HLA-specific alloantibodies and renal graft outcome. *Nephrol Dial Transplant* 2001; **16**: 897-904 [PMID: 11328893 DOI: 10.1093/ndt/16.5.897]

21   **Vasilescu ER**, Ho EK, de la Torre L, Itescu S, Marboe C, Cortesini R, Suciu-Foca N, Mancini D. Anti-HLA antibodies in heart transplantation. *Transpl Immunol* 2004; **12**: 177-183 [PMID: 14967316 DOI: 10.1016/j.trim.2003.08.002]

22   **Stegall MD**, Raghavaiah S, Gloor JM. The (re)emergence of B cells in organ transplantation. *Curr Opin Organ Transplant* 2010; **15**: 451-455 [PMID: 20613525 DOI: 10.1097/MOT.0b013e32833b9c11]

23   **Bartel G**, Regele H, Wahrmann M, Huttary N, Exner M, Hörl WH, Böhmig GA. Posttransplant HLA alloreactivity in stable kidney transplant recipients-incidences and impact on long-term allograft outcomes. *Am J Transplant* 2008; **8**: 2652-2660 [PMID: 18853952 DOI: 10.1111/j.1600-6143.2008.02428.x]

24   **Al-Lamki RS**, Bradley JR, Pober JS. Endothelial cells in allograft rejection. *Transplantation* 2008; **86**: 1340-1348 [PMID: 19034000 DOI: 10.1097/TP.0b01.3e3181891d8b]

25   **O'Leary JG**, Demetris AJ, Friedman LS, Gebel HM, Halloran PF, Kirk AD, Knechtle SJ, McDiarmid SV, Shaked A, Terasaki PI, Tinckam KJ, Tomlanovich SJ, Wood KJ, Woodle ES, Zachary AA, Klintmalm GB. The role of donor-specific HLA alloantibodies in liver transplantation. *Am J Transplant* 2014; **14**: 779-787 [PMID: 24580828 DOI: 10.1111/ajt.12667]

26   **Bishay ES**, Cook DJ, El Fettouh H, Starling RC, Young JB, Smedira NG, McCarthy PM. The impact of HLA sensitization and donor cause of death in heart transplantation. *Transplantation* 2000; **70**: 220-222 [PMID: 10919608]

27   **Bishay ES**, Smedira NG. Surgical management of massive atrial size mismatch in heart transplantation. *Ann Thorac Surg* 2000; **69**: 618-620 [PMID: 10735713 DOI: 10.1016/S0003-4975(99)01349-1]

28   **Böhmig GA**, Bartel G, Wahrmann M. Antibodies, isotypes and complement in allograft rejection. *Curr Opin Organ Transplant* 2008; **13**: 411-418 [PMID: 18685338 DOI: 10.1097/MOT.0b013e3283028312]

29   **Cornell LD**, Smith RN, Colvin RB. Kidney transplantation: mechanisms of rejection and acceptance. *Annu Rev Pathol* 2008; **3**: 189-220 [PMID: 18039144 DOI: 10.1146/annurev.pathmechdis.3.121806.151508]

30   **Ghasemian SR**, Light JA, Sasaki TA, Barhyte DY. Hyperacute rejection from antibody against class II HLA antigens. *Clin Transplant* 1998; **12**: 569-571 [PMID: 9850452]

31   **Grandtnerová B**, Javorský P, Kolácný J, Hovoricová B, Dědic P, Laca L. Treatment of acute humoral rejection in kidney transplantation with plasmapheresis. *Transplant Proc* 1995; **27**: 934-935 [PMID: 7879238]

32   **Lefaucheur C**, Suberbielle-Boissel C, Hill GS, Nochy D, Andrade J, Antoine C, Gautreau C, Charron D, Glotz D. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Contrib Nephrol* 2009; **162**: 1-12 [PMID: 19001809 DOI: 10.1159/000170788]

33   **Lobo PI**, Spencer CE, Stevenson WC, Pruett TL. Evidence demonstrating poor kidney graft survival when acute rejections are associated with IgG donor-specific lymphocytotoxin. *Transplantation* 1995; **59**: 357-360 [PMID: 7871565 DOI: 10.1097/00007890-199502150-00009]

34   **Terasaki PI**, Cai J. Human leukocyte antigen antibodies and chronic rejection: from association to causation. *Transplantation* 2008; **86**: 377-383 [PMID: 18698239 DOI: 10.1097/TP.0b013e31817c4cb8]

35   **El Fettouh HA**, Cook DJ, Bishay E, Flechner S, Goldfarb D, Modlin C, Dennis V, Novick AC. Association between a positive flow cytometry crossmatch and the development of chronic rejection in primary renal transplantation. *Urology* 2000; **56**: 369-372 [PMID: 10962296 DOI: 10.1016/S0090-4295(00)00657-9]

36   **Banasik M**, Boratyńska M, Kościelska-Kasprzak K, Mazanowska O, Bartoszek D, Zabińska M, Myszka M, Nowakowska B, Hałoń A, Szyber P, Patrzałek D, Klinger M. Long-term follow-up of non-HLA and anti-HLA antibodies: incidence and importance in renal transplantation. *Transplant Proc* 2013; **45**: 1462-1465 [PMID: 23726597 DOI: 10.1016/j.transproceed.2012.11.025]

37   **Banasik M**, Boratyńska M, Kościelska-Kasprzak K, Mazanowska O, Krajewska M, Zabińska M, Bartoszek D, Myszka M, Nowakowska B, Dawiskiba T, Lepiesza A, Chudoba P, Klinger M. The impact of de novo donor-specific anti-human leukocyte antigen antibodies on 5-year renal transplant outcome. *Transplant Proc* 2013; **45**: 1449-1452 [PMID: 23726594 DOI: 10.1016/j.transproceed.2012.12.026]

38   **Caro-Oleas JL**, González-Escribano MF, Gentil-Govantes MÁ, Acevedo MJ, González-Roncero FM, Blanco GB, Núñez-Roldán A. Clinical relevance of anti-HLA donor-specific antibodies detected by Luminex assay in the development of rejection after renal transplantation. *Transplantation* 2012; **94**: 338-344 [PMID: 22814330 DOI: 10.1097/TP.0b013e31825ace2c]

39   **Cooper JE**, Gralla J, Chan L, Wiseman AC. Clinical significance of post kidney transplant de novo DSA in otherwise stable grafts. *Clin Transpl* 2011: 359-364 [PMID: 22755431 DOI: 10.1111/j.1399-0012.2010.01305.x]

40   **Cooper JE**, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection episodes. *Transplantation* 2011; **91**: 1103-1109 [PMID: 21403588 DOI: 10.1097/TP.0b013e3182139da1]

41   **Everly MJ**, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, Catrou PG, Bolin P, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation* 2013; **95**: 410-417 [PMID: 23380861 DOI: 10.1097/TP.0b013e31827d62e3]

42   **Ginevri F**, Nocera A, Comoli P, Innocente A, Cioni M, Parodi A, Fontana I, Magnasco A, Nocco A, Tagliamacco A, Sementa A, Ceriolo P, Ghio L, Zecca M, Cardillo M, Garibotto G, Ghiggeri GM, Poli F. Posttransplant de novo donor-specific hla antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant* 2012; **12**: 3355-3362 [PMID: 22959074 DOI: 10.1111/j.1600-6143.2012.04251.x]

43   **Huang Y**, Ramon D, Luan FL, Sung R, Samaniego M. Incidences of preformed and de novo donor-specific HLA antibodies and their clinicohistological correlates in the early course of kidney transplantation. *Clin Transpl* 2012: 247-256 [PMID: 23721029 DOI: 10.1111/j.1399-0012.2011.01469.x]

44   **Jimenez J**, Young JB. Case 2: cardiogenic shock due to acute vascular rejection in a heart transplant recipient. *J Heart Lung Transplant* 2000; **19**: 817-818 [PMID: 11023298 DOI: 10.1097/00007890-200004271-00276]

45   **Mehra NK**, Siddiqui J, Baranwal A, Goswami S, Kaur G. Clinical relevance of antibody development in renal transplantation. *Ann N Y Acad Sci* 2013; **1283**: 30-42 [PMID: 23384346 DOI: 10.1111/nyas.12034]

46   **Adeyi OA**, Girnita AL, Howe J, Marrari M, Awadalla Y, Askar M, Martell J, Zeevi A, Shapiro R, Nalesnik M, Randhawa P, Demetris AJ, Duquesnoy RJ. Serum analysis after transplant nephrectomy reveals restricted antibody specificity patterns against structurally defined HLA class I mismatches. *Transpl Immunol* 2005; **14**: 53-62 [PMID: 15814283 DOI: 10.1016/j.trim.2005.01.001]

47   **Fuller A**, Profaizer T, Roberts L, Fuller TC. Repeat donor HLA-DR mismatches in renal transplantation: is the increased failure rate caused by noncytotoxic HLA-DR alloantibodies? *Transplantation* 1999; **68**: 589-591 [PMID: 10480424 DOI: 10.1097/00007890-199908270-00027]

48   **Lachmann N**, El Awar N, Salama A, Terasaki PI, Schonemann C. Single amino acid mismatches determine immuno-

genic HLA epitopes. *Tissue Antigens* 2008; **72**: 253-260

49   Laux G, Mansmann U, Deufel A, Opelz G, Mytilineos J. A new epitope-based HLA-DPB matching approach for cadaver kidney retransplants. *Transplantation* 2003; **75**: 1527-1532 [PMID: 12792509 DOI: 10.1097/01.TP.0000061759.57702.8A]

50   Lobashevsky AL, Senkbeil RW, Shoaf JL, Stephenson AK, Skelton SB, Burke RM, Deierhoi MH, Thomas JM. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients. *Hum Immunol* 2002; **63**: 364-374 [PMID: 11975980 DOI: 10.1016/S0198-8859(02)00371-3]

51   Lobashevsky A, Rosner K, Goggins W, Higgins N. Subtypes of immunoglobulin (Ig)-G antibodies against donor class II HLA and cross-match results in three kidney transplant candidates. *Transpl Immunol* 2010; **23**: 81-85 [PMID: 20304065 DOI: 10.1016/j.trim.2010.03.003]

52   Bosch A, Llorente S, Diaz JA, Salgado G, López M, Boix F, López-Hernández R, González-Soriano MJ, Campillo JA, Moya-Quiles MR, Perez-Lopez N, Minguela A, Jimeno L, Alvarez-López MR, Muro M. Low median fluorescence intensity could be a nonsafety concept of immunologic risk evaluation in patients with shared molecular eplets in kidney transplantation. *Hum Immunol* 2012; **73**: 522-525 [PMID: 22425738 DOI: 10.1016/j.humimm.2012.02.020]

53   Caro-Oleas JL, González-Escribano MF, González-Roncero FM, Acevedo-Calado MJ, Cabello-Chaves V, Gentil-Govantes MÁ, Núñez-Roldán A. Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation. *Nephrol Dial Transplant* 2012; **27**: 1231-1238 [PMID: 21810767 DOI: 10.1093/ndt/gfr429]

54   Dunn TB, Noreen H, Maurer D, Ozturk OG, Gillingham K, Matas AJ, Bray RA, Gebel HM. Impact of Low Level Donor Specific Antibodies in Renal Transplant Outcomes. *Hum Immunol* 2010; **71**: S25 [DOI: 10.1016/j.humimm.2010.06.059]

55   Eng HS, Bennett G, Bardy P, Coghlan P, Russ GR, Coates PT. Clinical significance of anti-HLA antibodies detected by Luminex: enhancing the interpretation of CDC-BXM and important post-transplantation monitoring tools. *Hum Immunol* 2009; **70**: 595-599 [PMID: 19527759]

56   Girnita AL, Brailey P, Alloway RR, Rike-Shields A, Sadaka B, Woodle ES. Early and Late Antibody Mediated Rejection Demonstrate Differential Responses to Proteasome Inhibitor-Based Therapy with Respect to Donor Specific Anti-HLA Antibody Specificity and Subtype. *Am J of Transplant* 2011; **11**: 171-172

57   Everly MJ. Summarizing the use of donor specific anti-HLA antibody monitoring in transplant patients. *Clin Transpl* 2011: 333-336 [PMID: 22755427 DOI: 10.1111/j.1399-0012.2010.01360.x]

58   Wu P, Everly MJ, Jin J, Mao Y, Chen J. Understanding the significance of low-level preformed donor-specific anti-HLA antibodies in renal transplant patients. *Clin Transpl* 2011: 365-368 [PMID: 22755432]

59   Aurora D, Balazs I, Ebner D, Hariharan J, Jiang N, Mostecki J. Detection of complement activating anti-HLA class I and II antibodies using Lifecodes Single Antigen assay. *Tissue Antigens* 2008; **71**: 289-290

60   Jordan SC, Vo A, Bunnapradist S, Toyoda M, Peng A, Puliyanda D, Kamil E, Tyan D. Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* 2003; **76**: 631-636 [PMID: 12973100]

61   Jordan SC, Vo A, Tyan D, Toyota M. Desensitization therapy with intravenous gammaglobulin (IVIG): applications in solid organ transplantation. *Trans Am Clin Climatol Assoc* 2006; **117**: 199-211; discussion 211 [PMID: 18528474]

62   Zachary AA, Montgomery RA, Ratner LE, Samaniego-Picota M, Haas M, Kopchaliiska D, Leffell MS. Specific and durable elimination of antibody to donor HLA antigens in

renal-transplant patients. *Transplantation* 2003; **76**: 1519-1525 [PMID: 14657698]

63   Cecka JM, Zhang Q, Reed EF. Preformed cytotoxic antibodies in potential allograft recipients: recent data. *Hum Immunol* 2005; **66**: 343-349 [PMID: 15866696 DOI: 10.1016/j.humimm.2005.01.030]

64   Claas FH, Doxiadis II. Management of the highly sensitized patient. *Curr Opin Immunol* 2009; **21**: 569-572 [PMID: 19682882 DOI: 10.1016/j.coi.2009.07.010]

65   Leffell MS, Zachary AA. Anti-allograft antibodies: some are harmful, some can be overcome, and some may be beneficial. *Discov Med* 2010; **9**: 478-484 [PMID: 20515617]

66   Zarkhin V, Li L, Sarwal M. "To B or not to B?" B-cells and graft rejection. *Transplantation* 2008; **85**: 1705-1714 [PMID: 18580460 DOI: 10.1097/TP.0b013e318177793e]

67   Jackson AM, Lucas DP, Melancon JK, Desai NM. Clinical relevance and IgG subclass determination of non-HLA antibodies identified using endothelial cell precursors isolated from donor blood. *Transplantation* 2011; **92**: 54-60 [PMID: 21516064 DOI: 10.1097/TP.0b013e31821b60e9]

68   Díaz I, Sánchez P, Alonso C, Valdés F. Immunological profile of patients awaiting a renal transplant. *Clin Transplant* 2004; **18**: 529-535 [PMID: 15344955 DOI: 10.1111/j.1399-0012.2004.00205.x]

69   Wasowska B, Baldwin WM, Howell DN, Sanfilippo F. The association of enhancement of renal allograft survival by donor-specific blood transfusion with host MHC-linked inhibition of IgG anti-donor class I alloantibody responses. *Transplantation* 1993; **56**: 672-680 [PMID: 7692630]

70   Jordan SC, Vo AA, Peng A, Toyoda M, Tyan D. Intravenous gammaglobulin (IVIG): a novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplant* 2006; **6**: 459-466 [PMID: 16468954 DOI: 10.1111/j.1600-6143.2005.01214.x]

71   Jordan SC, Toyoda M, Kahwaji J, Vo AA. Clinical aspects of intravenous immunoglobulin use in solid organ transplant recipients. *Am J Transplant* 2011; **11**: 196-202 [PMID: 21219579 DOI: 10.1111/j.1600-6143.2010.03400.x]

72   Morath C, Opelz G, Zeier M, Süsal C. Recent developments in desensitization of crossmatch-positive kidney transplant recipients. *Transplant Proc* 2012; **44**: 1648-1651 [PMID: 22841236 DOI: 10.1016/j.transproceed.2012.04.006]

73   Sagoo P, Perucha E, Sawitzki B, Tomiuk S, Stephens DA, Miqueu P, Chapman S, Craciun L, Sergeant R, Brouard S, Rovis F, Jimenez E, Ballow A, Giral M, Rebollo-Mesa I, Le Moine A, Braudeau C, Hilton R, Gerstmayer B, Bourcier K, Sharif A, Krajewska M, Lord GM, Roberts I, Goldman M, Wood KJ, Newell K, Seyfert-Margolis V, Warrens AN, Janssen U, Volk HD, Soulillou JP, Hernandez-Fuentes MP, Lechler RI. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J Clin Invest* 2010; **120**: 1848-1861 [PMID: 20501943 DOI: 10.1172/JCI39922]

74   Song EY, Lee YJ, Hyun J, Kim YS, Ahn C, Ha J, Kim SJ, Park MH. Clinical relevance of pretransplant HLA class II donor-specific antibodies in renal transplantation patients with negative T-cell cytotoxicity crossmatches. *Ann Lab Med* 2012; **32**: 139-144 [PMID: 22389881 DOI: 10.3343/alm.2012.32.2.139]

75   Zachary AA, Eng HS. Desensitization: achieving immune detente. *Tissue Antigens* 2011; **77**: 3-8 [PMID: 21155718 DOI: 10.1111/j.1399-0039.2010.01596.x]

76   Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; **280**: 735-739 [PMID: 4886455 DOI: 10.1056/NEJM196904032801401]

77   O'Rourke RW, Osorio RW, Freise CE, Lou CD, Garovoy MR, Bacchetti P, Ascher NL, Melzer JS, Roberts JP, Stock PG. Flow cytometry crossmatching as a predictor of acute rejection in sensitized recipients of cadaveric renal transplants. *Clin Transplant* 2000; **14**: 167-173 [PMID: 10770424 DOI: 10.1034/j.1399-0012.2000.140212.x]

78   Dragun D, Hegner B. Non-HLA antibodies post-trans-

plantation: clinical relevance and treatment in solid organ transplantation. *Contrib Nephrol* 2009; **162**: 129-139 [PMID: 19001820 DOI: 10.1159/000170845]

79　**Hetrick SJ**, Schillinger KP, Zachary AA, Jackson AM. Impact of pronase on flow cytometric crossmatch outcome. *Hum Immunol* 2011; **72**: 330-336 [PMID: 21262310 DOI: 10.1016/j.humimm.2011.01.005]

80　**Lobo PI**, Isaacs RB, Spencer CE, Pruett TL, Sanfey HA, Sawyer RG, McCullough C. Improved specificity and sensitivity when using pronase-digested lymphocytes to perform flow-cytometric crossmatch prior to renal transplantation. *Transpl Int* 2002; **15**: 563-569 [PMID: 12461661]

81　**Lobo PI**, Isaacs RB, Spencer CE, Pruett TL, Sanfey HA, Sawyer RG, McCullough C. Improved specificity and sensitivity when using pronase-digested lymphocytes to perform flow-cytometric crossmatch prior to renal transplantation. *Transplant Int* 2002; **15**: 563-569 [PMID: 12461661]

82　**Vaidya S**, Cooper TY, Avandsalehi J, Barnes T, Brooks K, Hymel P, Noor M, Sellers R, Thomas A, Stewart D, Daller J, Fish JC, Gugliuzza KK, Bray RA. Improved flow cytometric detection of HLA alloantibodies using pronase: potential implications in renal transplantation. *Transplantation* 2001; **71**: 422-428 [PMID: 11233905 DOI: 10.1097/00007890-200102150-00015]

83　**Shroyer TW**, Deierhoi MH, Mink CA, Cagle LR, Hudson SL, Rhea SD, Diethelm AG. A rapid flow cytometry assay for HLA antibody detection using a pooled cell panel covering 14 serological crossreacting groups. *Transplantation* 1995; **59**: 626-630 [PMID: 7878769]

84　**Fuggle SV**, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. *Transplantation* 2008; **86**: 384-390 [PMID: 18698240 DOI: 10.1097/TP.0b013e31817c90f5]

85　**McKenna RM**, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. *Transplantation* 2000; **69**: 319-326 [PMID: 10706035]

86　**Smith J**, Rose M. Transplantation Immunology: methods and Protocols. In: Hornik P, Rose M. Detection and clinical relevance of Antibodies After Transplantation. New Jersey: Totowa, NJ, 2009: 227-245 [DOI: 10.1385/1-59745-049-9:227]

87　**Buelow R**, Chiang TR, Monteiro F, Cornejo MC, Ellingson L, Claas F, Gaber O, Gelder F, Kotb M, Orosz C. Soluble HLA antigens and ELISA--a new technology for crossmatch testing. *Transplantation* 1995; **60**: 1594-1599 [PMID: 8545896 DOI: 10.1016/0198-8859(94)91782-5]

88　**Pei R**, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation* 2003; **75**: 43-49 [PMID: 12544869 DOI: 10.1097/00007890-200301150-00008]

89　**Reed EF**, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, Lunz J, Mohanakumar T, Nickerson P, Tambur AR, Zeevi A, Heeger PS, Gjertson D. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA-drilling down on key sources of variation. *Am J Transplant* 2013; **13**: 3050-3051 [PMID: 24103158 DOI: 10.1111/ajt.12462]

90　**Tait BD**, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, Reed EF, Bray RA, Campbell P, Chapman JR, Coates PT, Colvin RB, Cozzi E, Doxiadis II, Fuggle SV, Gill J, Glotz D, Lachmann N, Mohanakumar T, Suciu-Foca N, Sumitran-Holgersson S, Tanabe K, Taylor CJ, Tyan DB, Webster A, Zeevi A, Opelz G. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation* 2013; **95**: 19-47 [PMID: 23238534 DOI: 10.1097/TP.0b013e31827a19cc]

91　**Billen EV**, Christiaans MH, van den Berg-Loonen EM. Clinical relevance of Luminex donor-specific crossmatches: data from 165 renal transplants. *Tissue Antigens* 2009; **74**: 205-212 [PMID: 19497037 DOI: 10.1111/j.1399-0039.2009.01283.x]

92　**El-Awar NR**, Akaza T, Terasaki PI, Nguyen A. Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation* 2007; **84**: 532-540 [PMID: 17713439]

93　**Lobashevsky AL**, Higgins NG. Predictive Values (Pv) of Class I and Class Ii Antibodies (Ab) Detected by Luminex. *Hum Immunol* 2009; **70**: S32

94　**Mujtaba MA**, Goggins W, Lobashevsky A, Sharfuddin AA, Yaqub MS, Mishler DP, Brahmi Z, Higgins N, Milgrom MM, Diez A, Taber T. The strength of donor-specific antibody is a more reliable predictor of antibody-mediated rejection than flow cytometry crossmatch analysis in desensitized kidney recipients. *Clin Transplant* 2011; **25**: E96-102 [PMID: 20977497 DOI: 10.1111/j.1399-0012.2010.01341.x]

95　**Zeevi A**, Lunz J, Teuteberg J, Feingold B, Jelinek L, Michael S, Zaldonis D, Yoshiya T, Morrell M, Webber S. Does the Determination of Complement Binding Add to the Clinical Utility of Donor Specific Antibodies Detected by Luminex? *J of Heart and Lung Transplant* 2011; **30**: S228

96　**Amico P**, Hönger G, Steiger J, Schaub S. Utility of the virtual crossmatch in solid organ transplantation. *Curr Opin Organ Transplant* 2009; **14**: 656-661 [PMID: 19741534 DOI: 10.1097/MOT.0b013e328331c169]

97　**Tsapepas DS**, Vasilescu R, Tanriover B, Coppleson Y, Rekhtman Y, Hardy MA, Dube G, Crew RJ, Ratner LE, Cohen DJ, Mohan S. Preformed donor-specific antibodies and risk of antibody-mediated rejection in repeat renal transplantation. *Transplantation* 2014; **97**: 642-647 [PMID: 24637863 DOI: 10.1097/01.TP.0000440954.14510.6a]

98　**Brailey P**, Woodle ES, Alloway RR, Tevar AD, Cardi M, Portwood E, Mogilishetty G, Govil A, Rike AH, Walsh RC, Wall G, Girnita AL. Preformed Low Reactive Anti-HLA Antibodies Are Associated with Majority of Early Antibody-Mediated Rejection in Renal Transplantation. *Hum Immunol* 2010; **71**: S68 [DOI: 10.1016/j.humimm.2010.06.125]

99　**Eng HS**, Bennett G, Tsiopelas E, Lake M, Humphreys I, Bardy P, Coates PT, Russ G. Luminex R investigation of B-cell crossmatches: Low titer HLA donor-specific antibodies associated with inferior outcomes. *Hum Immunol* 2007; **68**: S31

100　**Leffell MS**. Real benefits from virtual crossmatches. *Transplantation* 2010; **89**: 138-139 [PMID: 20098274 DOI: 10.1097/TP.0b013e3181c92591]

101　**Lobashevsky AL**, Goggins W, Taber T, Higgins N, Duquesnoy R. Allelic mismatch at HLA-DRB3 locus resulted in production of a broad spectrum of alloantibodies. *Tissue Antigens* 2011; **77**: 377

102　**Mostecki J**, Hariharan J, Ebner D, Ray B, Balazs I. Detection of HLA Class I antibodies with recombinant Single Antigens using a Luminex-based assay. *Tissue Antigens* 2006; **67**: 553-555

103　**Claas FH**, Rahmel A, Doxiadis II. Enhanced kidney allocation to highly sensitized patients by the acceptable mismatch program. *Transplantation* 2009; **88**: 447-452 [PMID: 19696624]

104　**Arnold ML**, Doxiadis IIN, Kalden JR, Spriewald BM. Crossmatch results with sera containing HLA class II antibodies depend on the donor cell source. *Genes and Immunity* 2005; **6**: S67 [DOI: 10.1038/sj.gene.6364206]

105　**Lopez R**, Al Zahrani SJ, Awaji MI, Housawi AA, Saghier M, Khan FM, Berka N. Flow Crossmatch Bimodal Distribution for T Cell Treated with Pronase. *Hum Immunol* 2011; **72**: S154

106　**Billen EV**, Christiaans MH, Lee J, van den Berg-Loonen EM. Donor-directed HLA antibodies before and after transplant-ectomy detected by the luminex single antigen assay. *Transplantation* 2009; **87**: 563-569 [PMID: 19307795 DOI: 10.1097/TP.0b013e3181949e37]

107　**Loupy A**, Suberbielle-Boissel C, Hill GS, Lefaucheur C, Anglicheau D, Zuber J, Martinez F, Thervet E, Méjean A, Charron D, Duong van Huyen JP, Bruneval P, Legendre C, Nochy D. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant* 2009; **9**: 2561-2570 [PMID: 19775320]

108 **Vittoraki AG**, Skoumi DA, Apostolaki MD, Athanasopoulou MG, Georgoulia G, Iniotaki AG, St Paterakis G. Predicting Flow Cytometry Crossmatches Based on Luminex MFI Values. *Tissue Antigens* 2012; **79**: 489-495

109 **Zachary AA**, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in Luminex assays for HLA-specific antibodies: characteristics and resolution. *Hum Immunol* 2009; **70**: 496-501 [PMID: 19364516 DOI: 10.1016/j.humimm.2009.04.001]

110 **Lobashevsky AL**, Rosner K, Goggins W, Higgins NG. Do We Really Know Everything About Cross Match (Or Does IgG Subtype Matter)? *Hum Immunol* 2010; **71**: S41

111 **Gandhi MJ**, Degoey S, Falbo D, Jenkins S, Stubbs JR, Noreen H, Lorentzen DF, Lee J, Stegall M. Inter and intra laboratory concordance of HLA antibody results obtained by single antigen bead based assay. *Hum Immunol* 2013; **74**: 310-317 [PMID: 23238217 DOI: 10.1016/j.humimm.2012.12.003]

112 **Pereira S**, Perkins S, Lee JH, Shumway W, LeFor W, Lopez-Cepero M, Wong C, Connolly A, Tan JC, Grumet FC. Donor-specific antibody against denatured HLA-A1: clinically non-significant? *Hum Immunol* 2011; **72**: 492-498 [PMID: 21396421 DOI: 10.1016/j.humimm.2011.02.012]

113 **Grenzi PC**, de Marco R, Silva RZ, Campos EF, Gerbase-DeLima M. Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay. *Hum Immunol* 2013; **74**: 1300-1303 [PMID: 23831256 DOI: 10.1016/j.humimm.2013.06.035]

114 **Poli F**, Benazzi E, Innocente A, Nocco A, Cagni N, Gianatti A, Fiocchi R, Scalamogna M. Heart transplantation with donor-specific antibodies directed toward denatured HLA-A*02: 01: a case report. *Hum Immunol* 2011; **72**: 1045-1048 [PMID: 21888935 DOI: 10.1016/j.humimm.2011.08.001]

115 **Susal C**, Mahmoud K, Ovens J, Ruhenstroth A, Dohler B, Scherer S, Opelz G. Are Additional Antibodies Detected by Luminex Single Antigen Testing Clinically Relevant? A Collaborative Transplant Study Report. *Transpl Int* 2010; **23**: 36-37

116 **Woodle ES**, Girnita A, Brailey P, Sadaka B, Rike-Shields A, Alloway, RR, Wall G. Desensitization Results Are Influenced by the Nature of the HLA Ab Being Treated. *Transpl Int* 2011; **24**: 329

117 **Kosmoliaptsis V**, Chaudhry AN, Sharples LD, Halsall DJ, Dafforn TR, Bradley JA. Taylor CJ. HLA Class-I Alloantigen Immunogenicity Can be Predicted by Tertiary Structure and Electrostatic Charge Disparity. *Transpl Int* 2009; **22**: 69 [DOI: 10.1111/j.1432-2277.2009.00960.x]

118 **Kosmoliaptsis V**, Bradley JA, Taylor CJ. Structural limitations to the mimetic HLA epitope hypothesis. *Transplantation* 2009; **87**: 1262-1263; author reply 1263 [PMID: 19384177]

119 **Kosmoliaptsis V**, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ. Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigen-beads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies. *Transplantation* 2009; **87**: 813-820 [PMID: 19300182 DOI: 10.1097/TP.0b013e318199c581]

120 **Taylor CJ**, Kosmoliaptsis V, Summers DM, Bradley JA. Back to the future: application of contemporary technology to long-standing questions about the clinical relevance of human leukocyte antigen-specific alloantibodies in renal transplantation. *Hum Immunol* 2009; **70**: 563-568 [PMID: 19445992 DOI: 10.1016/j.humimm.2009.05.001]

121 **Freitas MC**, Rebellato LM, Ozawa M, Nguyen A, Sasaki N, Everly M, Briley KP, Haisch CE, Bolin P, Parker K, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI. The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation* 2013; **95**: 1113-1119 [PMID: 23514959 DOI: 10.1097/TP.0b013e3182888db6]

122 **Fontaine MJ**, Kuo J, Chen G, Galel SA, Miller E, Sequeira F, Viele M, Goodnough LT, Tyan DB. Complement (C1q) fixing solid-phase screening for HLA antibodies increases the availability of compatible platelet components for refractory patients. *Transfusion* 2011; **51**: 2611-2618 [PMID: 21615749 DOI: 10.1111/j.1537-2995.2011.03194]

123 **Lunz JG**, Webber S, Soltys K, Shapiro R, Teuteberg J, Feingold B, Morrell M, Jelinek L, Zeevi A. C1Q Binding Donor Specific Hla Antibody Monitoring by Luminex Single Antigen Assay Is Useful in Predicting Clinical Events. *Hum Immunol* 2011; **72**: S50

124 **Yabu JM**, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. *Transplantation* 2011; **91**: 342-347

125 **Gebel HM**, Liwski RS, Bray RA. Technical aspects of HLA antibody testing. *Curr Opin Organ Transplant* 2013; **18**: 455-462 [PMID: 23838651 DOI: 10.1097/MOT.0b013e32836361f1]

126 **Liwski R**, Lee S, Sperry R, Nickerson P, Bray R, Gebel H. The Anti-Human Globulin Enhanced C1Qscreen (Tm) Assay Improves the Detection of Complement Binding Donor Specific HLA Antibodies. *Hum Immunol* 2013; **74**: 48

127 **Bohmig GA**, Exner M, Watschinger B, Wenter C, Wahrmann M, Osterreicher C, Saemann MD, Mersich N, Horl WH, Zlabinger GJ, Regele H. C4d deposits in renal allografts are associated with inferior graft outcome. *Transplant Proc* 2001; **33**: 1151-1152

128 **Böhmig GA**, Exner M, Habicht A, Schillinger M, Lang U, Kletzmayr J, Säemann MD, Hörl WH, Watschinger B, Regele H. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol* 2002; **13**: 1091-1099 [PMID: 11912271]

129 **Collins AB**, Chicano SL, Cornell LD, Tolkoff-Rubin N, Goes NB, Saidman SL, Farrell ML, Cosimi AB, Colvin RB. Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO-compatible renal allografts. *Transplant Proc* 2006; **38**: 3427-3429 [PMID: 17175293 DOI: 10.1016/j.transproceed.2006.10.159]

130 **Govil A**, Arend LJ, Brailey P, Alloway RR, Mogilishetty G, Tevar AD, Roy-Chaudhury P, Woodle ES. DSA and C4d Deposition: Paradoxical Dissociation in Acute Pancreas Allograft Rejection. *Am J of Transplant* 2010; **10**: 89-91

131 **Habicht A**, Regele H, Exner M, Soleiman A, Horl WH, Watschinger B, Derfler K, Bohmig GA. A case of severe C4d-positive kidney allograft dysfunction in the absence of histomorphologic features of rejection. *Wien Klin Wochenschr* 2002; **114**: 945-948

132 **Hönger G**, Wahrmann M, Amico P, Hopfer H, Böhmig GA, Schaub S. C4d-fixing capability of low-level donor-specific HLA antibodies is not predictive for early antibody-mediated rejection. *Transplantation* 2010; **89**: 1471-1475 [PMID: 20395886 DOI: 10.1097/TP.0b013e3181dc13e7]

133 **Wahrmann M**, Bartel G, Exner M, Regele H, Körmöczi GF, Fischer GF, Böhmig GA. Clinical relevance of preformed C4d-fixing and non-C4d-fixing HLA single antigen reactivity in renal allograft recipients. *Transpl Int* 2009; **22**: 982-989 [PMID: 19619171 DOI: 10.1111/j.1432-2277.2007.00574.x]

134 **Loupy A**, Hill GS, Suberbielle C, Anglicheau D, Duong JP, Zuber J, Posson J, Mamzer MF, Martinez F, Thervet E, Bruneval P, Nochy D, Legendre C. Significance of Minimal and Focal C4d in Stable DSA plus Kidney Transplant Recipients. *Am J of Transplant* 2010; **11**: 56-65

135 **Loupy A**, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol* 2012; **8**: 348-357 [PMID: 22508180 DOI: 10.1038/nrneph.2012.81]

136 **Sutherland SM**, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. *Pediatr Transplant* 2012; **16**: 12-17 [PMID: 22093755 DOI:

10.1111/j.1399-3046.2011.01599.x]

137 **Duquesnoy RJ**, Marrari M, Mulder A, Mostecki J, Claas FHJ, van Balazs I. Human monoclonal antibody reactivity with HLA class I epitopes defined by pairs of mismatched eplets and self-eplets. *Tissue Antigens* 2011; **77**: 376-377 [PMID: 21063243 DOI: 10.1097/TP.0b013e3182007b74]

138 **Mongkolsuk T**, Ingsathit A, Worawichawong S, Jirasiritham S, Kitpoka P, Thammanichanond D. Shared molecular eplet stimulates acute antibody-mediated rejection in a kidney transplant recipient with low-level donor-specific antibodies: a case report. *Transplant Proc* 2014; **46**: 644-647 [PMID: 24656035 DOI: 10.1016/j.transproceed.2013.10.058]

139 **Marrari M**, Mostecki J, Mulder A, Claas F, Balazs I, Duquesnoy RJ. Human monoclonal antibody reactivity with human leukocyte antigen class I epitopes defined by pairs of mismatched eplets and self-eplets. *Transplantation* 2010; **90**: 1468-1472 [PMID: 21063243 DOI: 10.1097/TP.0b013e3182007b74]

140 **Duquesnoy RJ**, Marrari M, Mulder A, Claas FH, Mostecki J, Balazs I. Structural aspects of human leukocyte antigen class I epitopes detected by human monoclonal antibodies. *Hum Immunol* 2012; **73**: 267-277 [PMID: 22227099 DOI: 10.1016/j.humimm.2011.11.011]

141 **Duquesnoy RJ**, Marrari M, Mostecki J, Mulder A, Balazs I. Human Monoclonal Antibody Reactivity with Hla Class I Epitopes Defined by Eplet Pairs. *Hum Immunol* 2010; **71**: S77

142 **Zeevi A**, Marrari M, Feingold B, Webber S, Duquesnoy RJ. Human leukocyte antigen epitope analysis to assess complement- and non-complement-binding donor-specific antibody repertoire in a pediatric heart transplant recipient. *Hum Immunol* 2012; **73**: 48-51 [PMID: 22100305 DOI: 10.1016/j.humimm.2011.10.011]

143 **Duquesnoy RJ**, Marrari M, Jelenik L, Zeevi A, Claas FH, Mulder A. Structural aspects of HLA class I epitopes reacting with human monoclonal antibodies in Ig-binding, C1q-binding and lymphocytotoxicity assays. *Hum Immunol* 2013; **74**: 1271-1279 [PMID: 23770250 DOI: 10.1016/j.humimm.2013.05.016]

Complement cascade and kidney transplantation: The rediscovery of an ancient enemy

Alberto Mella, Maria Messina, Antonio Lavacca, Luigi Biancone

Alberto Mella, Maria Messina, Antonio Lavacca, Luigi Biancone, Renal Transplantation Unit "A. Vercellone", Division of Nephrology Dialysis and Transplantation, Department of Medical Sciences, Città della Salute e della Scienza Hospital and University of Torino, 88-10126 Turin, Italy

Author contributions: Mella A, Messina M, Lavacca A and Biancone L had contributed to this paper in conception and design, acquisition, analysis and interpretation of data, and drafting the article and revising it critically for important intellectual content; all authors approved this version to be published.

Correspondence to: Luigi Biancone, MD, PhD, Renal Transplantation Unit "A. Vercellone", Division of Nephrology Dialysis and Transplantation, Department of Medical Sciences, Città della Salute e della Scienza Hospital and University of Torino, Corso Bramante, 88-10126 Turin, Italy. luigi.biancone@unito.it
Telephone: +39-011-6335594 Fax: +39-011-6334990
Received: May 16, 2014   Revised: June 28, 2014
Accepted: September 6, 2014
Published online: September 24, 2014

Abstract

The identification of complement activity in serum and immunohistochemical samples represents a core element of nephropathology. On the basis of this observation, different experimental models and molecular studies have shown the role of this cascade in glomerular disease etiology, but the absence of inhibiting drugs have limited its importance. Since 2006, the availability of target-therapies re-defined this ancient pathway, and its blockage, as the new challenging frontier in renal disease treatment. In the graft, the complement cascade is able to initiate and propagate the damage in ischemia-reperfusion injury, C3 glomerulopathy, acute and chronic rejection, atypical hemolytic uremic syndrome and, probably, in many other conditions. The importance of complement-focused research is revealed by the evidence that eculizumab, the first complement-targeting drug, is now considered a valid option in atypical hemolytic uremic syndrome treatment but it is also under investigation in all the aforementioned conditions. In this review we evaluate the importance of complement cascade in renal transplantation diseases, focusing on available treatments, and we propose a speculative identification of areas where complement inhibition may be a promising strategy.

Core tip: Complement cascade is involved in different types of renal disease, from glomerulonephritides to pre-eclampsia, and the availability of new drugs, able to inhibit different steps of the cascade, re-defined this ancient pathway, and its blockage both in native and transplanted kidneys, as a new challenging frontier in renal disease treatment. In this review we evaluate the importance of complement cascade in renal transplantation diseases, focusing on available treatments, and we propose a speculative identification of areas where complement inhibition may be a promising strategy.

INTRODUCTION

Complement was initially identified by Ehrlich and Morgenroth in the 19th century as a factor that "complemented" the bactericidal action of immune cells[1]. Muller-Eberhard in 1960 described the cascade and hypothized its involvement in some different diseases[2,3]. Despite the overt demonstration of a complement activation in serum and immunohistochemical samples, the exact role of
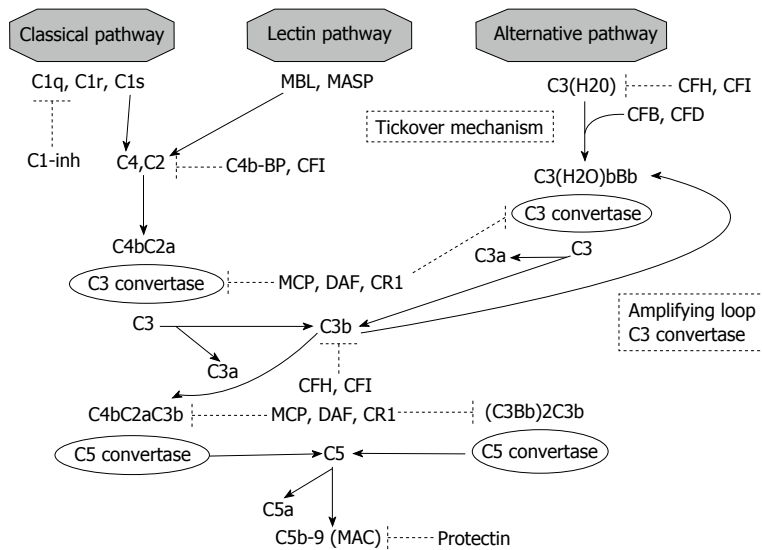
Figure 1 Complement cascade and regulatory proteins. MBL: Mannose binding protein; MASP: MBL-associated serine proteases; CFB: Complement factor B; CFD: Complement factor D; CFH: Complement factor H; CFI: Complement factor I; C1-inh: C1 inhibitor; C4b-BP: C4 binding protein; MCP: Membrane cofactor protein; DAF: Decay accelerating factor; MAC: Membrane attack complex.

this cascade remained unclear for a long time.

Recently, the increasing evidence of a key role of complement cascade in many renal diseases, from glomerulonephritides to pre-eclampsia[4-9], and the availability of new drugs able to inhibit different steps of the cascade, re-defined this ancient pathway, and its blockage, as the new challenging frontier in renal disease treatment.

In this review we evaluate the importance of complement cascade in renal transplantation diseases, with an initial general description and a subsequent in-depth evaluation. A separate section is dedicated to treatment, analyzing areas where complement inhibition may represent a promising strategy.

Description of complement cascade

The complement cascade represents a direct defense against bacterial infection and a "scavenger" for immune complexes and apoptotic cells[10].

As summarized in Figure 1, three pathways are involved in complement activation: (1) Classical pathway: C1q binds IgG and IgM, apoptotic cells, or acute-phase proteins promoting cascade progression; (2) Lectin pathways: two trigger proteins (mannose-binding lectin-MBL-and ficolins) activate complement after the adhesion to bacterial surface; and (3) Alternative pathway: in this important and well studied pathway, the first step is represented by the constant and spontaneous hydrolysis of C3.

In the classical pathway, the conformational change induced by C1q binding causes C1r and C1s activation. These proteases are both able to generate C4b from C4. After C2 cleavage and generation of C4b2a (a C3 convertase), the last step (C3 cleavage), generates the C5 convertase C4b2a3b[11,12].

In the lectin pathways, MBL interaction with one of its two associated serine proteases (MASPs-1 or MASP-2), promotes C3 cleavage[13,14].

Although the activation in previously reported pathways is due to a direct stimulation derived from binding on different epitopes, the alternative pathway is constitutively active, a condition called "tickover mechanism". In this manner, if necessary in response to an appropriate stimulus, the host enables a rapid and strong activation of the cascade.

As first step, the modification induced by factors B and D on hydrolyzed C3 creates C3(H₂O)Bb, a C3 convertase. C3bBb is the result, after C3 cleavage and C3b generation, of another interaction with factors B and D ("amplification loop"). C3bBb is also able to generate C3b and to enhance alternative pathway. The interaction of C3b with C3bBb is the final causative factor for alternative pathway C5 convertase formation (C3bBb3b)[13].

C5a, one of the product of C5 cleavage, is able to combine with final components of the cascade (C6, C7, C8, C9): this final step generates, in all three pathways, the membrane attack complex (MAC)[11,12].

Regulatory mechanism

The cascade regulation has a key role, because an uncontrolled activation, secondary to defects or mutations in regulatory proteins, is referred to be the triggering cause of some complement-mediated diseases (*i.e.*, atypical hemolytic syndrome).

The alternative pathway is the more controlled cascade, as predictable by the evidence of its continuous and spontaneous activation depending on C3 hydrolysis.

The regulatory proteins are divided in two types: fluid-phase and membrane-bound molecules (Figure 1): (1) The fluid-phase regulators are serum proteins. C1 inhibitor is able to arrest classical activation by blocking C1q dissociation, and, in high concentration, can inhibit both the lectin-binding and alternative pathways[11]. Factors H and I act a pivotal role cleaving C3b and conse-

quently regulating the alternative pathway. Factor I is also able to hydrolyze C4b in presence of C4 binding protein; and (2) The second type of regulatory proteins, not pathway specific, are membrane-bound regulators. In most cases, these molecule are anchored to the cell membrane through a glycophosphatidylinositol component. Factor I inhibits complement in association with two of them, membrane cofactor protein (MCP) and CR1; CD35 and complement receptor of immunoglobulin also accelerate C3 and C5 convertase degradation. CD55 enhances C3 convertase spontaneous degradation. Protectin (CD59) directly inhibits MAC formation[11,14].

Effects of complement cascade activation

Three different effectors are activated by complement cascade: (1) Anaphylatoxins (C3a and C5a): stimulators of different inflammatory pathways (tumor necrosis factor-α, upregulation of histamine, cytokine, chemokine, and eicosanoid production)[15]; (2) opsonins (C3b, iC3b, and C3d): these molecules are responsible for the "scavenger" ability of complement by direct binding to the surface of target cells and immune complexes facilitating their elimination[16]; and (3) MAC: C5b-9 generation on cell membrane leads to opsonization, and finally to osmotic lysis of pathogens and damaged cells[17].

COMPLEMENT-MEDIATED DISEASES IN KIDNEY TRANSPLANTATION

In this section, we summarized renal transplant diseases where, at the present time, complement activation shows its involvement. In every paragraph we enunciate the effect of complement inhibition in the mitigation of the damage, if present; a separate section is dedicated to available treatments and future perspectives.

Ischemia-reperfusion injury

An important limitation in long-term outcomes of cadaveric transplants is the inevitable ischemia-reperfusion injury. In the past decade, the use of preservation fluids and machine-reperfusion instruments have expanded the availability of organs reducing delayed graft function, but did not abrogate this kind of damage. The ischemia-reperfusion injury remains, for the same age and risk factors, the corner-stone which differentiate living from deceased donors.

As known, the reperfusion of the graft activates some different processes, such as release of reactive oxygen species, alteration of endothelial permeability and gene transcription. All these conditions ended on an inflammatory infiltrate and kidney injury[18,19].

The complement cascade is activated in the ischemia-reperfusion mediated damage. The alternative pathway seems to explain the major role, as suggested by the absence of antibody deposition in kidney biopsy after reperfusion in animal models[20]. As a further confirmation, C3 and CFB deficient mice are resistant to ischemia-reperfusion injury, despite C4 knockout mice suffered from

this damage[21]. C3 expression after reperfusion, probably related to methylation of *C3* gene promoter as observed after cold ischemia[22], is associated with a diminished graft survival[23].

Recent data have shown that a kidney from a mice with C3 deficiency is not affected from ischemia reperfusion injury after transplantation in a normal mice, and per contrast, the inverted transplant combination is associated with damage, suggesting the idea that the C3 production in the kidney, and not circulating C3, can be considered[24].

In a swine model, the presence of MASP and MBL deposits after ischemia-reperfusion suggests also an activation of lectine pathway[25] and a more complex overall picture.

In humans, increased serum C5a and C5aR expression in kidney biopsy are both found in brain-dead donors[26], and also a transient MAC-release is demonstrated after reperfusion[27].

The blockage of complement cascade shows a protective effect: small interfering RNA (siRNA) against C5a receptor, C5a antagonists and C1q inhibitors display a limitation of ischemia-reperfusion injury in different models[21,25].

Atypical hemolytic uremic syndrome

Microangiopathic hemolysis, thrombocytopenia and renal damage characterize atypical hemolytic uremic syndrome (aHUS)[28], a condition with poor prognosis both on native and transplanted kidneys. aHUS has also an important recurrence rate in the graft (from 60% to 100%)[29], and this evidence paves the way to the application of preventive strategies to mitigate/abrogate this damage.

In the past decade, the identification of alternative pathway role in aHUS pathogenesis[30] has completely changed the treatment and management of aHUS. It is now well-known that an overactivation of alternative pathway explains almost all cases of aHUS[29,31].

Patients with mutations on C3 or on regulatory proteins complement factor H (CFH), complement factor I (CFI), complement factor B (CFB) experienced high recurrence rates[31]. Mutations on MCP or on diacylglycerol kinase-ε, on the contrary, exposed patients to a very low rate of recurrence, because the graft expressed normal proteins[31,32].

Patients who had autoantibodies against regulatory protein (*i.e.*, anti-CFH antibodies) are in midstream: surprisingly cases of recurrent aHUS are observed even when the immunosuppressive regimen have successfully reduced the antibody titer[33].

The role of eculizumab in aHUS, both on treatment and prevention of recurrences, is discussed separately. Here we anticipate that all previously adopted strategies (plasma infusion, abrogation or minimization of calcineurine inhibitors (CNI)-for its potential inductive effect in aHUS) failed in treatment and prevention of aHUS[29]; also mammalian target of rapamycin inhibitors, proposed as alternative to CNI, are recognized as an independently

associated risk factors for aHUS[34].

C3 glomerulopathies

One of the most important innovation in nephropathology is the introduction of C3 glomerulopathy definition. This entity is intended as a condition in which the immunohistochemical samples show C3 glomerular accumulation with scanty immunoglobulin deposition[35], including deposit dense disease and membranoproliferative type I and type III in which the C3 deposition is isolated or predominant[36].

This pathological reclassification implies a new clinical insight: C3 glomerulopathy is a complement-mediated condition where alternative pathway activation plays, as in aHUS, a pivotal role. Genetic and serological tests revealed CFH, CFI, MCP, C3 and factor B mutations in a low but significant percentage of patients, and positive C3 nephritic factor (an immunoglobulin able to stabilize C3bBb reducing its inactivation)[37]. In most cases, with or without positive genetic/serological tests, elevated levels of soluble C5b-9 are demonstrable, surrounding evidence of a smoldering complement activation[36,37].

The recurrence rate of C3 glomerulopathy on renal transplantation could be approximately estimated on about 60%, as derived from two small case series of Servais *et al*[37] and Little *et al*[38] and confirmed in the recent paper of Zand *et al*[39].

The treatment, or the prevention, of the recurrence is far from being defined. Anti-cellular immunosuppression with rituximab, cyclofosfamide or mycophenolate mofetil with or without plasma therapy are ineffective both in native and transplanted kidneys[40,41]; as observed in aHUS, a post-treatment negativization of C3 nephritic factor is not sufficient to prevent a recurrence in the graft[41]. As for aHUS, the role of eculizumab in C3 glomerulopathies treatment is discussed in a separate section. We anticipate that no clinical trial have already evaluate the potential role of anti-C5 drugs in disease prevention, but some papers suggest promising effects of Eculizumab in recurrence treatment[41,42].

Other glomerulonephritides

The role of complement activation is demonstrated in some different glomerulonephritides.

In animal model of membranous glomerulonephritis, for example, nephrotic-range proteinuria was abrogated after complement deprivation[43]. Recent studies in humans showed a direct correlation between podocyte injury and complement activation[44]. Recently, antibodies against the phospholipase A2 receptors (PLA2R) were correlated with idiopathic membranous nephritis[45]: curiously, the anti-PLA2R antibodies are mainly an IgG4, which is the subtype with a low ability in classical pathway activation.

Complement cascade is also directly involved in systemic lupus erythematous (SLE). Patients with genetic deficiency in classical pathway proteins may develop SLE[46] and complement products excretion (C5a and MAC) increased during disease "flares"[47].

Recently, an complement involvement has been demonstrated in other antibody-mediated nephritis, *i.e.*, ANCA-vasculitis[48], antiphospholipid antibody syndrome[49] and crioglobulinemic disease[50]. As a consequence, complement inhibition is a key point of interest in these conditions.

Renal allograft rejection

Complement exerts an effect in the immune activity, and C5a-C5aR interaction plays the critical role. It has been shown that natural regulatory T cells express both C5aR and C3aR, and a blockage of these receptors in mice induces a tolerance profile in autoimmune condition and organ transplants[51]. It was also reported that anaphylatoxins can stimulate T-cell proliferation and activation[52]. Moreover, an indirect confirmation of the alternative pathway causative role is suggested by the evidence that a murine model with C4 deficiency transplanted with a kidney graft derived from a donor with the same defect experienced a T-cell mediated rejection[53].

The cascade involvement in acute antibody mediated rejection (ABMR) represents an interesting issue, both for therapeutic and diagnostic point-of-views.

Anti-Human Leukocyte Antigen antibodies, especially against donor specific molecules (anti-DSA), are identified as the pivotal cause[54] of ABMR, but, at the same time, a wide spectrum of lesions in presence of positive DSA tests has been reported, ranging from nearly normal histological samples to acute and rapidly destructive rejections[55]. So, the differentiation between "bad" or "not so bad" antibodies acquired a growing interest.

Moreover, some data show that the "bad" activity of antibodies can be also involved in previously considered "chronic" lesions (*i.e.*, transplant glomerulopathy)[56,57] and the application of acute rejection therapeutic approaches in these conditions seems to be able to abrogate or at least mitigate the progression to chronic allograft disfunction[58,59].

We know that antibodies can activate complement cascade both with classical and lectin pathway. This activation leads to C4 cleavage and C4d deposition on peritubular capillaries, which is considered a "footprint" of both acute and chronic ABMR[60].

Recently, patients with C1q-binding antibodies demonstrates a poorer outcome than patients with no-fixing antibodies at 1 and 5 years, with a significant difference in acute rejection rates and C4d positive staining[61]. This evidence definitely paves the way to evaluate the efficacy of complement blockage drugs to prevent or treat antibody-related diseases.

The reduction in C4d deposition with a better long term outcomes in patients with an increased expression of CD55 provides a further confirmation[62].

ANTI-COMPLEMENT STRATEGIES

Eculizumab

Eculizumab (Soliris®, Alexion Pharma) is an anti-C5 fully humanized monoclonal antibody able to abrogate terminal complement activation[63]. Eculizumab completely

changed the prognosis and the clinical course of paroxysmal nocturnal hemoglobinuria, a complement-dependent disease[63,64]. Efficacy, well-toleration, absence of major side-effects and the potential usefulness in all condition in which complement is involved increased the interest about this drug worldwide.

An important precaution is to adopt a combined anti-meningococcal vaccination and antibiotic treatment, because MAC-inhibition is associated with a "splenectomy-like" effect (and a consequent high risk of infection) and already available vaccines are unable to cover all meningococcal subgroups, as demonstrated by the development of meningococcal infection in a patient vaccinated before eculizumab treatment[65].

At the present time 50 studies are evaluating the effect of this drug in different subsets: more in detail 10 of these refers to transplant area, 15 to nephrologic diseases.

Regarding to C3 glomerulopathy, McCaughan *et al*[41] successfully treated a patient with a recurrence of deposit dense disease which had no previous response to rituximab and plasmapheresis despite a normalization in C3 nephritic factor titer; Bomback *et al*[42] in the first trial, demonstrated a diffuse but not consistent response: best outcomes are obtained in subjects with high levels of serum C5b-9, suggesting the idea that patients with a "burst" of complement are the best candidate to this therapy. In renal transplantation, eculizumab has been successfully adopted in desentitization protocols[66] and, as a rescue treatment, in some cases of ABMR[67,68].

Regulatory agencies in United States and Europe (Food and Drug Administration and European Medicine Agency) approved eculizumab in aHUS treatment on native kidneys after struggle results of some experiences[69,70]. Also in renal transplantation eculizumab is effective in aHUS treatment[71] and recurrence prevention[72,73].

Future perspectives

Some different strategies of complement cascade blockage are part of ongoing trials. One research hypothesis is to silence the expression of C5a receptor. Mice treated with an infusion of siRNA have a prolonged graft survival, with reduced inflammatory response on kidney biopsies[74].

In a murine model of crescentic glomerulonephritis CCX168, a small molecule able to inhibit C5a receptor, abrogates extracapillary proliferation[48]. Inhibition of antibody-mediated complement activation can be also provided by stimulating the natural inhibitory activity, or interfering with other parts of classical pathway.

Berinert® (CSL Bhering) is a concentrate of C1q esterase approved for the treatment of ereditary angio-edema[75]: a prospective and randomized trial in sensitized patients [NCT01134510] is investigating its effect in reducing antibody-mediated rejection rate.

Some authors proposed Miroc.cept (APT070), a derived form of complement receptor 1 able to attenuate myocardial and intestinal ischemia-reperfusion damage in rats[76], as a promising option for the reduction of delayed graft function.

Other molecules (a C3 cyclic peptide inhibitor - POT-4, Alcon-, an anti-C5 antibody-LFG316, Novartis-, and a C5 aptamer-ARC1905, Ophthotech) are under investigation in age-related macular degeneration.

CONCLUSION

In the past few years we acquired an enormous amount of evidences that undoubtedly confirmed the central role of complement cascade in different pathologies and an inhibitor of complement activation, Eculizumab, shows is safety and usefulness, also in renal transplantation. However, the issue is far from settled.

At the present time, the next step is, not any more, if the use of the drug is a successful strategy, but when we have to consider this therapy and, nevertheless, how long. For example, in aHUS, eculizumab prevents the recurrence when used in induction regimen but, as previously reported on native kidneys, the risk of relapses in cases of suspension or prolongation of the treatment over the defined period is intolerable[63]. Moreover, the treatment of the relapse with a readoption of eculizumab fails in some cases[69]. If the drug is started only at the time of the recurrence, the outcomes were strictly time-dependent, with poorer results in patient treated afterwards[62]. Similar data are emerging in the treatment of other conditions, as C3 glomerulopathy[36]. In theory, a suspension is a safe option only in those conditions where complement activation is a transient phenomenon, as in ischemia-reperfusion damage. In antibody-mediated damage the question is still debating.

As known, a limit of the lifelong approach is the cost-per-patient, so the adoption of instruments able to individuate the subset of patients where a minimization/interruption of the drug could be safe (*i.e.*, functional or serological tests of complement activation) is one of the future challenges.

In conclusion, the re-discovery of the key role of complement activation and the availability of new drugs with a direct ability in complement inhibition are modifying our treatment protocols and offer a new potential amelioration in graft and patient survival rates. Long term outcomes and randomized clinical trials for the new indications are desirable, in view of a better therapy individualization.

REFERENCES

1   **Ehrlich P**, Morgenroth J. Zur Theorie der Lysenwirkung. *Berlin Klin Wchsr* 1899; **36**: 6
2   **Morse JH**, Muller-eberhard HJ, Kunkel HG. Anti-nuclear factors and serum complement in systemic lupus erythematosus. *Bull N Y Acad Med* 1962; **38**: 641-651 [PMID: 14476340]
3   **Polley MJ**, Müller-Eberhard HJ. Chemistry and mechanism of action of complement. *Prog Hematol* 1966; **5**: 2-25 [PMID: 5337574]
4   **Seelen MA**, Trouw LA, Daha MR. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. *Curr Opin Nephrol Hypertens* 2003; **12**: 619-624

[PMID: 14564199]

5   **Ma H**, Sandor DG, Beck LH. The role of complement in membranous nephropathy. *Semin Nephrol* 2013; **33**: 531-542 [PMID: 24161038]

6   **Sethi S**, Nester CM, Smith RJ. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. *Kidney Int* 2012; **81**: 434-441 [PMID: 22157657 DOI: 10.1038/ki.2011.399]

7   **Lai KN**. Pathogenesis of IgA nephropathy. *Nat Rev Nephrol* 2012; **8**: 275-283 [PMID: 22430056 DOI: 10.1038/nrneph.2012.58]

8   **Caprioli J**, Noris M, Brioschi S, Pianetti G, Castelletti F, Bettinaglio P, Mele C, Bresin E, Cassis L, Gamba S, Porrati F, Bucchioni S, Monteferrante G, Fang CJ, Liszewski MK, Kavanagh D, Atkinson JP, Remuzzi G. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood* 2006; **108**: 1267-1279 [PMID: 16621965]

9   **Denny KJ**, Woodruff TM, Taylor SM, Callaway LK. Complement in pregnancy: a delicate balance. *Am J Reprod Immunol* 2013; **69**: 3-11 [PMID: 22925193 DOI: 10.1111/aji.12000]

10   **Walport MJ**. Complement. First of two parts. *N Engl J Med* 2001; **344**: 1058-1066 [PMID: 11287977]

11   **Ehrnthaller C**, Ignatius A, Gebhard F, Huber-Lang M. New insights of an old defense system: structure, function, and clinical relevance of the complement system. *Mol Med* 2011; **17**: 317-329 [PMID: 21046060 DOI: 10.2119/molmed.2010.00149]

12   **Markiewski MM**, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. *Am J Pathol* 2007; **171**: 715-727 [PMID: 17640961]

13   **Smith RJ**, Harris CL, Pickering MC. Dense deposit disease. *Mol Immunol* 2011; **48**: 1604-1610 [PMID: 21601923 DOI: 10.1016/j.molimm.2011.04.005]

14   **Berger SP**, Daha MR. Complement in glomerular injury. *Semin Immunopathol* 2007; **29**: 375-384 [PMID: 17901956]

15   **Liu ZM**, Zhu SM, Qin XJ, Cheng ZD, Liu MY, Zhang HM, Liu DX. Silencing of C5a receptor gene with siRNA for protection from Gram-negative bacterial lipopolysaccharide-induced vascular permeability. *Mol Immunol* 2010; **47**: 1325-1333 [PMID: 20138669 DOI: 10.1016/j.molimm.2009.11.001]

16   **He JQ**, Wiesmann C, van Lookeren Campagne M. A role of macrophage complement receptor CRIg in immune clearance and inflammation. *Mol Immunol* 2008; **45**: 4041-4047 [PMID: 18752851 DOI: 10.1016/j.molimm.2008.07.011]

17   **Rother RP**, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. *Nat Biotechnol* 2007; **25**: 1256-1264 [PMID: 17989688]

18   **Kosieradzki M**, Kuczynska J, Piwowarska J, Wegrowicz-Rebandel I, Kwiatkowski A, Lisik W, Michalak G, Danielewicz R, Paczek L, Rowinski WA. Prognostic significance of free radicals: mediated injury occurring in the kidney donor. *Transplantation* 2003; **75**: 1221-1227 [PMID: 12717206]

19   **Thurman JM**, Lucia MS, Ljubanovic D, Holers VM. Acute tubular necrosis is characterized by activation of the alternative pathway of complement. *Kidney Int* 2005; **67**: 524-530 [PMID: 15673300]

20   **Park P**, Haas M, Cunningham PN, Bao L, Alexander JJ, Quigg RJ. Injury in renal ischemia-reperfusion is independent from immunoglobulins and T lymphocytes. *Am J Physiol Renal Physiol* 2002; **282**: F352-F357 [PMID: 11788450]

21   **Quigg RJ**. Complement and the kidney. *J Immunol* 2003; **171**: 3319-3324 [PMID: 14500622]

22   **Parker MD**, Chambers PA, Lodge JP, Pratt JR. Ischemia-reperfusion injury and its influence on the epigenetic modification of the donor kidney genome. *Transplantation* 2008; **86**: 1818-1823 [PMID: 19104428 DOI: 10.1097/TP.0b013e31818fe8f9]

23   **Damman J**, Nijboer WN, Schuurs TA, Leuvenink HG, Morariu AM, Tullius SG, van Goor H, Ploeg RJ, Seelen MA. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. *Nephrol Dial Transplant* 2011; **26**: 2345-2354 [PMID: 21127132 DOI: 10.1093/ndt/gfq717]

24   **Vieyra MB**, Heeger PS. Novel aspects of complement in kidney injury. *Kidney Int* 2010; **77**: 495-499 [PMID: 20016462 DOI: 10.1038/ki.2009.491]

25   **Castellano G**, Melchiorre R, Loverre A, Ditonno P, Montinaro V, Rossini M, Divella C, Battaglia M, Lucarelli G, Annunziata G, Palazzo S, Selvaggi FP, Staffieri F, Crovace A, Daha MR, Mannesse M, van Wetering S, Paolo Schena F, Grandaliano G. Therapeutic targeting of classical and lectin pathways of complement protects from ischemia-reperfusion-induced renal damage. *Am J Pathol* 2010; **176**: 1648-1659 [PMID: 20150432 DOI: 10.2353/ajpath.2010.090276]

26   **van Werkhoven MB**, Damman J, van Dijk MC, Daha MR, de Jong IJ, Leliveld A, Krikke C, Leuvenink HG, van Goor H, van Son WJ, Olinga P, Hillebrands JL, Seelen MA. Complement mediated renal inflammation induced by donor brain death: role of renal C5a-C5aR interaction. *Am J Transplant* 2013; **13**: 875-882 [PMID: 23398742 DOI: 10.1111/ajt.12130]

27   **de Vries DK**, van der Pol P, van Anken GE, van Gijlswijk DJ, Damman J, Lindeman JH, Reinders ME, Schaapherder AF, Kooten Cv. Acute but transient release of terminal complement complex after reperfusion in clinical kidney transplantation. *Transplantation* 2013; **95**: 816-820 [PMID: 23348894 DOI: 10.1097/TP.0b013e31827e31c9]

28   **Noris M**, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361**: 1676-1687 [PMID: 19846853 DOI: 10.1056/NEJMra0902814]

29   **Le Quintrec M**, Zuber J, Moulin B, Kamar N, Jablonski M, Lionet A, Chatelet V, Mousson C, Mourad G, Bridoux F, Cassuto E, Loirat C, Rondeau E, Delahousse M, Frémeaux-Bacchi V. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. *Am J Transplant* 2013; **13**: 663-675 [PMID: 23356914 DOI: 10.1111/ajt.12077]

30   **Noris M**, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nat Rev Nephrol* 2012; **8**: 622-633 [PMID: 22986360 DOI: 10.1038/nrneph.2012.195]

31   **Noris M**, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. *Am J Transplant* 2010; **10**: 1517-1523 [PMID: 20642678 DOI: 10.1111/j.1600-6143.2010.03156.x]

32   **Lemaire M**, Frémeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, Fakhouri F, Taque S, Nobili F, Martinez F, Ji W, Overton JD, Mane SM, Nürnberg G, Altmüller J, Thiele H, Morin D, Deschenes G, Baudouin V, Llanas B, Collard L, Majid MA, Simkova E, Nürnberg P, Rioux-Leclerc N, Moeckel GW, Gubler MC, Hwa J, Loirat C, Lifton RP. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet* 2013; **45**: 531-536 [PMID: 23542698 DOI: 10.1038/ng.2590]

33   **Dragon-Durey MA**, Sethi SK, Bagga A, Blanc C, Blouin J, Ranchin B, André JL, Takagi N, Cheong HI, Hari P, Le Quintrec M, Niaudet P, Loirat C, Fridman WH, Frémeaux-Bacchi V. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol* 2010; **21**: 2180-2187 [PMID: 21051740 DOI: 10.1681/ASN.2010030315]

34   **Sartelet H**, Toupance O, Lorenzato M, Fadel F, Noel LH, Lagonotte E, Birembaut P, Chanard J, Rieu P. Sirolimus-induced thrombotic microangiopathy is associated with decreased expression of vascular endothelial growth factor in kidneys. *Am J Transplant* 2005; **5**: 2441-2447 [PMID: 16162193]

35   **Fakhouri F**, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. *Nat Rev Nephrol* 2010; **6**: 494-499 [PMID: 20606628 DOI: 10.1038/nrneph.2010.85]

36   **Pickering MC**, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Frémeaux-Bacchi V, Gale DP, Goicoechea de Jorge E, Griffin G, Harris

CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodríguez de Córdoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT. C3 glomerulopathy: consensus report. *Kidney Int* 2013; **84**: 1079-1089 [PMID: 24172683 DOI: 10.1038/ki.2013.377]

37   **Servais A**, Noël LH, Roumenina LT, Le Quintrec M, Ngo S, Dragon-Durey MA, Macher MA, Zuber J, Karras A, Provot F, Moulin B, Grünfeld JP, Niaudet P, Lesavre P, Frémeaux-Bacchi V. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int* 2012; **82**: 454-464 [PMID: 22456601 DOI: 10.1038/ki.2012.63]

38   **Little MA**, Dupont P, Campbell E, Dorman A, Walshe JJ. Severity of primary MPGN, rather than MPGN type, determines renal survival and post-transplantation recurrence risk. *Kidney Int* 2006; **69**: 504-511 [PMID: 16395262]

39   **Zand L**, Lorenz EC, Cosio FG, Fervenza FC, Nasr SH, Gandhi MJ, Smith RJ, Sethi S. Clinical findings, pathology, and outcomes of C3GN after kidney transplantation. *J Am Soc Nephrol* 2014; **25**: 1110-1117 [PMID: 24357668]

40   **Nester CM**, Smith RJ. Treatment options for C3 glomerulopathy. *Curr Opin Nephrol Hypertens* 2013; **22**: 231-237 [PMID: 23318699 DOI: 10.1097/MNH.0b013e32835da24c]

41   **McCaughan JA**, O'Rourke DM, Courtney AE. Recurrent dense deposit disease after renal transplantation: an emerging role for complementary therapies. *Am J Transplant* 2012; **12**: 1046-1051 [PMID: 22233157 DOI: 10.1111/j.1600-6143.2011.03923.x]

42   **Bomback AS**, Smith RJ, Barile GR, Zhang Y, Heher EC, Herlitz L, Stokes MB, Markowitz GS, D'Agati VD, Canetta PA, Radhakrishnan J, Appel GB. Eculizumab for dense deposit disease and C3 glomerulonephritis. *Clin J Am Soc Nephrol* 2012; **7**: 748-756 [PMID: 22403278 DOI: 10.2215/CJN.12901211]

43   **Camussi G**, Noble B, Van Liew J, Brentjens JR, Andres G. Pathogenesis of passive Heymann glomerulonephritis: chlorpromazine inhibits antibody-mediated redistribution of cell surface antigens and prevents development of the disease. *J Immunol* 1986; **136**: 2127-2135 [PMID: 3485141]

44   **Nangaku M**, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. *J Am Soc Nephrol* 2005; **16**: 1195-1204 [PMID: 15800119]

45   **Beck LH**, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009; **361**: 11-21 [PMID: 19571279 DOI: 10.1056/NEJMoa0810457]

46   **Pickering MC**, Walport MJ. Links between complement abnormalities and systemic lupus erythematosus. *Rheumatology* (Oxford) 2000; **39**: 133-141 [PMID: 10725062]

47   **Kusunoki Y**, Akutsu Y, Itami N, Tochimaru H, Nagata Y, Takekoshi Y, Sagawa A, Kataoka Y, Nagasawa S. Urinary excretion of terminal complement complexes in glomerular disease. *Nephron* 1991; **59**: 27-32 [PMID: 1944744]

48   **Xiao H**, Dairaghi DJ, Powers JP, Ertl LS, Baumgart T, Wang Y, Seitz LC, Penfold ME, Gan L, Hu P, Lu B, Gerard NP, Gerard C, Schall TJ, Jaen JC, Falk RJ, Jennette JC. C5a receptor (CD88) blockade protects against MPO-ANCA GN. *J Am Soc Nephrol* 2014; **25**: 225-231 [PMID: 24179165 DOI: 10.1681/ASN.2013020143]

49   **Lonze BE**, Zachary AA, Magro CM, Desai NM, Orandi BJ, Dagher NN, Singer AL, Carter-Monroe N, Nazarian SM, Segev DL, Streiff MB, Montgomery RA. Eculizumab prevents recurrent antiphospholipid antibody syndrome and enables successful renal transplantation. *Am J Transplant* 2014; **14**: 459-465 [PMID: 24400968 DOI: 10.1111/ajt.12540]

50   **Gupta N**, Wang ES. Long-term response of refractory primary cold agglutinin disease to eculizumab therapy. *Ann Hematol* 2014; **93**: 343-344 [PMID: 23722501 DOI: 10.1007/s00277-013-1800-7]

51   **Kwan WH**, van der Touw W, Paz-Artal E, Li MO, Heeger PS. Signaling through C5a receptor and C3a receptor diminishes function of murine natural regulatory T cells. *J Exp Med* 2013; **210**: 257-268 [PMID: 23382542 DOI: 10.1084/jem.20121525]

52   **Strainic MG**, Liu J, Huang D, An F, Lalli PN, Muqim N, Shapiro VS, Dubyak GR, Heeger PS, Medof ME. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. *Immunity* 2008; **28**: 425-435 [PMID: 18328742 DOI: 10.1016/j.immuni.2008.02.001]

53   **Lin T**, Zhou W, Farrar CA, Hargreaves RE, Sheerin NS, Sacks SH. Deficiency of C4 from donor or recipient mouse fails to prevent renal allograft rejection. *Am J Pathol* 2006; **168**: 1241-1248 [PMID: 16565498]

54   **Terasaki PI**, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant* 2004; **4**: 438-443 [PMID: 14961999]

55   **Colvin RB**. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007; **18**: 1046-1056 [PMID: 17360947]

56   **Einecke G**, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, Kaplan B, Halloran PF. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520-2531 [PMID: 19843030 DOI: 10.1111/j.1600-6143.2009.02799.x]

57   **Sis B**, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen LC, Solez K, Baldwin WM, Bracamonte ER, Broecker V, Cosio F, Demetris AJ, Drachenberg C, Einecke G, Gloor J, Glotz D, Kraus E, Legendre C, Liapis H, Mannon RB, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Rodriguez ER, Seron D, Seshan S, Suthanthiran M, Wasowska BA, Zachary A, Zeevi A. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant* 2010; **10**: 464-471 [PMID: 20121738 DOI: 10.1111/j.1600-6143.2009.02987.x]

58   **Billing H**, Rieger S, Ovens J, Süsal C, Melk A, Waldherr R, Opelz G, Tönshoff B. Successful treatment of chronic antibody-mediated rejection with IVIG and rituximab in pediatric renal transplant recipients. *Transplantation* 2008; **86**: 1214-1221 [PMID: 19005402 DOI: 10.1097/TP.0b013e3181880b35]

59   **Rostaing L**, Guilbeau-Frugier C, Fort M, Mekhlati L, Kamar N. Treatment of symptomatic transplant glomerulopathy with rituximab. *Transpl Int* 2009; **22**: 906-913 [PMID: 19453996]

60   **Feucht HE**, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Riethmüller G, Land W, Albert E. Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; **43**: 1333-1338 [PMID: 8315947]

61   **Loupy A**, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, Suberbielle C, Frémeaux-Bacchi V, Méjean A, Desgrandchamps F, Anglicheau D, Nochy D, Charron D, Empana JP, Delahousse M, Legendre C, Glotz D, Hill GS, Zeevi A, Jouven X. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med* 2013; **369**: 1215-1226 [PMID: 24066742 DOI: 10.1056/NEJMoa1302506]

62   **Brodsky SV**, Nadasdy GM, Pelletier R, Satoskar A, Birmingham DJ, Hadley GA, Obeidat K, Nadasdy T. Expression of the decay-accelerating factor (CD55) in renal transplants--a possible prediction marker of allograft survival. *Transplantation* 2009; **88**: 457-464 [PMID: 19696627 DOI: 10.1097/TP.0b013e3181b0517d]

63   **Hillmen P**, Young NS, Schubert J, Brodsky RA, Socié G, Muus P, Röth A, Szer J, Elebute MO, Nakamura R, Browne P, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik CF, Rother RP, Luzzatto L. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2006; **355**: 1233-1243 [PMID: 16990386]

64   **McKeage K**. Eculizumab: a review of its use in paroxysmal nocturnal haemoglobinuria. *Drugs* 2011; **71**: 2327-2345 [PMID: 22085388 DOI: 10.2165/11208300-000000000-00000]

65   **Struijk GH**, Bouts AH, Rijkers GT, Kuin EA, ten Berge IJ, Bemelman FJ. Meningococcal sepsis complicating eculizumab treatment despite prior vaccination. *Am J Transplant* 2013; **13**: 819-820 [PMID: 23289494 DOI: 10.1111/ajt.12032]

66   **Stegall MD**, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405-2413 [PMID: 21942930 DOI: 10.1111/j.1600-6143.2011.03757.x]

67   **Kocak B**, Arpali E, Demiralp E, Yelken B, Karatas C, Gorcin S, Gorgulu N, Uzunalan M, Turkmen A, Kalayoglu M. Eculizumab for salvage treatment of refractory antibody-mediated rejection in kidney transplant patients: case reports. *Transplant Proc* 2013; **45**: 1022-1025 [PMID: 23622614 DOI: 10.1016/j.transproceed.2013.02.062]

68   **Ghirardo G**, Benetti E, Poli F, Vidal E, Della Vella M, Cozzi E, Murer L. Plasmapheresis-resistant acute humoral rejection successfully treated with anti-C5 antibody. *Pediatr Transplant* 2014; **18**: E1-E5 [PMID: 24266980 DOI: 10.1111/petr.12187]

69   **Nester CM**, Brophy PD. Eculizumab in the treatment of atypical haemolytic uraemic syndrome and other complement-mediated renal diseases. *Curr Opin Pediatr* 2013; **25**: 225-231 [PMID: 23486421 DOI: 10.1097/MOP.0b013e32835df4a3]

70   **Legendre CM**, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, Bingham C, Cohen DJ, Delmas Y, Douglas K, Eitner F, Feldkamp T, Fouque D, Furman RR, Gaber O, Herthelius M, Hourmant M, Karpman D, Lebranchu Y, Mariat C, Menne J, Moulin B, Nürnberger J, Ogawa M, Remuzzi G, Richard T, Sberro-Soussan R, Severino B, Sheerin NS, Trivelli A, Zimmerhackl LB, Goodship T, Loirat C. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **368**: 2169-2181 [PMID: 23738544 DOI: 10.1056/NEJMoa1208981]

71   **Zuber J**, Le Quintrec M, Krid S, Bertoye C, Gueutin V, Lahoche A, Heyne N, Ardissino G, Chatelet V, Noël LH, Hourmant M, Niaudet P, Frémeaux-Bacchi V, Rondeau E, Legendre C, Loirat C. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012; **12**: 3337-3354 [PMID: 22958221 DOI: 10.1111/j.1600-6143.2012.04252.x]

72   **Weitz M**, Amon O, Bassler D, Koenigsrainer A, Nadalin S. Prophylactic eculizumab prior to kidney transplantation for atypical hemolytic uremic syndrome. *Pediatr Nephrol* 2011; **26**: 1325-1329 [PMID: 21556717 DOI: 10.1007/s00467-011-1879-9]

73   **Nester C**, Stewart Z, Myers D, Jetton J, Nair R, Reed A, Thomas C, Smith R, Brophy P. Pre-emptive eculizumab and plasmapheresis for renal transplant in atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2011; **6**: 1488-1494 [PMID: 21617085 DOI: 10.2215/CJN.10181110]

74   **Zheng X**, Zhang X, Feng B, Sun H, Suzuki M, Ichim T, Kubo N, Wong A, Min LR, Budohn ME, Garcia B, Jevnikar AM, Min WP. Gene silencing of complement C5a receptor using siRNA for preventing ischemia/reperfusion injury. *Am J Pathol* 2008; **173**: 973-980 [PMID: 18772341 DOI: 10.2353/ajpath.2008.080103]

75   **Craig TJ**, Bewtra AK, Bahna SL, Hurewitz D, Schneider LC, Levy RJ, Moy JN, Offenberger J, Jacobson KW, Yang WH, Eidelman F, Janss G, Packer FR, Rojavin MA, Machnig T, Keinecke HO, Wasserman RL. C1 esterase inhibitor concentrate in 1085 Hereditary Angioedema attacks--final results of the I.M.P.A.C.T.2 study. *Allergy* 2011; **66**: 1604-1611 [PMID: 21884533 DOI: 10.1111/j.1398-9995.2011.02702.x]

76   **Souza DG**, Esser D, Bradford R, Vieira AT, Teixeira MM. APT070 (Mirococept), a membrane-localised complement inhibitor, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury. *Br J Pharmacol* 2005; **145**: 1027-1034 [PMID: 15951831]

Kidney transplantation in patients with systemic lupus erythematosus

Sophia Lionaki, Chrysanthi Skalioti, John N Boletis

Sophia Lionaki, Chrysanthi Skalioti, John N Boletis, Nephrology Department and Transplantation Unit, Laiko Hospital, 11527 Athens, Greece
Author contributions: All authors contributed to this work.
Correspondence to: Sophia Lionaki, MD, Nephrology Department and Transplantation Unit, Laiko Hospital, 17 Ag. Thoma Street, 11527 Athens, Greece. sofia.lionaki@gmail.com
Telephone: +30-21-07456000

Abstract

Despite improvements in overall prognosis in lupus nephritis, 10%-30% of patients with proliferative renal involvement progress to end stage renal disease, according to the severity of the disease and associated socioeconomic factors. Kidney transplantation has been recognized as the most appropriate treatment for those patients, but several issues remain after renal function restoration in a lupus recipient. Among these are the fear of lupus nephritis recurrence in the graft, the choice of immunosuppressive therapy in cases of recurrent lupus for a patient who has already received a toxic and prolonged immunosuppressive course, and finally, the management of comorbidities to reduce associated morbidities in the long term. All the above topics are examined in this review, with the hope of providing a clear picture of data as illustrated in the current literature.

Key words: Kidney transplantation; Lupus; Recurrence

Core tip: Significant improvement has been made in the management of lupus nephritis, but 10%-30% of patients with proliferative renal involvement still progress to end stage renal disease, according to the severity of the disease and associated socioeconomic factors. Kid-

ney transplantation has been recognized as the most appropriate treatment for those patients, but several issues remain after renal function restoration in a lupus recipient. This review provides insights into topics such as the frequency of lupus nephritis recurrence in the graft, the choice of immunosuppressive therapy in such cases and the management of comorbidities to reduce associated morbidities in the long term.

INTRODUCTION

Although significant progress has been noted over the last few decades in the treatment of lupus nephritis, the incidence of end stage renal disease (ESRD) has increased significantly between 1982 and 2004; from 1.16 cases per million person-years in 1982 to 3.08 and 4.9 cases per million person-years in 1995 and 2004, respectively[1,2]. Kidney transplantation (KTX) is the treatment of choice for patients with incident ESRD. However, current practice for those who progress to ESRD as a result of exacerbation of lupus nephritis or newly diagnosed lupus with rapidly progressive renal disease is to start with hemodialysis (HD). The rationale is to suppress any residual lupus activity, to allow the disease to become quiescent, mostly in those patients who experience a rapid decline in renal function due to aggressive lupus. Remission of lupus overall is particularly important before proceeding to transplantation, and thus, all patients with recent significant renal or extra-renal activity and ESRD begin with HD. One potential benefit from this choice is the believed "burn-out" effect of this modality on the disease[3]. Secondly, 3 to 6 mo on dialysis, before proceed-

ing to transplantation, seem to be sufficient for renal function to recover in individuals with rapidly progressive glomerulonephritis due to lupus. In contrast, patients who are in complete remission for a considerable time period prior to ESRD, may also precede with preemptive KTX, if there is an appropriate living donor. This practice is supported by analysis of the United Network for Organ Sharing dataset from 1987 to 2009[4], which revealed that patients with lupus nephritis who received a kidney transplant preemptively, before the need for dialysis, presented better graft survival and a lower risk of recipient death. It was associated with superior patient and graft outcomes[4].

RENAL REPLACEMENT THERAPY FOR LUPUS PATIENTS WITH ESRD

Very few, small, retrospective studies have compared dialysis modalities to KTX in terms of cumulative survival and long-term clinical outcomes of patients. Goo *et al*[5] studied 45 systemic lupus erythematosus (SLE) patients who initiated HD, peritoneal dialysis (PD) or KTX, between 1990 and 2000, in Korea. Disease activity, defined by the SLE Disease Activity Index, was significantly higher after PD during the 4.5 years of follow-up (HD: 5.0 ± 3.6, PD: 7.4 ± 3.7, KTX: 2.2 ± 1.7), whereas survival rates were similar in the three groups[4]. Thirteen patients died during the observation period, mainly due to infections, but the type of renal replacement therapy they were under was not specified[5]. A clear superiority of KTX, in terms of survival and complication rates, has been shown in a retrospective multicenter study by Kang *et al*[6]. Ten-year survival in 59 individuals, who underwent KTX, PD or HD for lupus nephritis, was 90%, 81% and 55%, respectively. It should be noted however, that the transplantation group consisted of relatively younger patients. Lupus flare-up was absent among transplanted patients (0% *vs* 50% *vs* 14%) during the study period. Higher mortality rates were recorded in the HD group and they were attributed mainly to cardiovascular disease and malignancies. Only one transplanted patient died during the 10-year follow-up[6]. Current practice in the United States population is depicted in a large analysis by Costenbader *et al*[7], based on the United States Renal Data System. Between 1995 and 2006, 12344 patients with ESRD related to lupus nephritis were identified. Hemodialysis was the most commonly used renal replacement therapy with a significant increase during the study period (from 75.9% to 83.9%). Despite an increase in the number of living donors, KTX rates decreased markedly from 1995 to 2006, a fact that could be associated with donor organ shortage and the low socioeconomic status of several patients. Nevertheless, survival of patients undergoing KTX has shown absolutely no improvement over the 12 years of the study[7]. Patients suffering from ESRD secondary to lupus nephritis are younger[7,8] and severely immunocompromised due to the underlying disease and the prolonged use of immunosuppressive therapy, resulting

in several comorbidities. Thus, when planning the future of these patients in terms of renal replacement therapy, every effort should be made towards improving survival rates and quality of life.

Finally, PD is a better choice for initiating renal replacement therapy in patients with lupus and antiphospholipid syndrome, since access failure due to recurrent thrombosis is a major problem in this group of patients.

LUPUS ACTIVITY IN PATIENTS WITH ESRD

As mentioned above, from the time SLE patients progress to ESRD and start dialysis, clinical and serological activity of lupus is known to decrease. Longstanding clinical experience and research have shown that patients with renal failure resulting from lupus frequently experience a remission of their extra-renal manifestations and improvement in lupus serologic results with dialysis so that all immunosuppression can be withdrawn[3,9,10]. Fries *et al*[3] first described this quiescence of lupus in patients with ESRD and termed it "burnt-out lupus". Several studies have since reported significant improvement in all autoimmune phenomena following commencement of dialysis. Mojcik *et al*[11] found that the prevalence of patients with clinical lupus activity in the post-dialysis period diminished over time: 55%, 6.5% and 0% after 1, 5 and 10 years, respectively. They found that the serological activity in lupus was not necessarily correlated with clinical activity and that it was a more frequent condition present in 80%, 60% and 22% of the patients after 1, 5 and 10 years, respectively[11]. Although the causes of this phenomenon are not completely understood, it is repeatedly reported and in many cases associated with gradual or partial resolution of the extra-renal manifestations of lupus[12-14]. Less frequently, and typically in patients of black race, some investigators have reported continuation of lupus activity and occasionally exacerbation with the onset of ESRD[15]. However, these results are better understood if we consider that they refer to varying patient populations and genetic profiles.

INCIDENCE OF RECURRENT LUPUS NEPHRITIS IN THE ALLOGRAFT

The standard concern in lupus patients who undergo KTX is lupus nephritis recurrence in the graft. Variable rates of recurrent lupus nephritis (RLN) have been reported, ranging from 0% to 44%[16-33] with certain differences, *i.e.*, patients' characteristics, the era of immunosuppression and the indications for renal biopsies such as protocol biopsies or serial biopsies, accounting for this. Moreover, it is essential to distinguish clinically apparent RLN in the allograft from incident histopathological findings attributable to a lupus effect in the graft without any concurrent clinical, renal or extra-renal, symptoms or signs of lupus, and thus with questionable clinical impor-

tance. The setting of RLN in transplant patients generally include renal dysfunction, either an acute increase in serum creatinine, or a slow increase compared with the baseline value, or new onset proteinuria or glomerular hematuria or both.

Clinically apparent RLN is found at an incidence rate of 2%-11%[10,23-33]. The results from the largest cohort of patients indicate that RLN in the graft is uncommon[28]. Specifically, 2.44% of 6850 recipients with SLE, transplanted between 1987 and 2006, developed RLN[28]. Similarly, Stone *et al*[33] in an earlier period, showed a rate of RLN in the graft of 2%. Burgos *et al*[32] found an overall rate of RLN in 11% of 177 patients with SLE who underwent KTX. In our experience, RLN in the graft was documented in 7.7% of patients who underwent a graft biopsy according to clinical indication[28]. One of these patients in our population experienced graft loss due to lupus. Moroni *et al*[34] reported recurrence of lupus nephritis in 8.6% of the grafts, but no graft was lost because of RLN.

CLINICOPATHOLOGIC CORRELATIONS

Incidence rates of "subclinical" lupus nephritis in the allograft, *i.e.*, histopathological findings in protocol or serial biopsies, differed substantially from those which were performed solely according to clinical indication. The use of light microscopy alone, in the examination of biopsy specimens likely yielded a significantly lower rate of diagnosis of RLN. However, it was quickly recognized that transplant kidney biopsy specimens from patients with a history of ESRD as a result of lupus must additionally be evaluated by both immunofluorescence and electron microscopy. The diagnosis of lupus nephritis in the renal allograft should ideally be established after complete examination of the biopsy using the World Health Organization (WHO) or the International Society of Nephrology/Renal Pathology Society Histologic Classifications[35], including a positive immunofluorescence microscopy, and/or the presence of electron dense deposits in the electron microscopic assessment. In this regard, when both immunofluorescence and electron microscopy were used for the evaluation of renal biopsies, along with a more aggressive protocol of graft biopsies, it was shown that 30% of the patients experienced recurrence of lupus nephritis[26]. In the study by Goral *et al*[26], graft survival was not influenced by recurrent disease, and the authors recommended complete morphologic study of the specimen using immunofluoresce and electron microscopy[26]. Similarly, Nyberg *et al*[21], in an earlier study of 16 patients with SLE, had shown that 44% had biopsy-proven RLN. Interestingly, light microscopy findings in biopsy specimens obtained from these patients were not diagnostic for RLN, but it was diagnosed by the combined use of immunofluorescence and electron microscopy[20]. Ultrastructural evaluation with electron microscopy can be crucial in the diagnosis of patients with SLE, especially if they do not have any strong clinical manifestations and

serologic markers at the time of the kidney biopsy[26]. Norby *et al*[36], in a cross sectional study of lupus patients with surveillance biopsies after KTX, showed that subclinical recurrence was frequent, with 54% of the patients having RLN. The type and extent of renal involvement and activity is adequately characterized by electron microscopy in such cases[37]. Additionally, combined evaluation with immunofluorescence and electron microscopy may play a role in distinguishing RLN from other accompanying renal abnormalities, such as acute rejection, which may also associated with mild glomerulitis[37].

Time to RLN may also vary from days to decades after KTX. Goral *et al*[26], demonstrated that RLN can occur as early as 6 d after transplantation. Conversely, there are reports of very late recurrence, up to 16 years post transplantation[29]. In the same study, however, the median time to recurrence was 4.3 years post KTX[29]. Moreover, the histopathologic lesion may be different from the one in the native kidney, and most frequently is less severe[4,5,32]. Nevertheless, given the silent nature of many of the recurrences, it is impossible to determine the precise timing of recurrence, or the rate of recurrence in patients who did not undergo biopsies.

RISK FACTORS FOR RECURRENT LUPUS NEPHRITIS

The importance to practicing nephrologists of lupus recurrence in the renal graft is that these patients may have poorer outcomes compared with other kidney transplant recipients. However, a report of the American College of Surgeons/National Institute of Health Transplant Registry in 1975 opened the door to KTX for patients with lupus, as it was observed that they had outcomes comparable to those of non-lupus patients[16]. Following this observation, there have been numerous studies which have found that overall 5- and 10-year graft survival rates are similar[11,13,14,29,34,36,38-41] or comparable[25,29] among patients with lupus and those with other primary diseases. Recognized risk factors for allograft loss in lupus patients include black non-Hispanic ancestry, female gender, and young age[29,32]. Patients with antiphospholipid autoantibodies[34] and those receiving the kidney from living donors[36] also have a higher risk of recurrence. African American ethnicity was shown to be independently associated with RLN in the allograft and possibly with diminished survival[32]. Dooley *et al*[42] had earlier shown that, compared with Caucasians, lupus nephritis developed more frequently in African Americans (60% *vs* 25%). Furthermore, African Americans displayed a less favorable response to treatment and thus the disease progressed to ESRD at a higher rate[42]. Consistent with this, Contreras *et al*[29] found that black non-Hispanic and female recipients had, respectively, 1.88- and 1.70-fold increased risk for the development of RLN. The same study demonstrated that recipients younger than 33 years had a 1.69-fold increased risk for the development

of RLN. Onset of SLE and ESRD at a younger age in a black woman usually predicts a more aggressive disease[29]. Recurrent lupus nephritis and chronic rejection of the kidney were shown to be risk factors for allograft loss (HR = 2.48; 95%CI: 1.09-5.60 and HR = 2.72; 95%CI: 1.55-4.78, respectively) in the study by Burgos *et al*[32]. However, Stone *et al*[33] had found that RLN did not invariably result in allograft failure. The same study demonstrated that short time period of pre-transplantation dialysis, *i.e.*, less than 6 mo, had no adverse effect on KTX outcome in 10 of 11 studies that examined the relationship[33].

DIAGNOSIS AND TREATMENT OF RLN IN THE ALLOGRAFT

RLN in the allograft should be suspected in any patient who progresses to ESRD due to renal lupus, in the light of certain clinical and/or laboratory findings. Thus, new onset proteinuria or glomerular hematuria should directly lead to the suspicion of lupus nephritis in the allograft. However, rapid worsening of previously existing proteinuria should also raise the suspicion for RLN, especially with the coexistence of glomerular hematuria. The clinical presentation of increased serum creatinine is also typical of patients with RLN in the graft. However, among all transplant recipients who present with an elevated serum creatinine, there are certain other parameters that need to be excluded as possible contributors before considering a diagnosis of RLN. These include dehydration, toxic concentrations of serum calcineurin inhibitors, and obstructive uropathy. Diagnosis of RLN is made by biopsy and histopathologic evaluation by light microscopy, immunofluorescence and electron microscopy as discussed earlier. Measurement of serologic parameters, such as complement levels and titers of anti-double stranded DNA antibodies is not helpful in establishing the diagnosis in the allograft[24,33].

Immunosuppressive therapy

Kidney transplant recipients with recurrent lupus usually do not require any change in the immunosuppressive regimen, as they already receive maintenance therapy for the transplant. Most are shown to have mild lesions in the graft due to lupus, as demonstrated by surveillance biopsy studies[36]. Specifically, most of the patients had subclinical disease of class I or II in the biopsy[36]. On the other hand, the vast majority of patients had chronic allograft nephropathy (84%)[43], while they were also susceptible to calcineurin inhibitor toxicity, as are SLE patients who have not had transplants[42]. Besides, the major impediment to the goal of improved kidney transplant survival is a cumulative and progressive immune and non-immune injury[43,44]. In kidney transplant recipients with SLE, standard immunosuppression with a calcineurin inhibitor, mycophenolate mofetil and prednisone seems to protect against clinically overt recurrent disease, but not against chronic allograft nephropathy[36]. Importantly, indices of

lupus activity were found to be low in the study by Norby *et al*[36], and did not differentiate between patients with RLN and those without recurrence.

However, there are select patients who require additional immunosuppressive treatment. In terms of renal involvement, these mostly include patients with clinically evident disease and severe histopathologic lesions, consistent with WHO class III or IV[35] in the graft. Among patients who have a histologic diagnosis of recurrent lupus in the graft, along with rapid deterioration of renal function, all other factors associated with acute renal dysfunction in a kidney transplant recipient should be excluded at first, *i.e.*, acute rejection, chronic allograft nephropathy, calcineurin inhibitor toxicity. Following this workup, any lupus patient with new onset proteinuria or worsening proteinuria and/or hematuria in the presence of severe proliferative lesions in the graft biopsy requires the existing immunosuppressive regimen to be modified. Depending on the clinical picture and morphologic lesions, we use either higher doses of mycophenolate mofetil or (2-3 g/d), or initiate cyclophosphamide intravenously along with discontinuation of the current antimetabolite. Both of these options are always accompanied by glucocorticoids, usually pulses of methylprednisolone, 500-1000 mg/d for 3 consecutive days, which are followed by a tapering steroid regimen. We tend to choose cyclophosphamide in cases who present with rapid renal deterioration combined with a crescentic pattern in histology, and in all cases with severe extra renal disease, *i.e.*, pulmonary hemorrhage, central nervous system involvement, or any other life-threatening phenomenon attributable to lupus. Although there is not sufficient data to support the use of the aforementioned agents in the lupus transplant recipient with recurrence, this approach is based upon studies of patients with lupus nephritis involving the native kidney[45]. Similarly, the optimal scheme of cyclophosphamide for these patients, who already carry a considerable load of immunosuppression starting from their initial diagnosis of SLE, is not known. One reasonable option is the Euro lupus regimen, which consists of 6 intravenous pulses of 0.5 g cyclophosphamide[46]. Patients of black ethnicity may need more aggressive therapy, with monthly courses of cyclophosphamide of 0.5-1 g/m^2 body surface area. In any case, the leukocyte nadir is the main guide of therapy, *i.e.*, less than 4000/μL and absolute neutrophil count less than 1500/μL. However, the maximum dose of cyclophosphamide should not exceed 1 g/m^2 body surface area.

In cases of resistant RLN despite the use of mycophenolate mofetil and cyclophosphamide, the only treatment option is rituximab along with an increase in glucocorticoids. Nevertheless, there are no published data to support the use of rituximab in RLN among kidney transplant recipients, but only a few observational studies and case reports of the successful use of rituximab in patients who suffer from lupus nephritis in the native kidney and are resistant to mycophenolate mofetil and cyclophosphamide[45,47-49]. As the optimal scheme is not

known, one may use the same dose as for lupus nephritis in the native kidney, *i.e.*, 1 g given on days 1 and 15, or 375 mg/m² body surface area as given in antineutrophil cytoplasmic autoantibodies glomerulonephritis[50,51]. Yet, even with data on a considerable number of patients treated with rituximab, we should always keep in mind that the long-term toxicity has not been completely defined in the transplant population[51].

Non-immunosuppressive therapy

We treat all patients with histopathologic changes of RLN in the graft and protein excretion, typically more than 0.5 g/d with renin angiotensin system blockade. The notion for this approach is based upon studies in nontransplant patients with chronic kidney disease and proteinuria, where it has been shown that renin angiotensin system blockade decreases the progression of renal disease[52]. Anemia and hyperkalemia may be seen with the angiotensin converting enzymes inhibitors, but this issue is usually circumvented by the fact that transplant recipients are generally closely followed and such side effects are readily detected. Moreover, the long-term benefit from renin angiotensin system blockade outweighs the risk of experiencing such events.

MANAGEMENT OF COMORBIDITIES

Death with a functioning graft is a major cause of renal allograft loss in the general population. The same applies to recipients with ESRD due to lupus nephritis, and is mainly attributed to cardiovascular disease[53].

Cardiovascular disease

Although SLE predominantly affects young females of childbearing age, studies point out that the disease is characterized by an accelerated atherosclerotic mechanism, which increases cardiovascular morbidity and mortality[53,54]. Furthermore, the study by Costenbader *et al*[7], recorded an increased trend towards cardiovascular risk factors, namely smoking, obesity, diabetes mellitus and hypertension in incident SLE-related ESRD patients, in the years 1995-2006. Several small, mostly retrospective, single-center studies with limited numbers of patients indicate cardiovascular disease as the leading cause of morbidity and mortality in transplanted patients secondary to lupus nephritis[28,47,55]. A retrospective analysis of data from the United States Renal Data System and the United Network for Organ Sharing was conducted between 1990 and 1999. Among 2886 patients with lupus nephritis undergoing a renal transplant, cardiac events and cerebrovascular disease were the main causes of death. However, non-SLE recipients (*n* = 89958) exhibited a higher rate of these comorbidities, probably because they were older, with a higher prevalence of pre-existing cardiovascular disease and diabetes mellitus[55]. According to data from the National Transplant Center in Norway, 77 patients with SLE underwent first and subsequent KTX from 1972 to 2005. They were compared with 154

matched non-SLE transplanted patients. Norby *et al*[56] also found that the main cause of death was cardiovascular disease, with acute myocardial infarction as the major factor. Notably, death from cardiac-associated events occurred much earlier in SLE patients compared to the control group (median time: 3.9 years *vs* 13.0 years).

No specific data exist to date comparing morbidity between deceased and living donor KTX in this patient population, although living donation in these patients has been associated with improved patient and graft survival[41].

Other comorbid conditions

An area of major concern has always been a previous history of antiphospholipid antibody syndrome (APAS), or solely the presence of these antibodies in this patient population, because of the risk of graft or other vascular thrombosis. Vaidya *et al*[57], recently showed that the 10-year renal allograft survival is significantly lower among patients with APAS, compared with those who have only circulating antibodies. Careful monitoring is mandatory in order to avoid thrombotic episodes.

Infections (sepsis, pneumonia, viral infections, fungal infections, tuberculosis, urinary tract infections) have been reported as causes of morbidity and mortality after KTX due to lupus nephritis[6,28,47,53-55]. One could hypothesize that prolonged exposure to immunosuppressive agents prior to ESRD, as well after ESRD and KTX predisposes to infections. However, published data are contradictory as the prevalence of serious infections is not always higher in SLE recipients compared with non-SLE patients[6,57].

Malignancies, orthopedic complications, such as avascular necrosis of the femur head and osteoporosis have been rarely reported in various studies as late complications in kidney transplant SLE recipients[6,47].

REFERENCES

1   **Ward MM**. Changes in the incidence of end-stage renal disease due to lupus nephritis, 1982-1995. *Arch Intern Med* 2000; **160**: 3136-3140 [PMID: 11074743 DOI: 10.1001/archinte.160.20.3136]

2   **Ward MM**. Changes in the incidence of endstage renal disease due to lupus nephritis in the United States, 1996-2004. *J Rheumatol* 2009; **36**: 63-67 [PMID: 19004042]

3   **Fries JF**, Powers R, Kempson RL. Late-stage lupus nephropathy. *J Rheumatol* 1974; **1**: 166-175 [PMID: 4617003]

4   **Naveed A**, Nilubol C, Melancon JK, Girlanda R, Johnson L, Javaid B. Preemptive kidney transplantation in systemic lupus erythematosus. *Transplant Proc* 2011; **43**: 3713-3714 [PMID: 22172832 DOI: 10.1016/j.transproceed.2011.08.092]

5   **Goo YS**, Park HC, Choi HY, Kim BS, Park YB, Lee SK, Kang SW, Kim SI, Kim YS, Park KI, Lee HY, Han DS, Choi KH. The evolution of lupus activity among patients with endstage renal disease secondary to lupus nephritis. *Yonsei Med J* 2004; **45**: 199-206 [PMID: 15118989]

6   **Kang SH**, Chung BH, Choi SR, Lee JY, Park HS, Sun IO, Choi BS, Park CW, Kim YS, Yang CW. Comparison of clinical outcomes by different renal replacement therapy in patients with end-stage renal disease secondary to lupus nephritis. *Korean J Intern Med* 2011; **26**: 60-67 [PMID: 21437164 DOI: 10.3904/kjim.2011.26.1.60]

7   **Costenbader KH**, Desai A, Alarcón GS, Hiraki LT, Shaykev-

ich T, Brookhart MA, Massarotti E, Lu B, Solomon DH, Winkelmayer WC. Trends in the incidence, demographics, and outcomes of end-stage renal disease due to lupus nephritis in the US from 1995 to 2006. *Arthritis Rheum* 2011; **63**: 1681-1688 [PMID: 21445962 DOI: 10.1002/art.30293]

8 **Farrington K**, Rao R, Gilg J, Ansell D, Feest T. New adult patients starting renal replacement therapy in the UK in 2005 (chapter 3). *Nephrol Dial Transplant* 2007; **22** Suppl 7: vii11-vii29 [PMID: 17724040]

9 **Cheigh JS**, Stenzel KH, Rubin AL, Chami J, Sullivan JF. Systemic lupus erythematosus in patients with chronic renal failure. *Am J Med* 1983; **75**: 602-606 [PMID: 6353915 DOI: 10.1016/0002-9343(83)90440-0]

10 **Correia P**, Cameron JS, Ogg CS, Williams DG, Bewick M, Hicks JA. End-stage renal failure in systemic lupus erythematosus with nephritis. *Clin Nephrol* 1984; **22**: 293-302 [PMID: 6396007]

11 **Mojcik CF**, Klippel JH. End-stage renal disease and systemic lupus erythematosus. *Am J Med* 1996; **101**: 100-107 [PMID: 8686702 DOI: 10.1016/S0002-9343(96)00074-5]

12 **Cheigh JS**, Stenzel KH. End-stage renal disease in systemic lupus erythematosus. *Am J Kidney Dis* 1993; **21**: 2-8 [PMID: 8418620 DOI: 10.1016/S0272-6386(12)80712-8]

13 **Nossent HC**, Swaak TJ, Berden JH. Systemic lupus erythematosus after renal transplantation: patient and graft survival and disease activity. The Dutch Working Party on Systemic Lupus Erythematosus. *Ann Intern Med* 1991; **114**: 183-188 [PMID: 1984742 DOI: 10.7326/0003-4819-114-3-183]

14 **Ponticelli C**, Moroni G. Renal transplantation in lupus nephritis. *Lupus* 2005; **14**: 95-98 [PMID: 15732296 DOI: 10.1191/0961203305lu2067oa]

15 **Krane NK**, Burjak K, Archie M, O'donovan R. Persistent lupus activity in end-stage renal disease. *Am J Kidney Dis* 1999; **33**: 872-879 [PMID: 10213642 DOI: 10.1016/S0272-6386(99)70419-1]

16 Renal transplantation in congenital and metabolic diseases. A report from the ASC/NIH renal transplant registry. *JAMA* 1975; **232**: 148-153 [PMID: 804049 DOI: 10.1001/jama.1975.03250020022018]

17 **Amend WJ**, Vincenti F, Feduska NJ, Salvatierra O, Johnston WH, Jackson J, Tilney N, Garovoy M, Burwell EL. Recurrent systemic lupus erythematosus involving renal allografts. *Ann Intern Med* 1981; **94**: 444-448 [PMID: 7011137 DOI: 10.7326/0003-4819-94-4-444]

18 **Cameron JS**. Glomerulonephritis in renal transplants. *Transplantation* 1982; **34**: 237-245 [PMID: 6760479 DOI: 10.1097/00007890-198211000-00001]

19 **Coplon NS**, Diskin CJ, Petersen J, Swenson RS. The long-term clinical course of systemic lupus erythematosus in end-stage renal disease. *N Engl J Med* 1983; **308**: 186-190 [PMID: 6336825 DOI: 10.1056/NEJM198301273080403]

20 **Kotanko P**, Pusey CD, Levy JB. Recurrent glomerulonephritis following renal transplantation. *Transplantation* 1997; **63**: 1045-1052 [PMID: 9133463 DOI: 10.1097/00007890-199704270-00001]

21 **Nyberg G**, Blohmé I, Persson H, Olausson M, Svalander C. Recurrence of SLE in transplanted kidneys: a follow-up transplant biopsy study. *Nephrol Dial Transplant* 1992; **7**: 1116-1123 [PMID: 1336139]

22 **Roth D**, Milgrom M, Esquenazi V, Strauss J, Zilleruelo G, Miller J. Renal transplantation in systemic lupus erythematosus: one center's experience. *Am J Nephrol* 1987; **7**: 367-374 [PMID: 3324763 DOI: 10.1159/000167615]

23 **Bumgardner GL**, Mauer SM, Payne W, Dunn DL, Sutherland DE, Fryd DS, Ascher NL, Simmons RL, Najarian JS. Single-center 1-15-year results of renal transplantation in patients with systemic lupus erythematosus. *Transplantation* 1988; **46**: 703-709 [PMID: 3057693 DOI: 10.1097/00007890-198811000-00015]

24 **Stone JH**, Amend WJ, Criswell LA. Outcome of renal transplantation in ninety-seven cyclosporine-era patients with

systemic lupus erythematosus and matched controls. *Arthritis Rheum* 1998; **41**: 1438-1445 [PMID: 9704643]

25 **de Azevedo LS**, Romão Júnior JE, Ianhez LE, Saldanha LB, Sabbaga E. [Renal transplantation in patients with disseminated lupus erythematosus]. *AMB Rev Assoc Med Bras* 1988; **34**: 48-53 [PMID: 3072598]

26 **Goral S**, Ynares C, Shappell SB, Snyder S, Feurer ID, Kazancioglu R, Fogo AB, Helderman JH. Recurrent lupus nephritis in renal transplant recipients revisited: it is not rare. *Transplantation* 2003; **75**: 651-656 [PMID: 12640304 DOI: 10.1097/01.TP.0000053750.59630.83]

27 **Yu TM**, Chen YH, Lan JL, Cheng CH, Chen CH, Wu MJ, Shu KH. Renal outcome and evolution of disease activity in Chinese lupus patients after renal transplantation. *Lupus* 2008; **17**: 687-694 [PMID: 18625644 DOI: 10.1177/0961203308089439]

28 **Lionaki S**, Kapitsinou PP, Iniotaki A, Kostakis A, Moutsopoulos HM, Boletis JN. Kidney transplantation in lupus patients: a case-control study from a single centre. *Lupus* 2008; **17**: 670-675 [PMID: 18625640 DOI: 10.1177/0961203308089430]

29 **Contreras G**, Mattiazzi A, Guerra G, Ortega LM, Tozman EC, Li H, Tamariz L, Carvalho C, Kupin W, Ladino M, LeClercq B, Jaraba I, Carvalho D, Carles E, Roth D. Recurrence of lupus nephritis after kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1200-1207 [PMID: 20488956 DOI: 10.1681/ASN.2009101093]

30 **Weng F**, Goral S. Recurrence of lupus nephritis after renal transplantation: if we look for it, will we find it? *Nat Clin Pract Nephrol* 2005; **1**: 62-63 [PMID: 16932366 DOI: 10.1038/ncpneph0028]

31 **Choy BY**, Chan TM, Lai KN. Recurrent glomerulonephritis after kidney transplantation. *Am J Transplant* 2006; **6**: 2535-2542 [PMID: 16939521 DOI: 10.1111/j.1600-6143.2006.01502.x]

32 **Burgos PI**, Perkins EL, Pons-Estel GJ, Kendrick SA, Liu JM, Kendrick WT, Cook WJ, Julian BA, Alarcón GS, Kew CE. Risk factors and impact of recurrent lupus nephritis in patients with systemic lupus erythematosus undergoing renal transplantation: data from a single US institution. *Arthritis Rheum* 2009; **60**: 2757-2766 [PMID: 19714623 DOI: 10.1002/art.24776]

33 **Stone JH**, Amend WJ, Criswell LA. Outcome of renal transplantation in systemic lupus erythematosus. *Semin Arthritis Rheum* 1997; **27**: 17-26 [PMID: 9287386 DOI: 10.1016/S0049-0172(97)80033-9]

34 **Moroni G**, Tantardini F, Gallelli B, Quaglini S, Banfi G, Poli F, Montagnino G, Meroni P, Messa P, Ponticelli C. The long-term prognosis of renal transplantation in patients with lupus nephritis. *Am J Kidney Dis* 2005; **45**: 903-911 [PMID: 15861356 DOI: 10.1053/j.ajkd.2005.01.038]

35 **Weening JJ**, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004; **15**: 241-250 [PMID: 14747370 DOI: 10.1097/01.ASN.0000108969.21691.5D]

36 **Norby GE**, Strøm EH, Midtvedt K, Hartmann A, Gilboe IM, Leivestad T, Stenstrøm J, Holdaas H. Recurrent lupus nephritis after kidney transplantation: a surveillance biopsy study. *Ann Rheum Dis* 2010; **69**: 1484-1487 [PMID: 20498208 DOI: 10.1136/ard.2009.122796]

37 **Herrera GA**. The value of electron microscopy in the diagnosis and clinical management of lupus nephritis. *Ultrastruct Pathol* 1999; **23**: 63-77 [PMID: 10369101 DOI: 10.1080/019131299281725]

38 **Lochhead KM**, Pirsch JD, D'Alessandro AM, Knechtle SJ, Kalayoglu M, Sollinger HW, Belzer FO. Risk factors for renal allograft loss in patients with systemic lupus erythematosus. *Kidney Int* 1996; **49**: 512-517 [PMID: 8821838 DOI: 10.1038/ki.1996.73]

39 **Grimbert P**, Frappier J, Bedrossian J, Legendre C, Antoine C,

Hiesse C, Bitker MO, Sraer JD, Lang P. Long-term outcome of kidney transplantation in patients with systemic lupus erythematosus: a multicenter study. Groupe Cooperatif de Transplantation d'île de France. *Transplantation* 1998; **66**: 1000-1003 [PMID: 9808482 DOI: 10.1097/00007890-199810270 -00006]

40 **Bartosh SM**, Fine RN, Sullivan EK. Outcome after transplantation of young patients with systemic lupus erythematosus: a report of the North American pediatric renal transplant cooperative study. *Transplantation* 2001; **72**: 973-978 [PMID: 11571477 DOI: 10.1097/00007890-200109150-00047]

41 **Bunnapradist S**, Chung P, Peng A, Hong A, Chung P, Lee B, Fukami S, Takemoto SK, Singh AK. Outcomes of renal transplantation for recipients with lupus nephritis: analysis of the Organ Procurement and Transplantation Network database. *Transplantation* 2006; **82**: 612-618 [PMID: 16969282 DOI: 10.1097/01.tp.0000235740.56573.c6]

42 **Dooley MA**, Hogan S, Jennette C, Falk R. Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. Glomerular Disease Collaborative Network. *Kidney Int* 1997; **51**: 1188-1195 [PMID: 9083285 DOI: 10.1038/ki.1997.162]

43 **Nankivell BJ**, Chapman JR. Chronic allograft nephropathy: current concepts and future directions. *Transplantation* 2006; **81**: 643-654 [PMID: 16534463 DOI: 10.1097/01. tp.0000190423.82154.01]

44 **Cosio FG**, Gloor JM, Sethi S, Stegall MD. Transplant glomerulopathy. *Am J Transplant* 2008; **8**: 492-496 [PMID: 18294145 DOI: 10.1111/j.1600-6143.2007.02104.x]

45 **Ponticelli C**, Moroni G, Glassock RJ. Recurrence of secondary glomerular disease after renal transplantation. *Clin J Am Soc Nephrol* 2011; **6**: 1214-1221 [PMID: 21493742 DOI: 10.2215/CJN.09381010]

46 **Houssiau FA**, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido Ed Ede R, Danieli MG, Abramovicz D, Blockmans D, Mathieu A, Direskeneli H, Galeazzi M, Gül A, Levy Y, Petera P, Popovic R, Petrovic R, Sinico RA, Cattaneo R, Font J, Depresseux G, Cosyns JP, Cervera R. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002; **46**: 2121-2131 [PMID: 12209517 DOI: 10.1002/art.10461]

47 **Dong G**, Panaro F, Bogetti D, Sammartino C, Rondelli D, Sankary H, Testa G, Benedetti E. Standard chronic immunosuppression after kidney transplantation for systemic lupus erythematosus eliminates recurrence of disease. *Clin Transplant* 2005; **19**: 56-60 [PMID: 15659135 DOI: 10.1111/j.1399-00 12.2004.00297.x]

48 **Nyberg G**, Blohmé I, Svalander C, Persson H, Brynger H. Rejection and recurrence of SLE nephritis in cyclosporine-treated kidney transplant recipients. *Transplant Proc* 1987; **19**: 1637-1638 [PMID: 3274393]

49 **Kumano K**, Sakai T, Mashimo S, Endo T, Koshiba K, Elises JS, Iitaka K. A case of recurrent lupus nephritis after renal transplantation. *Clin Nephrol* 1987; **27**: 94-98 [PMID: 3549085]

50 **Stone JH**, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, Kallenberg CG, St Clair EW, Turkiewicz A, Tchao NK, Webber L, Ding L, Sejismundo LP, Mieras K, Weitzenkamp D, Ikle D, Seyfert-Margolis V, Mueller M, Brunetta P, Allen NB, Fervenza FC, Geetha D, Keogh KA, Kissin EY, Monach PA, Peikert T, Stegeman C, Ytterberg SR, Specks U. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010; **363**: 221-232 [PMID: 20647199 DOI: 10.1056/NEJMoa0909905]

51 **Brennan DC**, Glassock RJ, Bleyer AJ. American Society of Nephrology Quiz and Questionnaire 2012: Transplantation. *Clin J Am Soc Nephrol* 2013; **8**: 1267-1272 [PMID: 23539230 DOI: 10.2215/CJN.00430113]

52 **Verbeke F**, Lindley E, Van Bortel L, Vanholder R, London G, Cochat P, Wiecek A, Fouque D, Van Biesen W. A European Renal Best Practice (ERBP) position statement on the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for the management of blood pressure in non-dialysis-dependent chronic kidney disease: an endorsement with some caveats for real-life application. *Nephrol Dial Transplant* 2014; **29**: 490-496 [PMID: 24071661]

53 **Holdaas H**, Fellström B, Jardine AG, Holme I, Nyberg G, Fauchald P, Grönhagen-Riska C, Madsen S, Neumayer HH, Cole E, Maes B, Ambühl P, Olsson AG, Hartmann A, Solbu DO, Pedersen TR. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. *Lancet* 2003; **361**: 2024-2031 [PMID: 12814712 DOI: 10.1016/S0140-6736(03)13638-0]

54 **Roman MJ**, Shanker BA, Davis A, Lockshin MD, Sammaritano L, Simantov R, Crow MK, Schwartz JE, Paget SA, Devereux RB, Salmon JE. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med* 2003; **349**: 2399-2406 [PMID: 14681505 DOI: 10.1056/NEJMoa035471]

55 **Chelamcharla M**, Javaid B, Baird BC, Goldfarb-Rumyantzev AS. The outcome of renal transplantation among systemic lupus erythematosus patients. *Nephrol Dial Transplant* 2007; **22**: 3623-3630 [PMID: 17640941 DOI: 10.1093/ndt/gfm459]

56 **Norby GE**, Leivestad T, Mjøen G, Hartmann A, Midtvedt K, Gran JT, Holdaas H. Premature cardiovascular disease in patients with systemic lupus erythematosus influences survival after renal transplantation. *Arthritis Rheum* 2011; **63**: 733-737 [PMID: 21360503 DOI: 10.1002/art.30184]

57 **Vaidya S**. Ten-yr renal allograft survival of patients with antiphospholipid antibody syndrome. *Clin Transplant* 2012; **26**: 853-856 [PMID: 22507396 DOI: 10.1111/j.1399-0012.2012.0 1625.x]

MINIREVIEWS

Autoimmune diabetes recurrence should be routinely monitored after pancreas transplantation

La Salete Martins

La Salete Martins, Department of Nephrology, Renal and Pancreatic Transplant Units, Centro Hospitalar do Porto, Hospital de Santo António, 4099-001 Porto, Portugal
Author contributions: Martins LS was the sole author who conceived, drafted and approved the final version of the article to be published.
Correspondence to: La Salete Martins, MD, Department of Nephrology, Renal and Pancreatic Transplant Units, Centro Hospitalar do Porto, Hospital de Santo António, Lg Prof Abel Salazar, 4099-001 Porto, Portugal. lasalet@gmail.com
Telephone: +351-22-2077534 Fax: +351-22-2033189
Received: April 22, 2014   Revised: May 21, 2014
Accepted: July 17, 2014
Published online: September 24, 2014

Abstract

Autoimmune type 1 diabetes recurrence in pancreas grafts was first described 30 years ago, but it is not yet completely understood. In fact, the number of transplants affected and possibly lost due to this disease may be falsely low. There may be insufficient awareness to this entity by clinicians, leading to underdiagnosis. Some authors estimate that half of the immunological losses in pancreas transplantation are due to autoimmunity. Pancreas biopsy is the gold standard for the definitive diagnosis. However, as an invasive procedure, it is not the ideal approach to screen the disease. Pancreatic autoantibodies which may be detected early before graft dysfunction, when searched for, are probably the best initial tool to establish the diagnosis. The purpose of this review is to revisit the autoimmune aspects of type 1 diabetes and to analyse data about the identified autoantibodies, as serological markers of the disease. Therapeutic strategies used to control the disease, though with unsatisfactory results, are also addressed. In addition, the author's own experience with the prospective monitoring of pancreatic autoantibodies after transplantation and its correlation with graft outcome will be discussed.

Core tip: Recurrence of pancreatic autoantibodies after kidney-pancreas transplantation is a disturbing finding. It was estimated that half of the immunological losses of pancreas grafts may be due to autoimmunity. There is a rising investigational effort concerning this issue. At our unit, we have designed a protocol of prospective monitoring of pancreatic autoantibodies after transplantation. In our experience, patients with positive pancreatic autoantibodies, compared to negative patients, were more likely to present higher HbA1c and lower C-peptide levels. A review of the most important publications in this field, and about the interest of pancreatic autoantibodies monitoring after transplantation, was made.

Martins LS. Autoimmune diabetes recurrence should be routinely monitored after pancreas transplantation. *World J Transplant* 2014; 4(3): 183-187 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/i3/183.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i3.183

TYPE 1 DIABETES MELLITUS AND AUTOIMMUNITY

Type 1 diabetes mellitus (DM1), a disease with an evident underlying autoimmune process[1], may recur after pancreas transplantation. The first cases described by Sutherland et al[2] were documented only in patients who have received grafts from highly HLA-matched donors (siblings) and with minimized immunosuppression[3]. Few years later, diabetes recurrence was also documented in recipients of

pancreas grafts with HLA-mismatches with the donor and maintaining standard immunosuppression[4].

There is a recognized genetic susceptibility for DM1. The disease is strongly associated with *HLA* genes, specifically with alleles DR3 and DR4[5,6]; with polymorphisms of the proinsulin/insulin gene[7]; and with the PTP gene (*PTPN22*), a gene coding for a lymphocyte-specific tyrosine phosphatase[8]. However, only about 50% of HLA identical twins inheriting alleles DR3 and/or DR4 develop the disease[1,9]. It means that inheritance of the *HLA* gene is not a sufficient condition and susceptibility is most certainly polygenic.

It is not known which individuals are at higher risk for DM1 recurrence on pancreas graft and what are the important risk factors for the disease. Additionally, there is also no consensus about the best screening tests to identify patients at risk.

DIAGNOSIS OF AUTOIMMUNE DIABETES RECURRENCE ON PANCREAS GRAFTS

A pancreas graft biopsy showing an inflammatory T-cell infiltrate, specifically targeting the beta-cells (aspect designated as "insulitis") and sparing the exocrine tissue, remains the gold-standard for the diagnosis of DM1 recurrence[10]. However, it is not easy to justify such an invasive procedure, carrying a non neglectable risk of complications, in patients without a misfunctioning pancreas graft or, at least, without reliable data favouring the hypothesis of reactivated autoimmunity.

Serological markers of the autoimmune process, the islet cell autoantibodies (ICA)[4] have been proposed as a basic tool, the first screening test to identify the activity of autoimmune disease. The anti-glutamic acid decarboxylase (anti-GAD antibodies)[11]; the anti-insulin antibodies (IAA)[12]; the anti-IA2 (anti-tyrosine phosphatase) antibodies[13]; and the most recently described anti-ZnT8 (cation efflux zinc transporter) antibodies[14], have also been identified as autoimmune markers of DM1. The positivity for these immune humoral markers is considered a good predictor of the enhancement of autoimmune diabetes. The association of several markers (two or more) increases its predictive value[15]. As yet, pancreas biopsy is the confirmatory procedure when suspecting for recurrence on pancreas graft.

There is some controversy about the real role of these autoantibodies: do they have a direct participation in the process? Or are they surrogate markers, merely testifying the lesion? Although the pancreatic autoantibodies were not detected in a recent case documented with insulitis in the biopsy[16], they are usually present in the vast majority of the cases confirmed by biopsy.

The new onset or rising levels of these autoantibodies in pancreas transplant patients has been pointed out as a serious indicator of recurrence and progression of the disease. In fact, several studies reported worse pancreas outcome in patients with these humoral markers[10,17-20]. It has been suggested that half of the immune losses of

pancreas grafts may be due to autoimmunity[21]. Based on these data, monitorization of pancreatic autoantibodies has been recommended in all pancreas transplants[21] as a primary test to identify patients at risk for autoimmune graft loss. My personal opinion is concordant with these authors, stating that the disease may currently remain underdiagnosed. This may be the cause of pancreas graft failure in some cases with unclear etiology, probably because this is not sufficiently investigated.

IMMUNOSUPPRESSION AND AUTOIMMUNITY

Immunosuppressive protocols designed to prevent rejection in the pancreas transplant are not capable of containing autoimmunity[21]. This is a disturbing finding in organ transplantation. Remembering autoimmune disorders affecting the kidney, such as ANCA-associated vasculitis or lupus, they may relapse after kidney transplantation despite apparently adequate immunosuppression to control alloimmunity. One condition in kidney transplantation, which is quite similar to the pancreatic autoimmunity recurrence, is that observed in some patients with Alport syndrome: they may develop anti-glomerular basement membrane disease post-transplantation, after a new exposition to glomerular basement membrane antigens (type 4 collagen antigens), for which they were natively defective. Immune attack against the newly presented beta-cells may occur after a pancreas transplant.

The Miami group has tried to treat autoimmune relapse in pancreas transplants with anti-lymphocyte (anti-B and/or anti-T cell) therapies[21-23]. After a transient response in a few cases, autoimmune activity has recurred within a short period of time. At the time of the second recurrence they were able to identify the same clone of autoreactive GAD-specific T cells which has been found in the first recurrence. Pancreatic autoantibodies followed the reappearance of the T cells, with a new rise[23]. Therefore, it seems that immunosuppressive agents available at the moment cannot prevent this immune memory response. To date, there are no studies reporting effective and sustained treatment of pancreatic autoimmunity in DM1 patients with diabetes recurrence.

Efforts are needed to find therapeutic strategies to control this process. Can protocols used in kidney transplant hypersensitized patients be advantageous? Combined therapies, like plasmapheresis, immunoglobulin and rituximab have been successfully used in kidney transplants with HLA donor-specific antibodies; and also in systemic autoimmune diseases, such as lupus, with severe expression. The results from trials using new drugs (abatacept, etanercept, teplizumab, rituximab) have failed to prove long lived efficacy in native pancreas after DM1 onset[24]. However, intervention after clinical disease (tertiary intervention) may be too late, since overt disease corresponds to extensive beta-cell destruction. The most promising long-term results were achieved with hematopoietic stem-cell transplantation, in patients pre-

senting autoimmune markers, but before clinical diabetes (secondary intervention)[24]. Another serious worry are the important side effects of each drug. Toxicities and efficacy, if used in combination, remain to be assessed. We have learned the benefits of the association of complimentary therapies from the oncology research, allowing the use of smaller doses, with fewer side effects and with gains in terms of efficacy. Could this be an efficient strategy in pancreas transplant patients with recurred autoimmune diabetes?

OUR OWN EXPERIENCE

We have designed a prospective monitoring protocol of pancreatic autoantibodies after pancreas-kidney transplantation at our unit. Anti-GAD, IAA and ICA were analysed before or on admission for transplantation, at 6 and 12 mo, and at least once a year thereafter. In a cohort of 135 patients[25], 34.5% were positive for any of these antibodies before surgery, anti-GAD being the most prevalent (> 20%). Nearly half of these became negative within months or years, but in some others (previously negative) we verified a new appearance of anti-GAD antibodies. After a mean time of 6 years (ranging from 1 to 12 years), among the 78% of the patients with functioning pancreas, 44% are positive for at least one autoantibody. Anti-GAD remain the most common (31%). The frequency of patients with IAA before transplantation was surprisingly low, 3%, considering that patients under exogenous insulin may present anti-insulin antibodies. At our last follow-up the prevalence of IAA-positive is three-fold higher and none of the patients was under exogenous insulin. ICA, present in 10% of the patients before transplantation, tended to disappear and there was no new onset of these antibodies. Less than 3% maintain ICA positive now. In 10% of the patients more than one pancreatic autoantibody was present.

Pancreas graft survival was not significantly different in the group of patients with some positive pancreatic autoantibody, compared to the patients who were negative for these autoantibodies. The immunosuppression used in the positive and in the negative patients did not differ (tacrolimus and mycophenolic acid mostly used) and the frequency of patients withdrawn from steroids was also similar in both groups.

Positive patients for any pancreatic autoantibody tended to have a higher HLA-match with the donor, though not reaching statistical significance.

Concerning the glycemic control, our data are not so tranquilizing. The group of patients with at least one positive pancreatic autoantibody, compared to negative patients, was more likely to have higher HbA1c and lower C-peptide levels and this difference was statistically significant. And, more important, our results showed a more than 5 times higher probability to find positive autoimmunity among the pancreas transplants with normal-high HbA1c. Kidney graft function was similar in both groups, with or without pancreatic antibodies, which

strengths the argument that decline in glycemic control was not due to alloimmunity. In a former report from our centre[26], analysing the glycemic profile in both groups (positive and negative for pancreatic autoantibodies), the difference did not (yet) reach statistical significance. In fact, in this former study, a few number of patients with positive antibodies showed normal-high fasting glucose levels and the lowest C-peptide. Comparing results from our former study to the more recent one, it probably means that we are facing an evolutive process. The number of patients has almost doubled and the follow-up period is now much longer, and we could now associate pancreatic autoimmunity with less favourable glycemic profile. It will be interesting to analyse data with a more extended follow-up.

CONCLUSION

In conclusion, we think it is advisable to routinely monitor pancreatic autoantibodies after transplantation. There is substantial evidence that DM1 can reappear after pancreas transplantation, but may have been underdiagnosed for the last decades[21,27], mainly because it is a forgotten question. The awareness of the entity will certainly lead the majority of pancreas transplant units to search for the disease and to prospectively assess these antibodies. Pancreas graft biopsy remains the gold standard for the diagnosis of DM1 recurrence but, as it is an invasive procedure, it is not the ideal as a screening methodology, without other clinical or analytical (metabolic or immunological) data. On the other hand, pancreas biopsy only after impaired endocrine function is most of the times too late. Antibody monitoring is a non-invasive basic screening test, available in most units, and may bring the necessary information to proceed to pancreas biopsy before dysfunction. Islet autoantibodies are currently the most robust biomarker of DM1[28].

Additionally, pancreatic autoantibodies assessment may be of interest in other areas. The authors of a very recent study[29] have also proposed the use of the GAD-autoantibody status before pancreas-kidney transplantation as a guide to choose the kind of prophylactic antibody induction.

Positivity for pancreatic autoantibodies after transplantation may never occur, or it may be intermittent or persistent, not always correlated with graft function, at least in a short period of time. However, beta-cell destruction was documented in other cases and autoantibody rising may have preceded in several years the graft dysfunction, has it been searched for. An early diagnosis gives the clinician more time for some kind of intervention.

Another critical point is the lack of effectiveness of the treatments tried so far in DM1 patients. The recognition of the role of islet autoreactive CD4+ T cells[22,23] and CD8+ T cells[27,30] on beta-cell destruction, as well as all the targets of humoral activity[28], may lead to other treatment opportunities. Novel therapies, namely targeting proinsulin-reactive CD8+ T cells, were recently pro-

posed as a potential therapeutic approach[31].

In the meantime, we must improve our ability to make an early diagnosis and to increase our knowledge about all the processes of the disease. These may be some of the missing steps to find the most advantageous strategy to treat, or even to prevent, the autoimmune diabetes recurrence in pancreas grafts.

REFERENCES

1   **Atkinson MA**, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 1994; **331**: 1428-1436 [PMID: 7969282 DOI: 10.1056/NEJM199411243312107]

2   **Sutherland DE**, Sibley R, Xu XZ, Michael A, Srikanta AM, Taub F, Najarian J, Goetz FC. Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Trans Assoc Am Physicians* 1984; **97**: 80-87 [PMID: 6398950]

3   **Sutherland DE**, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. *Diabetes* 1989; **38** Suppl 1: 85-87 [PMID: 2642862 DOI: 10.2337/diab.38.1.S85]

4   **Bosi E**, Bottazzo GF, Secchi A, Pozza G, Shattock M, Saunders A, Gelet A, Touraine JL, Traeger J, Dubernard JM. Islet cell autoimmunity in type I diabetic patients after HLA-mismatched pancreas transplantation. *Diabetes* 1989; **38** Suppl 1: 82-84 [PMID: 2642861 DOI: 10.2337/diab.38.1.S82]

5   **Aly TA**, Ide A, Jahromi MM, Barker JM, Fernando MS, Babu SR, Yu L, Miao D, Erlich HA, Fain PR, Barriga KJ, Norris JM, Rewers MJ, Eisenbarth GS. Extreme genetic risk for type 1A diabetes. *Proc Natl Acad Sci USA* 2006; **103**: 14074-14079 [PMID: 16966600 DOI: 10.1073/pnas.0606349103]

6   **Tisch R**, McDevitt H. Insulin-dependent diabetes mellitus. *Cell* 1996; **85**: 291-297 [PMID: 8616883 DOI: 10.1016/S0092-8674(00)81106-X]

7   **Bell GI**, Horita S, Karam JH. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 1984; **33**: 176-183 [PMID: 6363172 DOI: 10.2337/diab.33.2.176]

8   **Smyth D**, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu-Tîrgoviste C, Savage DA, Dunger DB, Widmer B, Strachan DP, Ring SM, Walker N, Clayton DG, Twells RC, Gough SC, Todd JA. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004; **53**: 3020-3023 [PMID: 15504986 DOI: 10.2337/diabetes.53.11.3020]

9   **Redondo MJ**, Rewers M, Yu L, Garg S, Pilcher CC, Elliott RB, Eisenbarth GS. Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: prospective twin study. *BMJ* 1999; **318**: 698-702 [PMID: 10074012 DOI: 10.1136/bmj.318.7185.698]

10 **Tydén G**, Reinholt FP, Sundkvist G, Bolinder J. Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. *N Engl J Med* 1996; **335**: 860-863 [PMID: 8778604 DOI: 10.1056/NEJM199609193351205]

11 **Baekkeskov S**, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 1990; **347**: 151-156 [PMID: 1697648 DOI: 10.1038/347151a0]

12 **Ziegler AG**, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer U, Vordemann J, Lenz A, Standl E. On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. *Diabetologia* 1993; **36**: 402-408 [PMID: 8314444 DOI: 10.1007/BF00402275]

13 **Pietropaolo M**, Hutton JC, Eisenbarth GS. Protein tyrosine phosphatase-like proteins: link with IDDM. *Diabetes Care* 1997; **20**: 208-214 [PMID: 9118776 DOI: 10.2337/diacare.20.2.208]

14 **Wenzlau JM**, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci USA* 2007; **104**: 17040-17045 [PMID: 17942684 DOI: 10.1073/pnas.0705894104]

15 **Verge CF**, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 1996; **45**: 926-933 [PMID: 8666144 DOI: 10.2337/diab.45.7.926]

16 **Assalino M**, Genevay M, Morel P, Demuylder-Mischler S, Toso C, Berney T. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation in the absence of GAD and IA-2 autoantibodies. *Am J Transplant* 2012; **12**: 492-495 [PMID: 22151900 DOI: 10.1111/j.1600-6143.2011.03844.x]

17 **Esmatjes E**, Rodríguez-Villar C, Ricart MJ, Casamitjana R, Martorell J, Sabater L, Astudillo E, Fernández-Cruz L. Recurrence of immunological markers for type 1 (insulin-dependent) diabetes mellitus in immunosuppressed patients after pancreas transplantation. *Transplantation* 1998; **66**: 128-131 [PMID: 9679835 DOI: 10.1097/00007890-199807150-00022]

18 **Petruzzo P**, Andreelli F, McGregor B, Lefrançois N, Dawahra M, Feitosa LC, Dubernard JM, Thivolet C, Martin X. Evidence of recurrent type I diabetes following HLA-mismatched pancreas transplantation. *Diabetes Metab* 2000; **26**: 215-218 [PMID: 10880896]

19 **Braghi S**, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes* 2000; **49**: 218-224 [PMID: 10868938 DOI: 10.2337/diabetes.49.2.218]

20 **Thivolet C**, Abou-Amara S, Martin X, Lefrancois N, Petruzzo P, McGregor B, Bosshard S, Dubernard JM. Serological markers of recurrent beta cell destruction in diabetic patients undergoing pancreatic transplantation. *Transplantation* 2000; **69**: 99-103 [PMID: 10653387 DOI: 10.1097/00007890-200001150-00018]

21 **Pugliese A**, Reijonen HK, Nepom J, Burke GW 3rd. Recurrence of autoimmunity in pancreas transplant patients: research update. *Diabetes Manag* (Lond) 2011; **1**: 229-238 [PMID: 21927622 DOI: 10.2217/dmt.10.21]

22 **Laughlin E**, Burke G, Pugliese A, Falk B, Nepom G. Recurrence of autoreactive antigen-specific CD4+ T cells in autoimmune diabetes after pancreas transplantation. *Clin Immunol* 2008; **128**: 23-30 [PMID: 18455963 DOI: 10.1016/j.clim.2008.03.459]

23 **Vendrame F**, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, Diamantopoulos S, Standifer N, Geubtner K, Falk BA, Ichii H, Takahashi H, Snowhite I, Chen Z, Mendez A, Chen L, Sageshima J, Ruiz P, Ciancio G, Ricordi C, Reijonen H, Nepom GT, Burke GW, Pugliese A. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. *Diabetes* 2010; **59**: 947-957 [PMID: 20086230 DOI: 10.2337/db09-0498]

24 **Brooks-Worrell B**, Palmer JP. Prevention versus intervention of type 1 diabetes. *Clin Immunol* 2013; **149**: 332-338 [PMID: 23803322 DOI: 10.1016/j.clim.2013.05.018]

25 **Martins LS**, Henriques AC, Fonseca IM, Rodrigues AS, Oliverira JC, Dores JM, Dias LS, Cabrita AM, Silva JD, Noronha IL. Pancreatic autoantibodies after pancreas-kidney transplantation - do they matter? *Clin Transplant* 2014; **28**: 462-469 [PMID: 24655222 DOI: 10.1111/ctr.12337]

26   **Martins L**, Malheiro J, Henriques AC, Dias L, Dores J, Oliveira F, Seca R, Almeida R, Sarmento AM, Cabrita A, Teixeira M. Pancreas-kidney transplantation and the evolution of pancreatic autoantibodies. *Transplant Proc* 2009; **41**: 913-915 [PMID: 19376387 DOI: 10.1016/j.transproceed.2009.01.068]

27   **Abreu JR**, Roep BO. Immune monitoring of islet and pancreas transplant recipients. *Curr Diab Rep* 2013; **13**: 704-712 [PMID: 23943207 DOI: 10.1007/s11892-013-0399-3]

28   **Wenzlau JM**, Hutton JC. Novel diabetes autoantibodies and prediction of type 1 diabetes. *Curr Diab Rep* 2013; **13**: 608-615 [PMID: 23900975 DOI: 10.1007/s11892-013-0405-9]

29   **Ringers J**, van der Torren CR, van de Linde P, van der Boog PJ, Mallat MJ, Bonifacio E, Roep BO, de Fijter JW. Pretransplantation GAD-autoantibody status to guide prophylactic antibody induction therapy in simultaneous pancreas and kidney transplantation. *Transplantation* 2013; **96**: 745-752 [PMID: 23912172 DOI: 10.1097/TP.0b013e3182a012cc]

30   **Coppieters KT**, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med* 2012; **209**: 51-60 [PMID: 22213807 DOI: 10.1084/jem.20111187]

31   **Abreu JR**, Roep BO. Targeting proinsulin-reactive CD8+ T cells: a new direction for type 1 diabetes treatment. *Expert Rev Clin Immunol* 2013; **9**: 1001-1003 [PMID: 24168405 DOI: 10.1586/1744666X.2013.852958]

ORIGINAL ARTICLE

Impact of steroid maintenance on the outcomes in first-time deceased donor kidney transplant recipients: Analysis by induction type

Kalathil K Sureshkumar, Sabiha M Hussain, Ngoc L Thai, Tina Y Ko, Khaled Nashar, Richard J Marcus

Kalathil K Sureshkumar, Sabiha M Hussain, Tina Y Ko, Khaled Nashar, Richard J Marcus, Divisions of Nephrology and Hypertension, Department of Medicine, Allegheny General Hospital, Pittsburgh, PA 15212, United States
Ngoc L Thai, Abdominal Transplantation, Allegheny General Hospital, Pittsburgh, PA 15212, United States
Author contributions: Sureshkumar KK participated in research design, performance of the research, data analysis and writing of the manuscript; Hussain SM participated in research design and data analysis; Thai NL participated in research design and data analysis; Ko TY and Nashar K participated in data analysis; Marcus RJ participated in research design, performance of the research and data analysis.
Supported by Health Resources and Services Administration Contract 231-00-0115
Correspondence to: Kalathil K Sureshkumar, MD, FRCP (Glasgow), FASN, Division of Nephrology and Hypertension, Department of Medicine, Allegheny General Hospital, 320 East North Avenue, Pittsburgh, PA 15212,
United States. ksureshk@wpahs.org
Telephone: +1-412-3593319 Fax: +1-412-3594136
Received: March 24, 2014   Revised: June 5, 2014
Accepted: July 25, 2014
Published online: September 24, 2014

Abstract

AIM: To analyze the impact of steroid maintenance on the outcomes in kidney transplant recipients stratified by induction agent received.

METHODS: Patients who underwent first-time deceased donor kidney transplantation between 2000 and 2008 after receiving induction therapy with rabbit-antithymocyte globulin (r-ATG), alemtuzumab or an interleukin-2 receptor blocker (IL-2B) and discharged on a calcineurin inhibitor (CNI)/mycophenolate mofetil (MMF)-regimen along with or without steroids were identified from the Organ Procurement and Transplant Network/United Network of Organ Sharing database.

For each induction type, adjusted overall and death-censored graft as well as patient survivals were compared between patients discharged on steroid *vs* no steroid. Among r-ATG induced patients, analysis was repeated after splitting the group into low and high immune risk groups.

RESULTS: Among the 37217 patients included in the analysis, 17863 received r-ATG (steroid = 13001, no-steroid = 4862), 3028 alemtuzumab (steroid = 852, no-steroid = 2176) and 16326 IL-2B (steroid = 15008, no-steroid = 1318). Adjusted overall graft survival was inferior (HR = 1.16, 95%CI: 1.06-1.27, *P* = 0.002) with similar death-censored graft survival (HR = 0.99, 95%CI: 0.86-1.14, *P* = 0.86) for steroid *vs* no-steroid groups in r-ATG induced patients. Both adjusted overall and death-censored graft survivals for steroid *vs* no-steroid groups were similar in alemtuzumab (HR = 0.92, 95%CI: 0.73-1.15, *P* = 0.47 and HR = 0.87, 95%CI: 0.62-1.22, *P* = 0.43 respectively) and IL-2B (HR = 1.05, 95%CI: 0.91-1.21, *P* = 0.48 and HR = 0.94, 95%CI: 0.75-1.18, *P* = 0.60 respectively) induced groups. Adjusted patient survivals were inferior for steroid *vs* no-steroid groups in r-ATG induced (HR = 1.31, 95%CI: 1.15-1.49, *P* < 0.001) but similar in alemtuzumab (HR = 1.02, 95%CI: 0.75-1.38, *P* = 0.92) and IL-2B (HR = 1.17, 95%CI: 0.97-1.40, *P* = 0.10) induced patients. Among the r-ATG induced group there were 4346 patients in the low immune risk and 13517 patients in the high immune risk group. Adjusted overall graft survivals were inferior for steroid *vs* no steroid groups in both low immune (HR = 1.34, 95%CI: 1.09-1.64, *P* = 0.001) and high immune (HR = 1.18, 95%CI: 1.07-1.30, *P* = 0.005) risk groups. Adjusted death-censored graft survivals for steroid *vs* no steroid groups were similar in both low (HR = 1.06, 95%CI: 0.78-1.45, *P* = 0.70) and high (HR = 1.04, 95%CI: 0.98-1.20, *P* = 0.60) immune risk groups. Adjusted patient survivals were inferior for steroid *vs* no steroid groups in both low immune (HR =

1.54, 95%CI: 1.18-2.02, $P < 0.001$) and high immune (HR = 1.32, 95%CI: 1.16-1.51, $P = 0.002$) risk groups. Overall, there were significantly higher deaths from infections and cardiovascular causes in patients maintained on steroids.

CONCLUSION: Our study showed an association between steroid addition to a CNI/MMF-maintenance regimen and increased death with functioning graft in patients receiving r-ATG induction for first-time deceased donor kidney transplantation.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Induction agent; Steroid maintenance; Graft failure risk; Patient death risk; High immune risk

Core tip: This study critically looked at outcomes in a large recent cohort of first time deceased donor kidney transplant recipients from the Organ Procurement and Transplant Network/United Network of Organ Sharing database to assess the impact of triple maintenance immunosuppression after receiving various induction therapies. In multivariate analysis, we found an increased risk for death with functioning graft when steroid maintenance was added to calcineurin inhibitor/mycophenolate mofetil based regimen in patients who received powerful induction with rabbit-antithymocyte globulin (r-ATG), an effect that persisted even when patients were split into high and low immune risk groups. Based on these finding we feel that, one has to be cautious while maintaining intense immunosuppression by adding steroid to a calcineurin inhibitor/mycophenolate mofetil regimen in kidney transplant recipients who were selected for r-ATG induction.

INTRODUCTION

Corticosteroid has been an integral part of maintenance immunosuppression from the dawn of clinical kidney transplantation. Chronic steroid use can contribute to worsening of hypertension as well as dyslipidemia, and development of new onset diabetes mellitus, all risk factors for cardiovascular (CV) disease. Steroid therapy can also increase susceptibility to infections and accelerates bone loss. In one survey, when kidney transplant recipients were asked which drug they would like to discontinue, majority chose prednisone[1]. Attempts to develop steroid- free immunosuppression for kidney transplant recipients started nearly three decades ago but the enthusiasm waned when results of studies including the

Canadian multicenter randomized clinical trial showed increased risk for acute rejection (AR) and graft loss associated with steroid withdrawal[2-4]. The availability of more potent maintenance immunosuppressive agents such as tacrolimus and mycophenolate mofetil (MMF) as well as induction agents like rabbit-antithymocyte globulin (r-ATG) resulted in a resurgence of the interest in early steroid withdrawal during the last decade. For instance, a recent registry analysis showed that the percentage of kidney transplant recipients discharged following the initial transplant admission on a steroid-free maintenance immunosuppression increased from 3.7% in the year 2000 to 32.5% as of 2006[5].

Perioperative induction therapy was utilized in greater than 70% of kidney transplant recipients in the United States during the last decade[6]. Calcineurin inhibitor (CNI)/MMF with or without steroid was the most commonly employed maintenance immunosuppression over the same time period. One could speculate that the degree of immunosuppression rendered by triple immunosuppression following powerful induction might be excessive in some patients with the possibility of adverse outcomes. The risk-benefit evaluation of steroid maintenance in kidney transplant recipients who receive powerful induction with agents such as r-ATG and potent maintenance immunosuppression with CNI/MMF would be beneficial. We aimed to compare the outcomes for steroid *vs* no steroid addition in patients who underwent a first deceased donor kidney (DDK) transplantation after receiving different induction agents and maintained on CNI/MMF based regimen.

MATERIALS AND METHODS

The study was performed in accordance with the ethical standards laid down by the Declaration of Helsinki as well as Declaration of Istanbul and was approved by the Institutional Review Board. Patients $\geq$ 18 years of age who underwent a first-time DDK transplantation between January 1, 2000 and December 31, 2008 after receiving induction therapy with r-ATG, alemtuzumab or an interleukin-2 receptor blocker (IL-2B) agent (basiliximab or daclizumab) and discharged on a CNI/MMF based maintenance immunosuppression regimen with or without steroids were identified from the Organ Procurement and Transplant Network/United Network of Organ Sharing database. A DDK transplant was considered as a first transplant if there was no history of previous kidney transplant for the patient in the database. For each induction type, patients were divided into two groups: those who underwent early steroid withdrawal before the hospital discharge categorized as early steroid withdrawal (ESW) group and those who were discharged on steroid maintenance. The latter group was designated as chronic steroid maintenance (CSM) group. This is an intention to treat analysis using the maintenance immunosuppression regimen at the time of discharge from the initial transplant hospitalization as the basis for defining the groups. Changes in maintenance immunosuppression that oc-
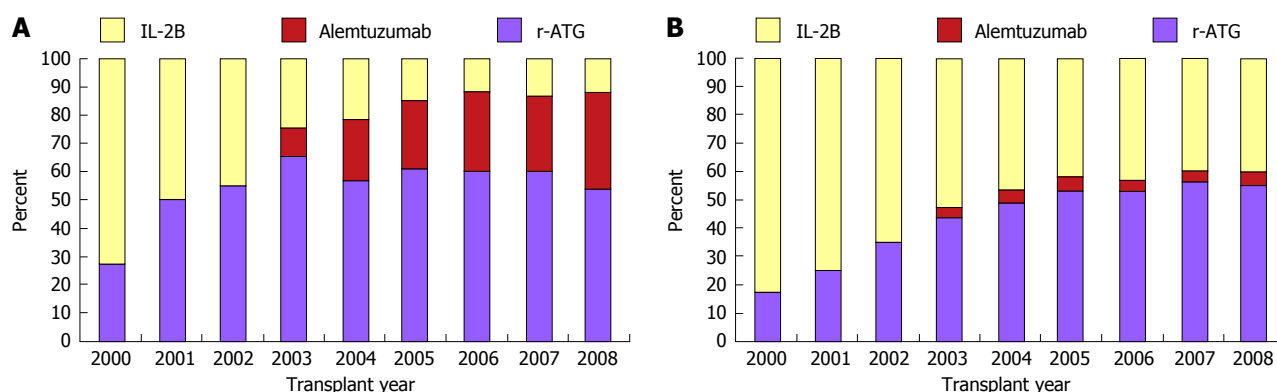
Figure 1 Trends in the use of induction agents. A: Steroid free group; B: Steroid-maintenance group; r-ATG: Rabbit antithymocyte globulin; IL-2B: Interleukin-2 receptor blocker.

curred after initial discharge were not used to classify study subjects. Patients were excluded from the analysis if they underwent live donor kidney or multi-organ transplants or received no induction, more than one induction or a different induction agent.

Demographic data for patients who received different induction agents were collected. Graft was considered failed when one of the following occurred: need for maintenance dialysis, re-transplantation or patient death. Over all and death-censored graft as well as patient survivals were compared between ESW and CSM groups for each induction type after adjusting for pre-specified variables. An adjusted model was used in the analysis to account for substantial variations in the demographic features for ESW *vs* CSM in each induction type. Covariates that can adversely impact graft outcome included in the model were donor related factors: age, gender, expanded criteria donor (ECD) kidney, donation after cardiac death (DCD) kidney, death from cerebrovascular accident(CVA); recipient related factors: age, African American race, diabetes mellitus, dialysis duration, peak panel reactive antibody (PRA) titer, number of human leukocyte antigen (HLA) mismatches; and transplant related factors: cold ischemia time (CIT), delayed graft function (DGF, defined as the need for dialysis within the first week after transplantation), 12 mo AR, and transplant year. The type of CNI agent used was not included in the model since most patients were discharged on tacrolimus. A further analysis was performed to compare the overall and death-censored graft as well as patient survivals between ESW and CSM groups for the r-ATG induced patients by splitting them into high and low immune risk groups. Patients were considered high immune risk if they met any of the following: African American recipient, peak PRA > 20%, CIT > 24 h, ECD kidney recipient, DCD kidney recipient, or developed DGF.

Statistical analysis
Group comparisons were done utilizing 2-tailed t-test for continuous variables and χ^2 test for categorical variables. Mean ± SD, median with range or percentage was used to express values. For the purpose of analysis, the risk

factors were considered absent when data for them were missing. About 20%-25% of the data were missing for the variable "treated acute rejection" but for the remainder of the variables used in the analysis, only fewer than 2% of the data were missing. Adjusted (multivariate, after correcting for the confounding variables listed above) over all and death-censored graft as well as patient survivals were calculated and were compared between CSM *vs* ESW groups within each induction type using a Cox regression model. This was done for the whole group, as well as high and low immune risk groups (defined above) among the r-ATG induced patients. HR and 95%CI were calculated. A *P*-value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 14.

RESULTS
Demographic characteristics
Median follow-up in months with range by induction type were as follows: r-ATG: 35.3 (21.8-55.1), alemtuzumab: 25.0 (13.3-46.0) and IL-2B agent: 46.4 (23.8-72.4). Pattern of induction therapy over the study period are shown in Figure 1. Alemtuzumab was used as an induction agent for the first time in 2003. Since then its use has gradually increased in ESW group but somewhat unchanged in CSM group. IL-2B induction was decreasingly utilized from 2000 to 2008 which was more marked in the ESW group. In the year 2008, roughly 50% patients received induction with r-ATG, 40% with alemtuzumab and 10% with an IL-2B agent in the ESW group. During the same year, about 55% of patients received r-ATG, 5% received alemtuzumab and 40% an IL-2B agent induction in the CSM group.

There were 37217 patients who received a first DDK transplant during the study period of January 2000 to December 2008 after receiving induction with r-ATG, alemtuzumab or an IL-2B agent followed by maintenance with CNI/MMF based regimen. Out of this, 8356 (22.5%) patients underwent early steroid withdrawal and 28861 (77.5%) patients were discharged on maintenance steroid. Distribution of patients by induction type and

Table 1 Demographic features						
	r-ATG		Alemtuzumab		IL-2 receptor blocker	
	Steroid (*n* = 13001)	No steroid (*n* = 4862)	Steroid (*n* = 852)	No steroid (*n* = 2176)	Steroid (*n* = 15008)	No steroid (*n* = 1318)
Donor age (yr)	39 ± 17	39 ± 17	41 ± 18	40 ± 17	37 ± 17	37 ± 17
Donor gender (M/F) (%)	59/41	60/40	61/39	58/42	60/40	60/40
Donor death from CVA (%)	43	40[b]	41	43	40	36[b]
ECD kidney (%)	19	19	26	22[b]	15	16
DCD kidney (%)	9.8	8.9	14.1	10.9[a]	5.3	6.7[a]
Recipient age (yr)	52 ± 13	53 ± 13[d]	52 ± 13	53 ± 12	52 ± 13	54 ± 15[d]
Recipient gender (M/F) (%)	57/53	63/37[d]	56/44	60/40[a]	63/37	64/36
African American race (%)	37	26[d]	33	33	26	20[d]
Diabetes (%)	34	20[d]	31	38[b]	34	34
Pre-txp dialysis (%)	91	88[b]	88	88	91	87[d]
Dialysis duration (mo)	48 ± 35	43 ± 36[d]	47 ± 37	50 ± 34	43 ± 33	41 ± 32
Peak PRA (%)	18 ± 31	10 ± 22[d]	19 ± 31	14 ± 27[d]	9 ± 22	8 ± 20
HLA mismatches	3.9 ± 1.8	3.8 ± 1.8[a]	3.9 ± 1.8	3.7 ± 1.7[b]	3.6 ± 1.9	3.5 ± 2.0
Cold ischemia (h)	18.3 ± 8.2	18.5 ± 8.8	20.8 ± 8.7	20.5 ± 9.2	17.8 ± 7.9	17.4 ± 8.5
Delayed graft function (%)	28.9	19.5[d]	29.1	21.2[d]	20.2	16.5
Discharged on tacrolimus (%)	81	79[b]	70	98[d]	67	78[d]

[a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$, all comparisons are steroid *vs* no steroid groups. CVA: Cerebro vascular accident; DCD: Donation after cardiac death; ECD: Expanded criteria donor; HLA: Human leukocyte antigen; PRA: Panel reactive antibody; r-ATG: Rabbit antithymocyte globulin; IL-2: Interleukin-2; M: Male; F: Female.

stratified by steroid use are shown in Table 1. Among all patients, 17863 (48%) received induction with r-ATG (steroid = 13001, no steroid = 4862), 3028 (8%) received alemtuzumab (steroid = 852, no steroid = 2176) and 16326 (44%) an IL-2B agent (steroid = 15008, no steroid = 1318). Among the r-ATG induced patients, there was a higher prevalence of females, African Americans, diabetics, tacrolimus use, pre-transplant dialysis requirement and duration, elevated PRA titer, donor death from CVA and development of DGF in CSM group. Recipient age was higher in ESW group. Among patients who received alemtuzumab, there was higher prevalence ECD/DCD kidney use, female gender, high peak PRA, HLA mismatch and DGF in the CSM group whereas recipient diabetes and tacrolimus use was higher in the ESW group. Among IL-2B induced patients, there was a higher prevalence of African Americans, dialysis requirement and duration, donor death from CVA and DGF development in CSM group while ESW group had higher recipient age, DCD kidney and tacrolimus use.

Impact of steroid use on graft survival by induction type

Adjusted overall and death censored graft survivals for CSM *vs* ESW groups for the three induction agents are shown in Figure 2. The adjusted overall graft survival was significantly inferior for CSM *vs* ESW groups in patients who underwent induction with r-ATG (HR = 1.16, 95%CI: 1.06-1.27, $P = 0.002$). However, adjusted death-censored graft survivals were similar between the groups. In alemtuzumab induced patients, there were no significant differences in both adjusted overall (HR = 0.92, 95%CI: 0.73-1.15, $P = 0.47$) and death-censored (HR = 0.87, 95%CI: 0.62-1.22, $P = 0.43$) graft survivals for CSM *vs* ESW groups. Similarly, adjusted overall (HR = 1.05, 95%CI: 0.91-1.21, $P = 0.48$) and death-censored

(HR = 0.94, 95%CI: 0.75-1.18, $P = 0.60$) graft survivals were similar for CSM *vs* ESW groups in IL-2B induced patients.

A further analysis was performed by splitting the r-ATG induced patients into low and high immune risk groups as explained in the methods section. There were 4346 patients in the low immune risk and 13517 patients in the high immune risk group. Adjusted overall and death-censored graft survivals for low and high immune risk groups are shown in Figure 3. Adjusted overall graft survivals were inferior for CSM *vs* ESW groups in both low immune (HR = 1.34, 95%CI: 1.09-1.64, $P = 0.001$) and high immune (HR = 1.18, 95%CI: 1.07-1.30, $P = 0.005$) risk patients. Adjusted death-censored graft survivals for CSM *vs* ESW groups were similar in both low (HR = 1.06, 95%CI: 0.78-1.45, $P = 0.70$) and high (HR = 1.04, 95%CI: 0.98-1.20, $P = 0.60$) immune risk groups.

Impact of steroid maintenance on patient survival by induction type

Adjusted patient survivals for CSM *vs* ESW for the three different induction agents are shown in Figure 2. Patient survivals were inferior for CSM *vs* ESW groups in r-ATG induced (HR = 1.31, 95%CI: 1.15-1.49, $P < 0.001$) but similar in alemtuzumab (HR = 1.02, 95%CI: 0.75-1.38, $P = 0.92$) and IL-2B (HR = 1.17, 95%CI: 0.97-1.40, $P = 0.10$) induced patients. In r-ATG induced patients, adjusted patient survivals were inferior for CSM *vs* ESW groups in both low immune (HR = 1.54, 95%CI: 1.18-2.02, $P < 0.001$) and high immune (HR = 1.32, 95%CI: 1.16-1.51, $P = 0.002$) risk groups (Figure 3). We further analyzed the causes of recipient death for CSM *vs* ESW groups in r-ATG induced patients (Table 2). There were significantly higher deaths from infections and CV causes in CSM groups.
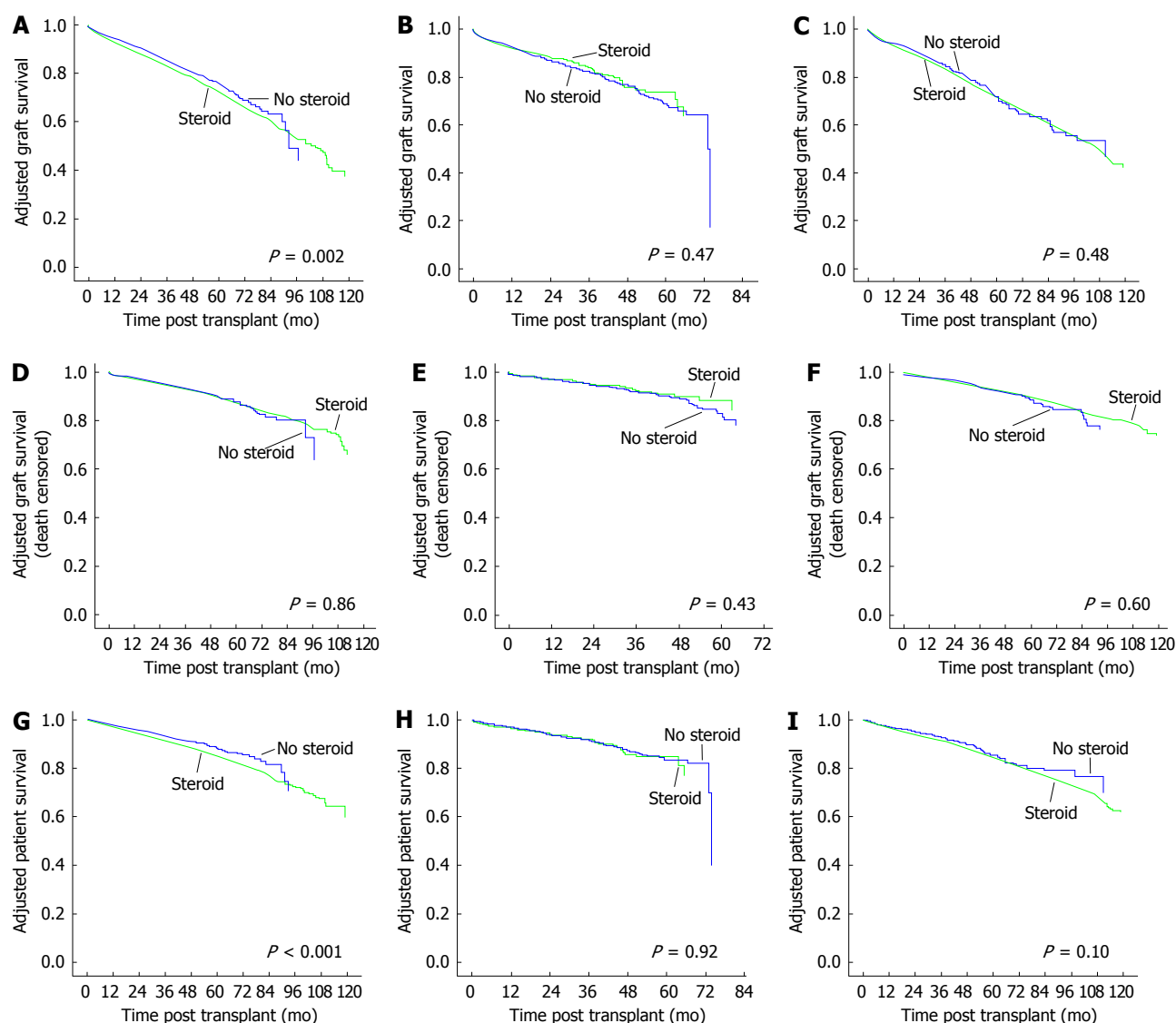
Figure 2 Adjusted over-all graft (A, B and C), death-censored graft (D, E and F) and patient (G, H and I) survivals for early steroid withdrawal *vs* chronic steroid maintenance groups. A: Adjusted over-all graft rabbit antithymocyte globulin (r-ATG); B: Adjusted over-all graft alemtuzumab; C: Adjusted over-all graft interleukin-2 receptor blocker (IL-2B); D: Death-censored graft r-ATG; E: Death-censored graft alemtuzumab; F: Death-censored graft IL-2B; G: Patient r-ATG; H: Patient alemtuzumab; I: Patient IL-2B.

Table 2 Cause of death in rabbit antithymocyte globulin induced patients

Cause of death	CSM group n = 13001 (%)	ESW group n = 4862 (%)	*P*-value
Cardiovascular	410 (3.2)	97 (2.0)	< 0.0001
Infections	313 (2.4)	42 (0.9)	< 0.0001
Malignancy	148 (1.1)	55 (1.1)	0.97
Other causes	916 (7)	206 (4.2)	< 0.0001

CSM: Chronic steroid maintenance; ESW: Early steroid withdrawal.

DISCUSSION

Our study showed an association between increased overall graft failure as well as patient death risks and steroid addition to a CNI/MMF maintenance regimen in patients undergoing a first DDK transplantation following induction with r-ATG. Similar death-censored graft survival for steroid *vs* no-steroid maintenance in these patients suggests increased death with functioning graft in the steroid maintenance group. There were increased patient deaths from CV and infectious causes for steroid *vs* no-steroid groups.

Current data suggest that corticosteroids could be discontinued safely during the first week after transplantation in patients who are at low immunological risk and receive induction therapy[7]. Studies of early steroid withdrawal have shown outcomes comparable to steroid maintenance regimens[8-14]. Seven year results from a large prospective study performed within the frame work of the Collaborative Transplant Study involving predominantly Caucasian kidney transplant recipients showed a benefit for steroid withdrawal in terms of graft, patient and death-censored graft survival[15]. A five year prospective trial demonstrated similar graft survival and function as well as patient survival in kidney transplant recipients
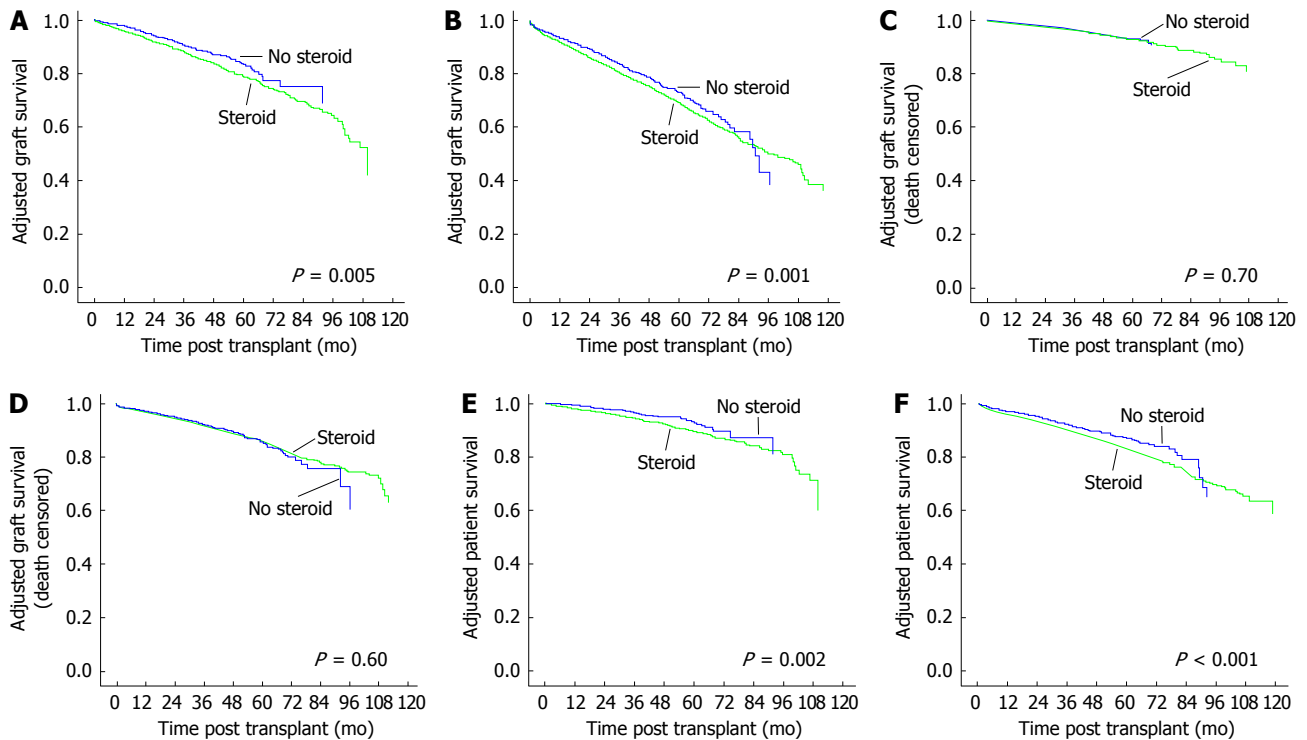
Figure 3 Adjusted over-all graft (A and B), death-censored graft (C and D) and patient (E and F) survivals in rabbit antithymocyte globulin induced patients. A: Adjusted over-all graft low-risk; B: Adjusted over-all graft high-risk; C: Death-censored graft low-risk; D: Death-censored graft high-risk; E: Patient low-risk; F: Patient high-risk.

randomized to ESW *vs* CSM following induction with either r-ATG or IL-2B and tacrolimus/MMF maintenance[16]. There was increased incidence of mild biopsy proven AR in ESW group but favorable effects on CV risk factors including serum triglyceride levels, new onset diabetes after transplantation (NODAT) requiring insulin and weight gain. A meta-analysis involving 34 studies and 5637 patients showed a small increase in AR rates in steroid avoidance/withdrawal group, without any measurable adverse effect on graft or patient survival, while enjoying significant benefits in terms of CV risk factors[17]. A large single center retrospective analysis involving 1241 primary living and DDK transplant recipients showed similar patient, graft, death-censored graft, and AR free survival rates at 10 years for rapid steroid discontinuation group compared to historic controls on maintenance steroids[18]. All patients received induction with r-ATG and maintenance therapy consisting of a CNI agent along with either MMF or sirolimus. In the subgroup of patients who received DDK transplants, patient, graft and death-censored graft survivals were significantly higher in the ESW group. There were significant reductions in the incidences of NODAT, cataracts and avascular necrosis in rapid steroid discontinuation group. This study compared prednisone related side effects to historic control group maintained on relatively higher doses of prednisone (0.1-0.15 mg/kg). Our study showed superior patient and overall graft but not death-censored graft survival in the ESW groups indicating that the beneficial effects appear to be related to lower rate of death with functioning graft in ESW group.

The polyclonal antibody r-ATG is a powerful depleting induction agent commonly used in kidney transplant recipients. r-ATG contains antibodies to several human T-cell surface antigens including major histocompatibility antigens and causes apoptosis and cell lysis. r-ATG causes severe lymphocyte depletion with prolonged immunosuppressive effect with the drug detectable in blood at 90 d after initiation of therapy[19]. Our study showed inferior adjusted overall graft and patient survival in kidney transplant recipients continued on steroid maintenance after r-ATG induction when compared to patients in whom steroid was discontinued on hospital discharge. One could speculate that inferior outcomes in the CSM group could possibly be related to the enhanced immunosuppression related to the steroid containing triple immunosuppression following powerful induction with r-ATG. Similar death-censored graft survival between CSM and ESW groups along with the finding of significantly higher deaths from infectious and CV causes in CSM group is in support of this speculation. A highly significant association between maintenance steroid dose and death with functioning graft caused by CV disease or infection beyond the first year following DDK transplantation was reported recently in a study involving close to 42000 patients[20]. Such an association was observed with neither tacrolimus nor mycophenolic acid in that study. Adverse metabolic profile in patients on steroid therapy as shown in previous studies mentioned before could be contributing to CV complications and enhanced immunosuppression to infectious complications. These findings persisted when r-ATG induced patients in our study were split into

high and low immune risk groups indicating that any potential benefits of steroid maintenance in terms of reducing immunological allograft injury even in high immune risk subgroup appear to be overshadowed by CV and infectious complications. These adverse outcomes associated with CSM after r-ATG induction was not seen in patients who were maintained on steroid following induction with IL-2B agent or alemtuzumab. IL-2B is a non-depleting monoclonal antibody with lesser degrees of immunosuppression. Alemtuzumab is a depleting monoclonal antibody typically administered as a single dose intra-operatively and can cause prolonged lymphopenia. It is not clear why inferior outcomes seen with CSM *vs* ESW following r-ATG induction was not observed with alemtuzumab. Alemtuzumab induced patients generally tolerate only lower doses of antiproliferative agent and many of them could have been taken off MMF completely due to low white blood cell counts[21]. This may decrease the degree of immunosuppression despite being on steroid maintenance. It should be noted that there were only 852 patients in the alemtuzumab group who were discharged on steroid maintenance thus limiting power of the comparison. A previous registry analysis showed superior outcomes with r-ATG induction when compared to induction with either alemtuzumab or IL-2B in DDK transplant recipients discharged on a steroid-free CNI/MMF regimen[21].

Large number of patients in a national cohort including several transplant centers and inclusion of high immune risk patients adds to the validity of the current analysis. One could argue that r-ATG induction and steroid maintenance is generally used in high risk patients with expectedly poor outcomes. Classification of r-ATG induced patients into high and low immune risk groups and the finding of inferior outcomes associated with CSM in both groups tend to mollify this criticism. Our findings are consistent with a previous registry analysis by Luan *et al*[5] which showed improved graft and patient survivals at years 1 and 4 post transplantation for patients in whom steroid was discontinued on discharge. That study evaluated both living and DDK transplant recipients and included first and subsequent transplants. We believe our findings are important since r-ATG is a commonly used induction agent and majority of the kidney transplant recipients are discharged on maintenance steroids. For instance, in the current analysis, 48% of patients received r-ATG out of which 73% were discharged on a steroid maintenance regimen. About 35% of all the study population received r-ATG induction followed by steroid maintenance.

Our study is not without limitations. Retrospective design can confirm association but cannot prove causation. Residual confounding likely exists despite utilizing an adjusted model and lack of granularity on some important details in the registry could likely have an effect on the outcomes tested. Center-specific differences in practice patterns lead to selection bias regarding different induction agents and steroid maintenance. Some early steroid withdrawal protocols discontinue steroids at

seven days post-transplant and some others at day five. Discharging these patients from the hospital before the steroid withdrawal is complete would wrongly categorize them as being on steroids. Details on the doses of induction and maintenance immunosuppressive agents were not available which may have impact on the outcomes. Information on daily or cumulative doses of steroids used would be very helpful while analyzing the negative effects of steroids over long periods of time but unfortunately as mentioned dosing information was not available in the database. Data regarding changes in maintenance immunosuppressive regimen since initial hospital discharge were not available. This could be important since as many as 30% of patients initially discharged on no steroids may have been restarted on CSM mostly after rejection episodes. But this should in fact have diminished the strength of observed associations. Some patients initially discharged on steroid containing regimen may have undergone later steroid withdrawal since some transplant centers withdraw steroids late after transplantation. Despite these misclassifications, there is likely minimal impact on the results because of the non-differential influence of misclassification which tend to deflate the results toward the null[22]. The possibility of a type 1 error cannot be completely excluded.

In summary, our study showed an association of adverse outcomes with steroid addition to a CNI/MMF maintenance regimen in patients who received r-ATG induction for a first DDK transplantation. One has to be cautious while maintaining intense immunosuppression by adding steroid to a CNI/MMF regimen in kidney transplant recipients who were selected for r-ATG induction. Since r-ATG is a commonly used induction agent and majority of patients are maintained on steroid, this important observation needs to be further evaluated in future studies.

ACKNOWLEDGMENTS

COMMENTS

Background
Analysis of the outcomes in kidney transplant recipients who were maintained on steroid and stratified by the induction type.

Innovations and breakthroughs
Enhanced immunosuppression with steroid maintenance in kidney transplant recipients who receive powerful induction therapy with rabbit-antithymocyte globulin may be associated with adverse outcomes.

Applications
In clinical transplantation.

Peer review
The manuscript clearly describes the current situation about this problem representing therefore a subject of interest to the reader.

REFERENCES

1   **Prasad GV**, Nash MM, McFarlane PA, Zaltzman JS. Renal transplant recipient attitudes toward steroid use and ste-

roid withdrawal. *Clin Transplant* 2003; **17**: 135-139 [PMID: 12709080 DOI: 10.1034/j.1399-0012.2003.00034.x]

2   **Sinclair NR**. Low-dose steroid therapy in cyclosporine-treated renal transplant recipients with well-functioning grafts. The Canadian Multicentre Transplant Study Group. *CMAJ* 1992; **147**: 645-657 [PMID: 1521210]

3   **Hricik DE**, O'Toole MA, Schulak JA, Herson J. Steroid-free immunosuppression in cyclosporine-treated renal transplant recipients: a meta-analysis. *J Am Soc Nephrol* 1993; **4**: 1300-1305 [PMID: 8130356]

4   **Kasiske BL**, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 2000; **11**: 1910-1917 [PMID: 11004223]

5   **Luan FL**, Steffick DE, Gadegbeku C, Norman SP, Wolfe R, Ojo AO. Graft and patient survival in kidney transplant recipients selected for de novo steroid-free maintenance immunosuppression. *Am J Transplant* 2009; **9**: 160-168 [PMID: 18976304 DOI: 10.1111/j.1600-6143.2008.02442.x]

6   **Cai J**, Terasaki PI. Induction immunosuppression improves long-term graft and patient outcome in organ transplantation: an analysis of United Network for Organ Sharing registry data. *Transplantation* 2010; **90**: 1511-1515 [PMID: 21057388 DOI: 10.1097/TP.0b013e3181fecfcb]

7   **Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group**. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

8   **Vincenti F**, Monaco A, Grinyo J, Kinkhabwala M, Roza A. Multicenter randomized prospective trial of steroid withdrawal in renal transplant recipients receiving basiliximab, cyclosporine microemulsion and mycophenolate mofetil. *Am J Transplant* 2003; **3**: 306-311 [PMID: 12614286 DOI: 10.1034/j.1600-6143.2003.00005.x]

9   **Matas AJ**, Kandaswamy R, Gillingham KJ, McHugh L, Ibrahim H, Kasiske B, Humar A. Prednisone-free maintenance immunosuppression-a 5-year experience. *Am J Transplant* 2005; **5**: 2473-2478 [PMID: 16162197 DOI: 10.1111/j.1600-6143.2005.01051.x]

10   **Rostaing L**, Cantarovich D, Mourad G, Budde K, Rigotti P, Mariat C, Margreiter R, Capdevilla L, Lang P, Vialtel P, Ortuño-Mirete J, Charpentier B, Legendre C, Sanchez-Plumed J, Oppenheimer F, Kessler M. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807-814 [PMID: 15818323 DOI: 10.1097/01.TP.0000154915.20524.0A]

11   **Kumar MS**, Heifets M, Moritz MJ, Saeed MI, Khan SM, Fyfe B, Sustento-Riodeca N, Daniel JN, Kumar A. Safety and efficacy of steroid withdrawal two days after kidney transplantation: analysis of results at three years. *Transplantation* 2006; **81**: 832-839 [PMID: 16570004 DOI: 10.1097/01.tp.0000203558.34739.c6]

12   **Vincenti F**, Schena FP, Paraskevas S, Hauser IA, Walker RG, Grinyo J. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. *Am J Transplant* 2008; **8**: 307-316 [PMID: 18211506 DOI: 10.1111/j.1600-6143.2007.02057.x]

13   **Pascual J**, Zamora J, Galeano C, Royuela A, Quereda C. Steroid avoidance or withdrawal for kidney transplant recipients. *Cochrane Database Syst Rev* 2009; (**1**): CD005632 [PMID: 19160257]

14   **Schiff J**, Cole EH. Renal transplantation with early steroid withdrawal. *Pediatr Nephrol* 2009; **24**: 243-251 [PMID: 18535842 DOI: 10.1007/s00467-008-0876-0]

15   **Opelz G**, Döhler B, Laux G. Long-term prospective study of steroid withdrawal in kidney and heart transplant recipients. *Am J Transplant* 2005; **5**: 720-728 [PMID: 15760395 DOI: 10.1111/j.1600-6143.2004.00765.x]

16   **Woodle ES**, First MR, Pirsch J, Shihab F, Gaber AO, Van Veldhuisen P. A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early (7 day) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. *Ann Surg* 2008; **248**: 564-577 [PMID: 18936569]

17   **Knight SR**, Morris PJ. Steroid avoidance or withdrawal after renal transplantation increases the risk of acute rejection but decreases cardiovascular risk. A meta-analysis. *Transplantation* 2010; **89**: 1-14 [PMID: 20061913 DOI: 10.1097/TP.0b013e3181c518cc]

18   **Rizzari MD**, Suszynski TM, Gillingham KJ, Dunn TB, Ibrahim HN, Payne WD, Chinnakotla S, Finger EB, Sutherland DE, Kandaswamy R, Najarian JS, Pruett TL, Kukla A, Spong R, Matas AJ. Ten-year outcome after rapid discontinuation of prednisone in adult primary kidney transplantation. *Clin J Am Soc Nephrol* 2012; **7**: 494-503 [PMID: 22282482 DOI: 10.2215/CJN.08630811]

19   **Deeks ED**, Keating GM. Rabbit antithymocyte globulin (thymoglobulin): a review of its use in the prevention and treatment of acute renal allograft rejection. *Drugs* 2009; **69**: 1483-1512 [PMID: 19634926 DOI: 10.2165/00003495-200969110-00007]

20   **Opelz G**, Döhler B. Association between steroid dosage and death with a functioning graft after kidney transplantation. *Am J Transplant* 2013; **13**: 2096-2105 [PMID: 23750878 DOI: 10.1111/ajt.12313]

21   **Sureshkumar KK**, Thai NL, Hussain SM, Ko TY, Marcus RJ. Influence of induction modality on the outcome of deceased donor kidney transplant recipients discharged on steroid-free maintenance immunosuppression. *Transplantation* 2012; **93**: 799-805 [PMID: 22290269 DOI: 10.1097/TP.0b013e3182472898]

22   **Copeland KT**, Checkoway H, McMichael AJ, Holbrook RH. Bias due to misclassification in the estimation of relative risk. *Am J Epidemiol* 1977; **105**: 488-495 [PMID: 871121]

ORIGINAL ARTICLE

Intra-articular transplantation of porcine adipose-derived stem cells for the treatment of canine osteoarthritis: A pilot study

Shen-Yang Tsai, Yun-Ching Huang, Ling-Ling Chueh, Lih-Seng Yeh, Ching-Shwun Lin

Shen-Yang Tsai, Lih-Seng Yeh, Institute of Veterinary Clinical Sciences, School of Veterinary Medicine, National Taiwan University, Taipei 10617, Taiwan

Yun-Ching Huang, Division of Urology, Department of Surgery, Chang Gung Memorial Hospital, Chia-Yi 60003, Taiwan

Ling-Ling Chueh, Graduate Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei 10617, Taiwan

Ching-Shwun Lin, Department of Urology, School of Medicine, University of California, San Francisco, CA 94143-0738, United States

Author contributions: All authors contributed to this work.

Supported by The National Taiwan University

Correspondence to: Dr. Ching-Shwun Lin, Department of Urology, School of Medicine, University of California, 533 Parnassus Ave, San Francisco, CA 94143-0738, United States. clin@urology.ucsf.edu

Telephone: +1-415-4763800   Fax: +1-415-4763803

Received: December 21, 2013   Revised: March 16, 2014

Accepted: June 27, 2014

Published online: September 24, 2014

Abstract

AIM: To test whether intra-articular injection of porcine adipose-derived stem cells (ADSCs) can treat canine osteoarthritis (OA).

METHODS: To enroll in this study dogs must have stifle joint OA that had lasted ⩾ 3 mo and been treated with OA medication without significant improvement. Three dogs fulfilled these criteria and were thus subjects for ADSCs treatment. ADSCs were isolated from abdominal adipose tissue of a 2-mo-old female Yorkshire pig. Their stem cell marker expression was examined by immunofluorescence staining. For treatment, 5 million ADSCs were injected into the diseased joint of each dog. In the next 48 h, the patient was observed for signs of inflammatory and allergic reactions. The patient was then discharged to the owner and, at 2, 6, and 12 wk, followed up with orthopedic assessment, owner questionnaire, X-ray imaging, and force-plate gait analysis.

RESULTS: Porcine ADSCs expressed mesenchymal stem cell markers CD90 and CD105. Injection of porcine ADSCs into canine stifle joints did not cause any inflammatory or allergic reactions. Orthopedic evaluation found improvements in two dogs, particularly at the longest time point. Owners' evaluation found increased capacity and decreased pain in all three dogs' activities such as walking and running. Radiographic evaluation did not find statistically significant differences before and after treatment. Force-plate analysis found significant improvements in all three dogs after treatment.

CONCLUSION: Xenotransplantation of ADSCs for the treatment of OA is feasible. Further studies are needed to validate this novel treatment modality, which can then be implemented for the routine treatment of OA in veterinary medicine.

Core tip: Intra-articular injection of autologous adipose-derived stem cells (ADSCs) has been shown to be effective in treating osteoarthritis in dogs. However, most veterinary clinics lack the equipment and expertise for ADSC isolation. The present study showed that intra-articular injection of porcine ADSCs into the diseased stifle joint of three dogs improved their mobility and activity and lessened their pain. The injection of porcine ADSCs elicited no signs of inflammation or immunologi-

cal reaction. Thus, porcine ADSCs can substitute for autologous canine ADSCs, and such a treatment strategy can drastically reduce treatment cost.

INTRODUCTION

Osteoarthritis (OA), also known as degenerative joint disease, is the most common cause of chronic pain in companion animals and afflicts more than 10 million dogs in the United States[1]. Current treatments, which commonly involve behavior modification and use of non-steroidal anti-inflammatory drugs (NSAIDs), are non-curative and are rather aimed at alleviating discomfort, decelerating tissue damage, and/or improving joint functionality[2]. However, chronic use of NSAIDs is associated with gastric ulceration/perforation and renal and hepatic damages[2]. As such, alternative treatment modalities without these adverse side effects are highly desirable.

Pathologically OA is characterized by the progressive degradation of articular cartilage, subchondral bone remodeling, and synovial inflammation[3,4]. While the exact cause of these pathological changes remains unknown, it is believed to involve the overproduction of pro-inflammatory cytokines (*e.g.*, interleukin-1β), tissue-destructive proteinases (*e.g.*, MMP-13), and catabolic mediators (*e.g.*, nitric oxide) from chondrocytes and synovial cells[3,4]. As such, current pharmaceutical development for OA treatment largely aimed at preventing the production or action of these harmful molecules[3,4].

Mesenchymal stem/stromal cells (MSCs), first identified in bone marrow, are now found in virtually all tissues[5]. They are classified as multipotent, that is, able to differentiate into several cell lineages such as osteocytes, chondrocytes, adipocytes, and myocytes. This multipotency was originally the scientific basis for using MSCs as therapeutic agents for a wide variety of diseases, but decade-long research has resulted in a shift of emphasis from differentiation to paracrine actions as the main mechanism for MSC's therapeutic efficacy[6]. Specifically, when transplanted into diseased tissues, MSCs communicate with local cells through secretion of a wide array of cytokines and growth factors[7]. They can also promote the migration of endogenous repairing cells to injury sites and suppress immunoreactions[7]. Together, these actions help restore physiological balance and enhance healing. In veterinary medicine, MSCs have been used to treat not only OA but also tendon injury, bone fracture, spinal cord injury, and liver disease[8].

Adipose-derived stem/stromal cells (ADSCs) are MSCs isolated from adipose tissue, and their therapeutic potential has been demonstrated in numerous preclinical and clinical studies[9-11]. Due to their abundant tissue source, low donor site morbidity, and ease of isolation, ADSCs have become increasingly the preferred MSCs for therapeutic application[9]. In veterinary medicine, there have been numerous claims in the mainstream media and in the Internet about ADSC's "miraculous" therapeutic efficacy. In the scientific literature, there have been four studies that used ADSCs to treat canine OA[12-15], and the results all indicated ADSC's efficacy in ameliorating OA symptoms.

The isolation of ADSCs from patients requires certain equipment, reagents, and expertise that are lacking in most veterinary clinics. As such, clinical application of autologous ADSCs in veterinary medicine is currently a costly and time-consuming process. On the other hand, if the therapeutic ADSCs can be obtained in a ready-for-injection form, their clinical applicability will increase substantially. To this end, it has been shown that ADSCs possess immunosuppressive capability and can be xenogeneically transplanted in immunocompetent recipients without the use of immunosuppressants[16]. Therefore, we hypothesized that ADSCs can be applied in a xenogeneic fashion, thereby eliminating the veterinarian's burden to perform adipose tissue harvest and ADSC isolation. In the present study we show that porcine ADSCs can effectively treat canine OA.

MATERIALS AND METHODS

Stem cell preparation

ADSCs were isolated as described previously[17] from the abdominal fat of a 2-mo-old female Yorkshire pig. Briefly, the adipose tissue was rinsed with phosphate-buffered saline (PBS) containing 1% penicillin and streptomycin, minced into small pieces, and then incubated in a solution containing 0.075% collagenase type I A (Sigma-Aldrich, St. Louis, MO, United States) for 1 h at 37 ℃ with vigorous shake. The top lipid layer was removed and the remaining liquid portion was centrifuged at 220 g for 10 min at room temperature. The pellet was treated with 160 mmol/L NH₄Cl for 10 min to lyse red blood cells. The remaining cells were suspended in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), filtered through a 40-μm cell strainer (BD Biosciences, Bedford, MA, United States), and plated at a density of 1×10^6 cells in a 10-cm dish. After reaching 80% confluence, the cells were harvested, aliquoted, and stored in liquid nitrogen at a density of 5×10^5 cells per mL of freezing media (DMEM, 20% FBS, and 10% DMSO) per vial. One week prior to the scheduled stem cell (SC) injection, the frozen cells were thawed and cultured until the necessary cell number for intra-articular injection was reached.

Immunofluorescence staining

ADSCs (1×10^5 cells/well) were seeded on coverslips and placed in 6-well plates with 3 mL/well of DMEM and 10% FBS. After 24 h of incubation, the cells were

Figure 1 Gait analysis platform. The platform was a 10 m × 1.22 m × 0.19 m wooden walkway and in the middle of which embedded a pressure sensor. During the analysis the dog was leash-walked by its owner from one end toward the other end of the walkway. The walking speed was recorded by two sets of photoelectric cells. The walk was repeated until three valid speeds were recorded for each leg.

fixed in cold methanol for 5 min at 4 ℃, permeabilized with 0.05% Triton X-100 for 5 min, and blocked with 5% normal equine serum in PBS for 1 h at room temperature. The cells were then incubated with a primary antibody for 1 h at room temperature. After washing with PBS three times, the cells were incubated with FITC-conjugated secondary antibody for 1 h at room temperature. After three washes with PBS, the cells were further stained with 4-, 6-diamidino-2-phenylindole (DAPI, for nuclear staining) for 5 min. The stained cells were examined with Nikon Eclipse E600 fluorescence microscope and photographed with Retiga 1300 Q-imaging camera using the ACT-1 software (Nikon Instruments Inc., Melville, NY). Primary antibodies were against CD34 (sc7324), CD105 (sc18838), calponin (ab46794), SSEA-1 (ab16285), Oct4 (ab19857), and CD90 (ab225). Antibodies with "sc" and "ab" catalog numbers were purchased from Santa Cruz Biotech (Santa Cruz, CA, United States) and Abcam Inc. (Cambridge, MA, United States), respectively.

Patients

To enroll in this study the dogs must have chronic and stable OA of the stifle joints as defined by exhibition of the following symptoms: chronic lameness, exercise intolerance, difficulty getting up after a prolonged rest, and pain in the diseased limb when maneuvered. In addition, the disease must have lasted 3 mo or longer and been treated with OA medication without significant improvement. A total of three dogs fulfilled these criteria and thus enrolled in this study.

To exclude the involvement of systemic diseases, patients underwent physical examination and hematologic evaluation, including blood cell count, serum albumin, total protein, glucose, liver index, and kidney index. To affirm OA in the stifle joint, X-ray imaging was conducted to identify osteophyte formation and subchondral bone sclerosis/remodeling. Exclusion criteria included history of surgery, autoimmune or inflammatory arthritis, and

other systemic diseases or non-orthopedic disorders (such as neurological diseases) that could affect gait and thus led to misdiagnosis.

To avoid the effects of OA medications on SC treatment evaluation, patients were required to stop taking steroids and non-steroidal analgesics for 3 and 2 wk, respectively, prior to SC injection. Use of these medications was also prohibited during the treatment and follow-up periods (3 mo total). In addition, patients were required to maintain stable body weight, as fluctuation in body weight might affect SC treatment evaluation.

Prior to SC injection, the owner of each patient dog was informed of the potential benefits and risks of this treatment. The owner was then required to sign a consent form, agreeing to closely observe the patient during the entire treatment and follow-up periods, to report any unusual behavior or symptom, and to return to the clinic for checkup at specified times.

Treatment

Anesthesia was induced by *iv* injection of propofol (4 mg/kg) and maintained by isoflurane inhalation. Physiological monitoring included heart rate, electrocardiography, blood oxygen, blood pressure, and respiration. After shaving and disinfection of the stifle joint area, the anesthetized dog was placed on its side with the affected limb facing up. A 1-inch-long 24G needle attached to a syringe was inserted through the joint capsule lateral to the patellar ligament and toward the femoral condyle. A small amount of synovial fluid was withdrawn for analysis to rule out non-OA inflammation or autoimmune disease. Stem cells (5 million in 1 mL of PBS) were then injected *via* the same needle. The patient was allowed to wake up from anesthesia and further observed for approximately 45 min before being discharged with 3 d of cephalexin (20 mg/kg orally). Phone calls were made to the owner 6, 24, and 48 h later to inquire about any adverse reactions.

Evaluation

Treatment efficacy was evaluated at 2, 6, and 12 wk by orthopedic assessment, owner questionnaire, X-ray imaging, and force-plate gait analysis. Orthopedic assessment was conducted according to McCarthy *et al*[18] and used a scoring system shown in Table 1. Owner's questionnaire and radiographic assessment were conducted according to Moreau *et al*[19] and used scoring systems shown in Table 2. Force-plate analysis was conducted according to Moreau *et al*[19]. In this study the force plate was a 10 m × 1.2 m × 0.19 m wooden walkway and in the middle of which embedded a pressure sensor (model OR6-7-1000, Advanced Mechanical Technology, Inc. Watertown, MA, United States) (Figure 1). During each analysis the dog was leash-walked by its owner from one end toward the other end of the walkway. The walking speed (between 1.9 and 2.2 m/s) was recorded by two sets of photoelectric cells, and a difference of < 0.2 m/s between the two recorded speeds was considered a valid speed. The walk was repeated until three valid speeds were recorded for each leg. During each

Table 1 Clinical scoring system for evaluation of canine osteoarthritis

Criterion	Clinical evaluation	Score
Lameness	Walks normally	1
	Slightly lame when walking	2
	Moderately lame when walking	3
	Severely lame when walking	4
	Reluctant to rise and will not walk more than five paces	5
Joint mobility	Full range of motion	1
	Mild limitation (10%-20%) in range of motion; no crepitus	2
	Mild limitation (10%-20%) in range of motion; with crepitus	3
	Moderate limitation (20%-50%) in range of motion; with crepitus	4
	Severe limitation (> 50%) in range of motion; with crepitus	5
Pain on palpation	None	1
	Mild signs: dog turns head in recognition	2
	Moderate signs: dog pulls limb away	3
	Severe signs: dog vocalizes or becomes aggressive	4
	Dog will not allow palpation	5
Weight-bearing	Equal on all limbs standing and walking	1
	Normal standing; favors affected limb when walking	2
	Partial weight-bearing standing and walking	3
	Partial weight-bearing standing; non-weight-bearing walking	4
	Non-weight-bearing standing and walking	5
Overall score of clinical condition	Not affected	1
	Mildly affected	2
	Moderately affected	3
	Severely affected	4
	Very severely affected	5

walk the values of peak vertical force (PVF) and vertical impulse (VI) were obtained. PVF, normalized for body weight, is expressed as N kg⁻ in percentile. VI, normalized for time and body weight, is expressed as Nskg⁻. Values obtained in the three trials (walks with valid speed) were averaged for statistical analysis.

Statistical analysis
Data was analyzed with SPSS Statistics (SPSS Inc., Chicago, IL, United States of America), using one-way ANOVA followed by Tukey-Kramer post hoc comparison. Statistical significance was set at $P < 0.05$.

RESULTS

Stem cell characterization
Porcine ADSCs were immunostained for smooth muscle marker calponin, hematopoietic stem cell marker CD34, embryonic stem cell markers Oct4 and SSEA-1, and mesenchymal stem cell markers CD90 and CD105. The results shown in Figure 2 are consistent with ADSCs of other mammalian species[16,20].

Patient characteristics
Three dogs, all having unilateral stifle OA, were enrolled in this study. Their characteristics are listed in Table 3.

Synovial fluid analysis
Synovial fluid analysis (Table 4) indicated that the three canine patients' ailing stifle joints were not due to non-OA inflammation or autoimmune disease.

Orthopedic assessment
On a scale of 1 to 5, with 1 being the best, the patient's OA symptoms such as lameness, joint mobility, pain on palpation, and weight bearing, were evaluated. The results presented in Figure 3 indicate an overall improvement.

Owner's assessment
At 6, 24, and 48 h after SC injection, the owners were contacted by phone. At 6 h, all three owners reported more pronounced lameness than before SC injection. However, at 24 and 48 h, all three owners reported having returned to at least the pre-treatment level of weight bearing. The owners also reported no sign of inflammation on the treated joint, nor any sign of allergic reaction.

For long-term follow-up, the owners were asked to evaluate their pets' ability to perform activities (*e.g.*, walking, running, and jumping) and degree of discomfort. The scoring was on based on scales of 1 to 5 and 1 to 4 (1 being the best) for activity and discomfort, respectively. The results presented in Figure 3 indicate an overall improvement.

Radiographic assessment
X-ray images of the diseased stifle joint of each dog were evaluated by one radiologist and four surgeons. The scores of these 5 independent evaluations were averaged and presented in Figure 4. Overall, there was no significant difference before and after treatment.

Force-plate analysis
As shown in Figure 5 and Table 5, both PVF and VI values were significantly higher (better joint function) at all three time points after SC injection when compared to before injection. Furthermore, in cases 2 and 3, the highest values occurred at the 12th week.

DISCUSSION
Several studies have demonstrated the efficacy of intra-articular MSC injection for the treatment of OA[7,21]. In veterinary medicine there have been four such studies using ADSCs to treat canine OA. First, Black *et al*[13] conducted a randomized, double-blinded, and placebo-controlled study that involved 21 dogs with chronic OA of the hip. A minimum of 23 g of adipose tissue was excised from each dog and shipped to the principal authors' company Vet-Stem for ADSC isolation. The isolated cells were then shipped back to the veterinary clinic, where the veterinarian performed the intra-articular injection. Dogs in the treatment group were each injected with 4.2 to 5 million of autologous ADSCs whereas dogs in the control group PBS. Therapeutic efficacy was evaluated by

Table 2 Scoring system for owner's evaluation of their dog's activity and signs of pain					
	Walking	**Getting up**[1]	**Running**	**Climbing stairs**	**Playing or exercising**
Activities					
No difficulty	1	1	1	1	1
Slight and occasional difficulty	2	2	2	2	2
Frequent slight difficulty	3	3	3	3	3
Constant and obvious difficulty	4	4	4	4	4
Unable to perform	5	5	5	5	5
Signs of pain					
No sign of pain	1	1	1	1	1
Occasional pain, but with no link to a specific activity	2	2	2	2	2
Pain after this activity	3	3	3	3	3
Constant pain	4	4	4	4	4

[1]From prolonged rest.
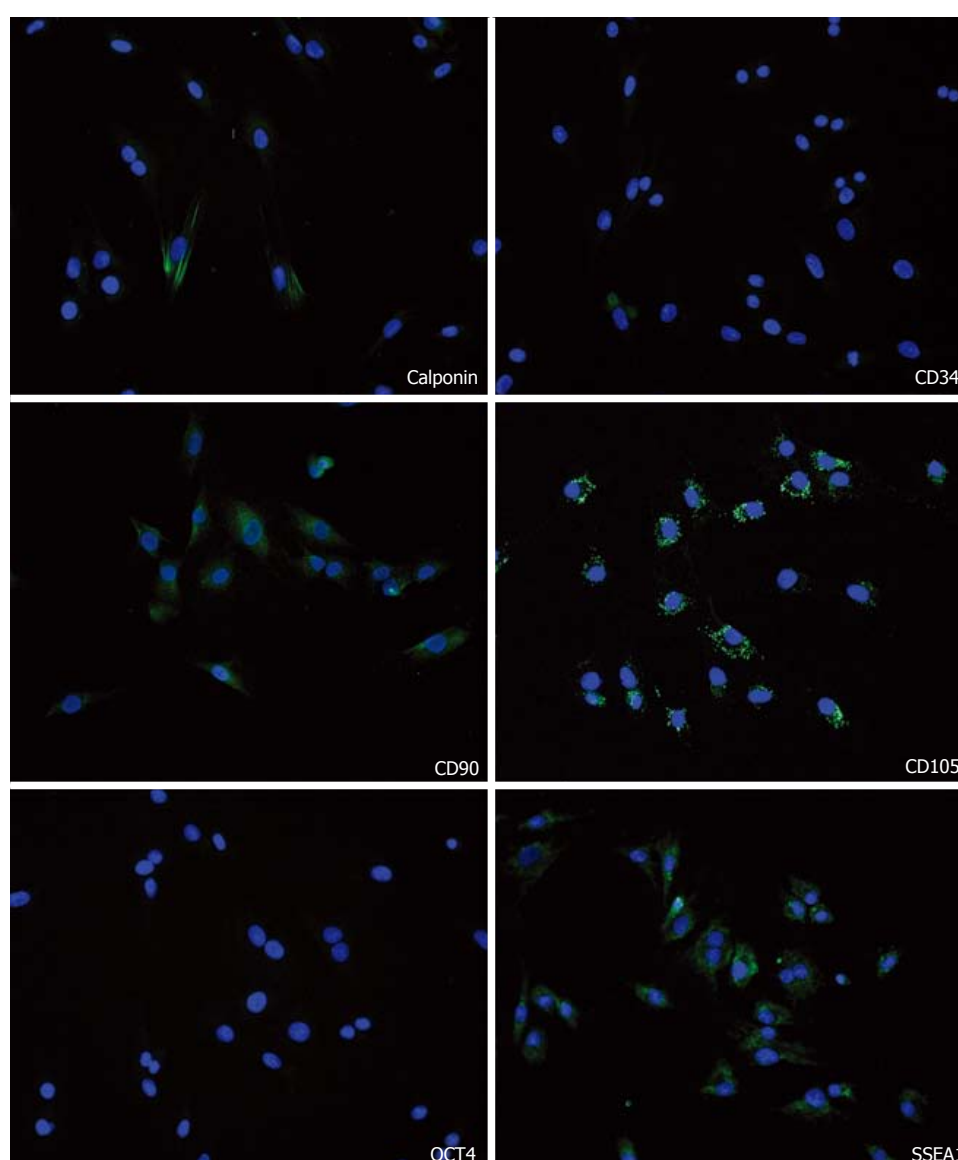
Figure 2 Stem cell marker expression. Porcine adipose-derived stem cells were stained for the indicated cell markers (green) and nuclei (blue).

orthopedic assessment and owner questionnaire at 1, 2, and 3 mo post-treatment. The results show that ADSC therapy significantly improved scores for lameness, pain, and range of motion.

In a second study, also by Black *et al*[12], 14 dogs were treated with ADSCs for chronic OA of the elbow. The
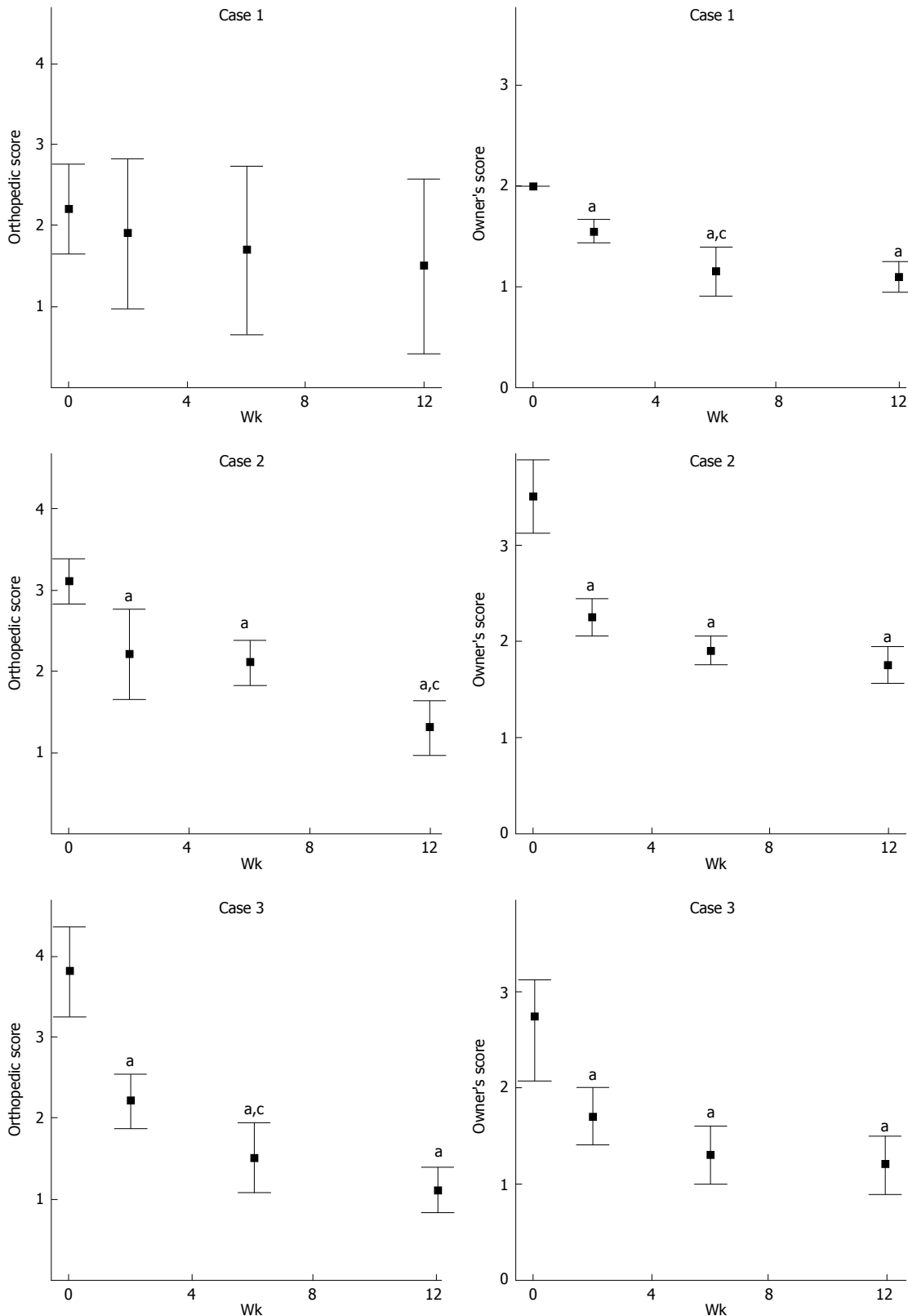
Figure 3 Orthopedic and owner's evaluation. Dogs (cases 1 to 3) were evaluated by veterinarians and owners for stifle joint function and pain according to the scoring criteria listed in Tables 1 and 2. Data presented are the average and range of these scores. [a]*P* < 0.05, orthopedic score after stem cells injection *vs* pre-treatment (week 0); [c]*P* < 0.05, orthopedic score after stem cells injection *vs* the previous time point. Lower scores indicate better joint function or less pain.

preparation of ADSCs was similar to the first study, and 3 to 5 million autologous ADSCs were injected into each OA elbow. Treatment evaluation was conducted the same way as before, and the results again indicated ADSC's therapeutic efficacy. One notable difference this time is that all recruited dogs were treated with ADSCs, citing
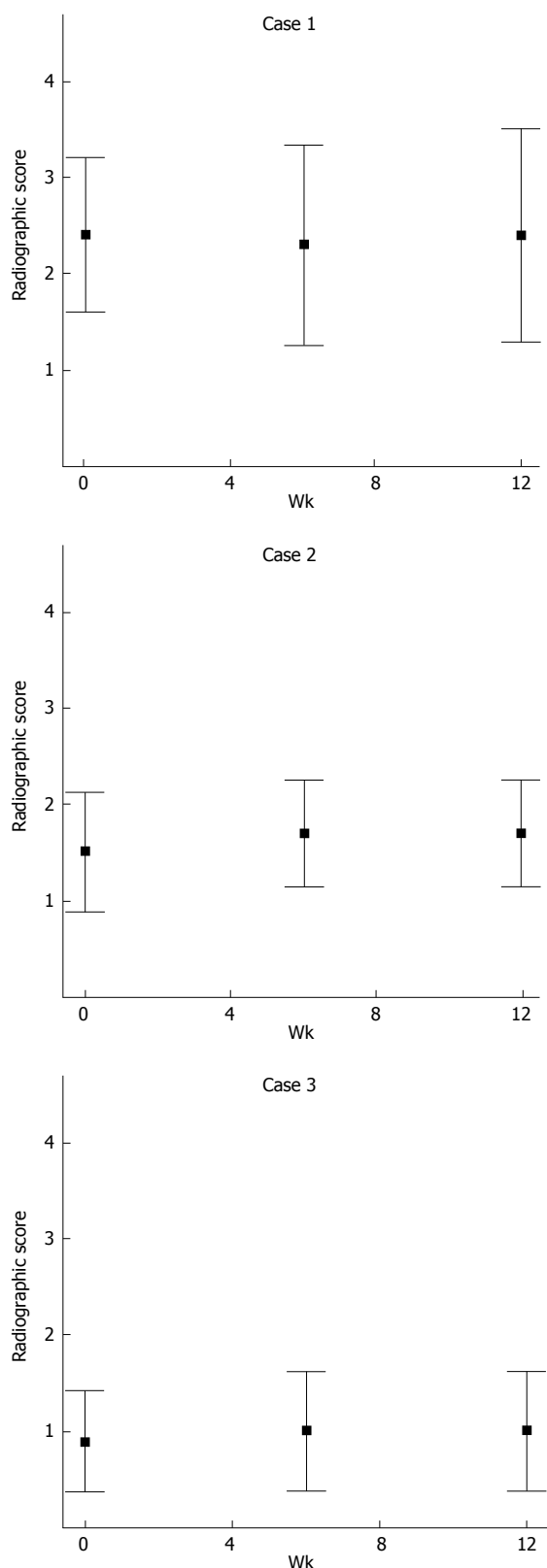
Figure 4 Radiographic evaluation. X-ray images of the diseased stifle joint of each dog (cases 1 to 3) were evaluated by 5 investigators independently according to the scoring criteria listed in Table 3. Data presented are the average and range of these scores.

Table 3 Patient characteristics

	Case 1	Case 2	Case 3
Breed	Labrador Retriever	Golden Retriever	Beagle
Age (yr)	2	5	2
Sex	Male	Female	Male
Weight (kg)	34	35	14
Joint affected	Left stifle	Left stifle	Right stifle
Disease duration	> 1 yr	5 mo	6 mo

Table 4 Synovial fluid analysis

	Case 1	Case 2	Case 3
Color	Light yellow	Light yellow	Light yellow
Turbidity	Clear	Clear	Clear
Viscosity	> 2.5 cm	> 2.5 cm	< 2.5 cm
Nucleated cell count (/μL)	2160	1088	1433
Neutrophil	2%	2%	10%
Monocyte	22%	0%	20%
Lymphocyte	75%	93%	68%
Macrophage	11%	5%	2%

injection of 3 to 5 million autologous ADSCs and evaluated one month later by veterinarian and owner assessments. The results show that all 4 dogs had improved joint functionality and reduced lameness.

The forth study used force-plate analysis to assess the effectiveness of autologous ADSCs in treating hip OA in 8 dogs[15]. The results show that both PVF and VI were significantly improved at 180 d post-treatment.

While the abovementioned studies all demonstrated ADSC's therapeutic efficacy, the adoption of this novel OA treatment into clinical practice faces many challenges, including: (1) most veterinary clinics lack the equipment and expertise for ADSC isolation; (2) excision of adipose tissue causes donor site morbidity; (3) individually-made ADSC isolation is costly and time-consuming; and (4) at least two veterinarian appointments are needed for adipose tissue procurement and ADSC injection. However, these obstacles can be overcome if the therapeutic ADSCs are from a xenogeneic source, and to this end, numerous studies have demonstrated the feasibility of xenotransplantation with ADSCs[16].

In the present study, the injection of porcine ADSCs into the stifle joint of 3 dogs did not cause any inflammatory or allergic reaction, and its therapeutic efficacy was comparable to the aforementioned autologous treatments. As the radiographic assessment, which was conducted independently by 5 investigators, did not find structural improvement in the treated joints, it appears that the improved scores in force-plate, orthopedic, and owner's assessments were due to ADSC's anti-inflammatory and/or immunomodulatory effects. Similar negative radiographic findings in OA patients treated with NSAIDs have been reported previously[22]. The present study is also similar to the three aforementioned veterinarian studies in that the presence of the injected ADSCs in the treated joint could not be confirmed due to the fact that these patients are companion dogs, not experimental canine models, and as

owners' objection to placebo treatment.

The third study involved 4 dogs with chronic OA of the elbow[14]. The dogs were treated with intra-articular
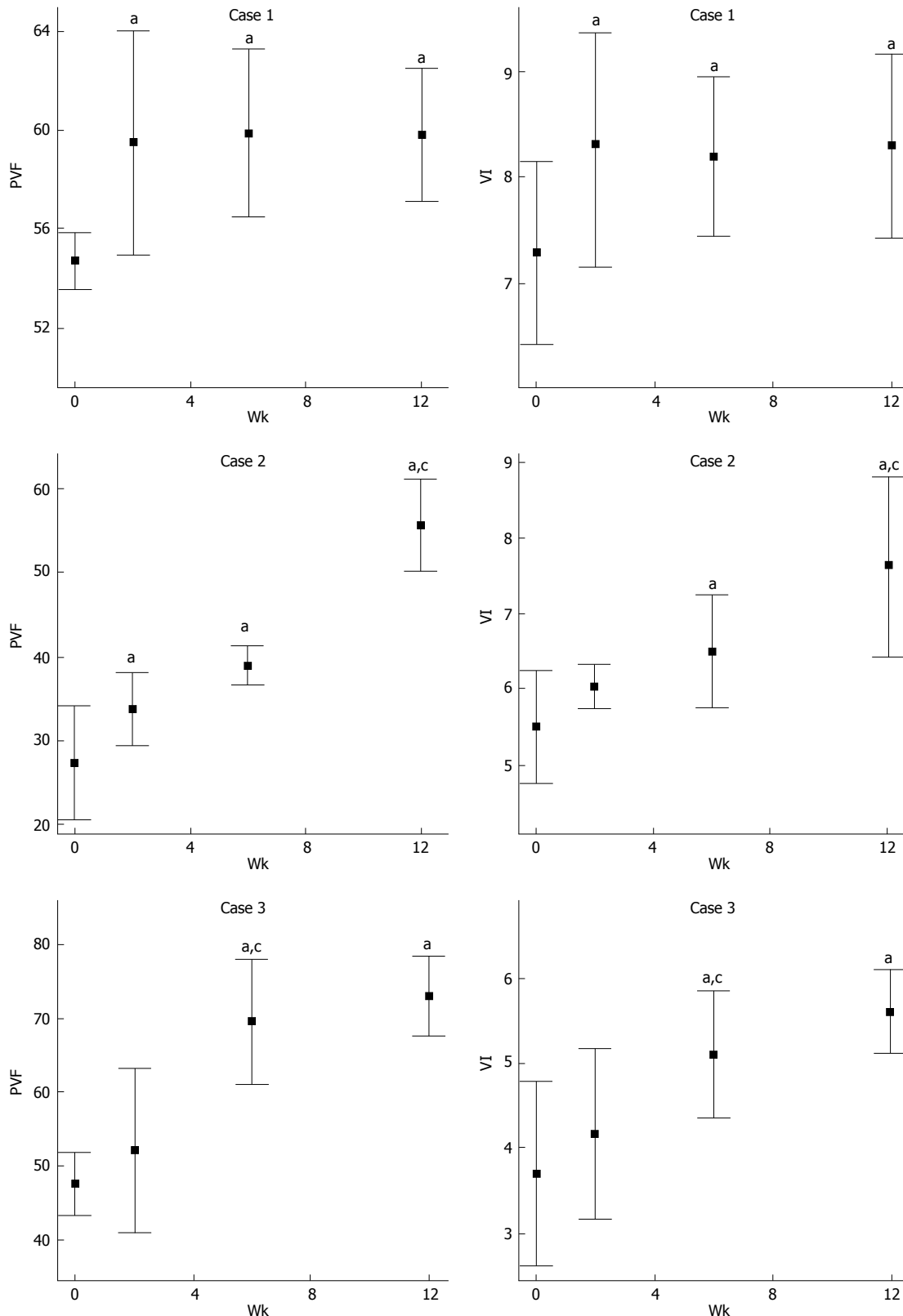
Figure 5 Force-plate evaluation. Dogs (cases 1 to 3) were evaluated by force-plate gait analysis as shown in Figure 1. Values of PVF and VI were obtained from three walks with valid speed. Both values were normalized for body weight; VI was further normalized for time. Data presented are the average and range of values obtained from three walks with valid speed. Unit for PVF is N kg⁻ in percentile (kg is dog's body weight in kilogram). Unit for VI is N s kg⁻ (s is time in second). [a]$P < 0.05$, the week after stem cells injection *vs* week 0 (pre-treatment); [c]$P < 0.05$, the week after stem cells injection *vs* the previous time point. Higher scores indicate better joint function. PVF: Peak vertical force; VI: Vertical impulse.

such, could not be sacrificed for histological examination. The present study is pilot in nature and was intended

as a proof of concept. When further validated in trials with larger case numbers, it can potentially lead to the de-

Table 5 Ground reaction force for all three dogs at all time points

	Pre-injection	2 wk post-injection	6 wk post-injection	12 wk post-injection
Case 1				
PVF-experimental limb	54.72	59.52	59.84	59.76
PVF-contralateral limb	66.02	63.53	65.89	68.67
VI-experimental limb	7.3	8.3	8.4	8.3
VI-contralateral limb	8.4	9.7	9.2	9.5
Case 2				
PVF-experimental limb	27.42	33.77	39	55.62
PVF-contralateral limb	73.43	68.33	77.8	73.92
VI-experimental limb	5.5	6.1	6.5	7.7
VI-contralateral limb	11	11.1	13.3	11.5
Case 3				
PVF-experimental limb	47.59	52.13	69.51	73.02
PVF-contralateral limb	109.72	105.24	85.95	87.29
VI-experimental limb	3.7	4.2	5	5.6
VI-contralateral limb	9	9.3	8.2	8.4

PVF: Peak vertical force (% body weight); VI: Vertical impulse (% body weight × time).

velopment of a simple and inexpensive treatment for OA and other degenerative diseases in veterinary medicine. However, owner's reluctance to participate and/or objection to placebo treatment will remain the most challenging issues going forward with such trials. It is thus our hope that the publication of this pilot study will encourage more participation from both veterinarians and pet owners.

COMMENTS

Background
Osteoarthritis (OA) is a prevailing canine disease that negatively impacts the patient's quality of life. Current treatments for OA most commonly involve the use of non-steroidal anti-inflammatory drugs that can cause gastric ulceration and renal and hepatic damages. As such, alternative treatments without these adverse side effects are highly sought after. Toward this end, intra-articular injection of autologous adipose-derived stem cells (ADSCs) has been shown to be highly effective. However, the isolation of ADSCs from patients requires certain equipment, reagents, and expertise that are lacking in most veterinary clinics. Thus, the present study aimed at testing whether ADSCs can be applied in a xenogeneic fashion. In particular, porcine ADSCs, which can be manufactured on a commercial scale, were shown to be highly effective in treating canine OA.

Research frontiers
Mesenchymal stem/stromal cells (MSCs), including ADSCs, have been consistently shown to have the ability to suppress immunity. Therefore, when transplanted into an allogeneic or xenogeneic recipient, they are well tolerated and able to perform functions similar to autologously transplanted MSCs, such as the secretion of anti-inflammatory and immunomodulatory molecules. These unique properties of MSCs are apparently responsible for the therapeutic efficacy of porcine ADSCs for canine OA, as demonstrated in the present study.

Innovations and breakthroughs
Previous studies have shown that intra-articular injection of autologous ADSCs was efficacious in treating canine OA. However, such a treatment is time-consuming and burdensome for both the veterinarian and the pet owner. In contrast, by using porcine ADSC instead of autologous ADSC, as demonstrated in the present study, intra-articular injection of ADSC will become an inexpensive and expeditious treatment for canine OA.

Applications
From the authors' results, it appears that intra-articular injection of porcine ADSCs is a safe and effective treatment for canine OA. However, due to the small sample size in the current study, this innovative therapeutic approach will require further validation with treatment on additional patients. It is also imperative that long-term safety and effectiveness be demonstrated before this treatment modality can become a routine veterinary practice.

Terminology
Xenotransplantation is transplantation of tissue or cell from one species to another. Force-plate analysis employs a plate or platform in which an embedded pressure sensor measures the ground reaction forces generated by a walking animal. A limb with OA generates less ground reaction forces than a normal limb, and as OA symptoms improve, so do ground reaction forces.

Peer review
This paper investigates the effect of intra-articular injection of adipose-derived stem cells for treating canine OA. The aim of the study is highly important and authors do a good job to describe its significance in the paper. Overall, the manuscript is well-written.

REFERENCES

1   **Johnston SA**. Osteoarthritis. Joint anatomy, physiology, and pathobiology. *Vet Clin North Am Small Anim Pract* 1997; **27**: 699-723 [PMID: 9243777]

2   **Rychel JK**. Diagnosis and treatment of osteoarthritis. *Top Companion Anim Med* 2010; **25**: 20-25 [PMID: 20188335 DOI: 10.1053/j.tcam.2009.10.005]

3   **Kapoor M**, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011; **7**: 33-42 [PMID: 21119608 DOI: 10.1038/nrrheum.2010.196]

4   **Bijlsma JW**, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. *Lancet* 2011; **377**: 2115-2126 [PMID: 21684382 DOI: 10.1016/S0140-6736(11)60243-2]

5   **da Silva Meirelles L**, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* 2006; **119**: 2204-2213 [PMID: 16684817 DOI: 10.1242/jcs.02932]

6   **Baraniak PR**, McDevitt TC. Stem cell paracrine actions and tissue regeneration. *Regen Med* 2010; **5**: 121-143 [PMID: 20017699 DOI: 10.2217/rme.09.74]

7   **Nöth U**, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. *Nat Clin Pract Rheumatol* 2008; **4**: 371-380 [PMID: 18477997 DOI: 10.1038/ncprheum0816]

8   **Ribitsch I**, Burk J, Delling U, Geißler C, Gittel C, Jülke H, Brehm W. Basic science and clinical application of stem cells in veterinary medicine. *Adv Biochem Eng Biotechnol* 2010; **123**: 219-263 [PMID: 20309674 DOI: 10.1007/10_2010_66]

9   **Zuk PA**. The adipose-derived stem cell: looking back and looking ahead. *Mol Biol Cell* 2010; **21**: 1783-1787 [PMID: 20375149 DOI: 10.1091/mbc.E09-07-0589]

10 **Lin G**, Wang G, Banie L, Ning H, Shindel AW, Fandel TM,

Lue TF, Lin CS. Treatment of stress urinary incontinence with adipose tissue-derived stem cells. *Cytotherapy* 2010; **12**: 88-95 [PMID: 19878076 DOI: 10.3109/14653240903350265]

11   **Lin G**, Xin Z, Zhang H, Banie L, Wang G, Qiu X, Ning H, Lue TF, Lin CS. Identification of active and quiescent adipose vascular stromal cells. *Cytotherapy* 2012; **14**: 240-246 [PMID: 22070603 DOI: 10.3109/14653249.2011.627918]

12   **Black LL**, Gaynor J, Adams C, Dhupa S, Sams AE, Taylor R, Harman S, Gingerich DA, Harman R. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Vet Ther* 2008; **9**: 192-200 [PMID: 19003780]

13   **Black LL**, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. *Vet Ther* 2007; **8**: 272-284 [PMID: 18183546]

14   **Guercio A**, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, Di Bella S, Piccione G. Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int* 2012; **36**: 189-194 [PMID: 21936851 DOI: 10.1042/CBI20110304]

15   **Vilar JM**, Morales M, Santana A, Spinella G, Rubio M, Cuervo B, Cugat R, Carrillo JM. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res* 2013; **9**: 131 [PMID: 23819757 DOI: 10.1186/1746-6148-9-131]

16   **Lin CS**, Lin G, Lue TF. Allogeneic and xenogeneic transplantation of adipose-derived stem cells in immunocompetent recipients without immunosuppressants. *Stem Cells Dev* 2012; **21**: 2770-2778 [PMID: 22621212 DOI: 10.1089/scd.2012.0176]

17   **Lin G**, Garcia M, Ning H, Banie L, Guo YL, Lue TF, Lin CS. Defining stem and progenitor cells within adipose tissue. *Stem Cells Dev* 2008; **17**: 1053-1063 [PMID: 18597617 DOI: 10.1089/scd.2008.0117]

18   **McCarthy G**, O'Donovan J, Jones B, McAllister H, Seed M, Mooney C. Randomised double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulfate for the treatment of dogs with osteoarthritis. *Vet J* 2007; **174**: 54-61 [PMID: 16647870 DOI: 10.1016/j.tvjl.2006.02.015]

19   **Moreau M**, Dupuis J, Bonneau NH, Desnoyers M. Clinical evaluation of a nutraceutical, carprofen and meloxicam for the treatment of dogs with osteoarthritis. *Vet Rec* 2003; **152**: 323-329 [PMID: 12665145 DOI: 10.1136/vr.152.11.323]

20   **Lindroos B**, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev* 2011; **7**: 269-291 [PMID: 20853072 DOI: 10.1007/s12015-010-9193-7]

21   **Coleman CM**, Curtin C, Barry FP, O'Flatharta C, Murphy JM. Mesenchymal stem cells and osteoarthritis: remedy or accomplice? *Hum Gene Ther* 2010; **21**: 1239-1250 [PMID: 20649459 DOI: 10.1089/hum.2010.138]

22   **Dieppe P**, Cushnaghan J, Jasani MK, McCrae F, Watt I. A two-year, placebo-controlled trial of non-steroidal anti-inflammatory therapy in osteoarthritis of the knee joint. *Br J Rheumatol* 1993; **32**: 595-600 [PMID: 8339132 DOI: 10.1093/rheumatology/32.7.595]

RETROSPECTIVE STUDY

D-MELD risk capping improves post-transplant and overall mortality under markov microsimulation

Jeffrey B Halldorson, Robert L Carithers Jr, Renuka Bhattacharya, Ramasamy Bakthavatsalam, Iris W Liou, Andre A Dick, Jorge D Reyes, James D Perkins

Jeffrey B Halldorson, Division of Transplant Surgery, Department of Surgery, University of California, San Diego, CA 92103-8401, United States
Robert L Carithers Jr, Renuka Bhattacharya, Iris W Liou, Division of Transplantation, Department of Surgery, University of Washington, Seattle, WA 98195, United States
Ramasamy Bakthavatsalam, Andre A Dick, Jorge D Reyes, James D Perkins, Division of Gastroenterology, Department of Medicine, University of Washington, Seattle, WA 98195, United States
Author contributions: Halldorson JB, Carithers Jr RL, Reyes JD and Perkins JD designed the research; Halldorson JB and Perkins JD performed the research; Halldorson JB, Carithers Jr RL, Bhattacharya R, Bakthavatsalam R, Liou IW, Dick AA, Reyes JD and Perkins JD performed acquisition and interpretation of data; Halldorson JB wrote the manuscript; Carithers Jr RL, Bhattacharya R, Bakthavatsalam R, Liou IW, Dick AA, Reyes JD and Perkins JD provided assistance drafting the manuscript, provided critical reading leading to revisions of the manuscript and gave final approval of the version to be published.
Correspondence to: Jeffrey B Halldorson, MD, Division of Transplant Surgery, Department of Surgery, University of California, Suite 2-286, 200 West Arbor Drive, #8401, San Diego, CA 92103-8401, United States. jhalldorson@ucsd.edu
Telephone: +1-619-5433493 Fax: +1-619-5437785
Received: January 9, 2014   Revised: July 9, 2014
Accepted: July 18, 2014
Published online: September 24, 2014

Abstract

AIM: To hypothesize that the product of calculated Model for End-Stage Liver Disease score excluding exception points and donor age (D-MELD) risk capping ± Rule 14 could improve post liver transplant and overall survival after listing.

METHODS: Probabilities derived from the United Network for Organ Sharing database between 2002 and 2004 were used to simulate potential outcomes for all patients listed for transplantation. The Markov simulation was then modified by screening matches using a 1200 or 1600 D-MELD risk cap ± allowing transplants for Model for End-Stage Liver Disease (MELD) ⩽ 14 (Rule 14). The differential impact of the rule changes was assessed.

RESULTS: The Markov simulation accurately reproduced overall and post transplant survival. A 1200 D-MELD risk cap improved post-transplant survival. Both the 1200 and 1600 risk caps improved overall survival for waitlisted patients. The addition of Rule 14 further improved post transplant and overall survival by redistribution of donor livers to recipients in higher MELD subgroups. The mechanism for improved overall and post-transplant survival after listing was due to shifting a larger percentage of transplants to the moderate MELD score subgroup (MELD 15-29) while also ensuring that high MELD recipients have livers of high quality to achieve excellent post transplant survival.

CONCLUSION: A 1200 D-MELD risk cap + Rule 14 provided the greatest overall benefit primarily by focusing liver transplantation towards the moderate MELD recipient.

Core tip: Optimal matching between donor livers and recipients balances recipient need with optimal utility. The product of calculated Model for End-Stage Liver Disease score excluding exception points and donor age (D-MELD) risk cap uniquely utilizes ethically neutral donor/recipient factors while maintaining predictive power, making it useful for donor/recipient matching

paradigms aimed at improving utility. Described is a novel utilization of the D-MELD risk cap as an aid for donor/recipient matching. Markov modeling suggests that this paradigm improves outcomes both by decreasing futile transplantations but also by focusing the majority of transplantation on moderate Model for End-Stage Liver Disease (MELD) recipients while continuing to provide younger donor livers for the fewer number of patients transplanted at high MELD.

INTRODUCTION

As a response to waitlist mortality from the cadaveric donor shortage, the once homogenous pool of younger, donation after brain death donors has now become a heterogeneous mix of standard and extended criteria donors. By far the most significant change is greater use of older donors aged greater than 50 who now may comprise over half of liver donor populations[1-3]. The older donor has now become a dominant risk factor for poorer graft and patient survival after liver transplantation[4,5].

The older donor pool in combination with sickest first allocation policies based on Model for End-Stage Liver Disease (MELD) scoring often produces match offers between higher risk donors and the sickest patients. Although it can be demonstrated that the sickest (highest MELD) patients derive survival benefit from transplantation even when matching with higher risk donors, overall survival is inferior to that of lower risk donor/recipient combinations[6]. While good results can be obtained with older liver donors, high risk donor/recipient matches have poorer survival and therefore decrease the utility obtained from the scarce resource of donor livers for transplantation[7].

Since post transplant outcome is not formally considered in current allocation policy, matching higher risk older donors with appropriate risk recipients is often improvised at the time of liver offer. With intent to maximize the benefit from the severely limited medical resource of cadaveric organs, modifications have been called for to optimize matching between donors and recipients at the time of allocation[8-10].

One proposed modification raises the minimal MELD score for listing to 14 (Rule 14) with the intent to ensure that recipients are sick enough to realize survival benefit from transplantation. Prior modeling work has demonstrated Rule 14 to improve overall survival for the population of waitlisted patients, with a particular benefit for patients listed at low MELD who may have higher expected survival without transplantation[11].

Another potential area to maximize utility in liver transplantation is decreasing the number of transplantations with low expected post transplant survival. Survival after transplantation is largely dependent on the risk combination between donor quality and recipient medical condition, and survival is poorer when higher risk donors are placed into the sickest recipients[12]. Since the dominant donor risk factor is age, and recipient medical condition is accurately measured by MELD score, Model for End-Stage Liver Disease score excluding exception points and donor age (D-MELD) (the product of donor age and recipient MELD excluding United Network for Organ Sharing exception points) was developed as an ethically appropriate tool to improve donor/recipient matching during allocation[13]. As a product of two continuous variables, D-MELD produces a risk gradient from which a cap can be instituted to decrease the rate of futile donor/recipient matches. Importantly, D-MELD was designed to avoid controversial discriminator variables such as gender, recipient age, race or subjective assessments of "organ quality" that make other donor/recipient matching systems impractical or ethically controversial[14]. Prior work demonstrated D-MELD scores greater than 1200 or 1600 to be significantly associated with poorer post transplant survival[13].

Based on D-MELD risk cap thresholds ± Rule 14, the experimental model evaluates the potential utility of modifications in donor/recipient matching as follows: (1) donor/recipient matches with D-MELD risk caps above threshold (*e.g.*, D-MELD scores greater than 1200 or 1600) would continue waiting for a better match; (2) Matches below the D-MELD threshold (*e.g.*, D-MELD scores less than 1600) would then be offered based on urgency prioritized by MELD score; and (3) A minimum MELD score of 14 would be required for listing. We hypothesize that elimination of donor/recipient matching at high D-MELD and low MELD would focus the majority of liver matches in waitlisted patients with moderate (15-29) MELD scores, improving both post transplant survival and pre-transplant mortality by reducing the need for terminal disease progression in order to obtain an offer.

While it is intuitive that a risk cap could improve post transplant outcomes, an overly restrictive limit might result in prolonged waiting times for the sickest patients, thereby increasing waitlist deaths and outweighing any gains from increased post transplant survival. In addition, redistribution of older livers originally designated for high MELD recipients could potentially worsen post transplant survival for lower MELD subpopulation recipients, lessening the expected benefit from transplantation.

Markov decision analysis can be used to estimate the cumulative impact of decisions involving many potential outcome states in a complex system[15]. In order to understand the potential impact of proposed allocation changes, a Markov microsimulation was designed based on outcomes from all patients listed for liver transplantation between 2002-2004 and followed until 2008. The impact of both more (D-MELD 1200) and less (D-
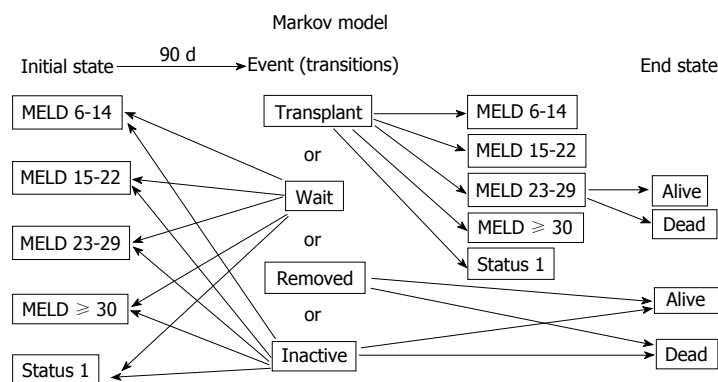
Figure 1 Schematic of Markov model states and transitions. MELD: Model for End-Stage Liver Disease.

MELD 1600) restrictive risk caps with and without Rule 14 was examined for its potential impact on post transplant survival, overall survival and MELD subgroup at transplantation.

MATERIALS AND METHODS

To model the multiple potential sequential events following liver transplant listing, we devised a Markov model assigning potential states as derived from the database (Figure 1). Event probabilities and expected survival probabilities for different end states were derived from records of all adult patients listed between January 1, 2002 and December 31, 2004, as recorded in the scientific registry of transplant recipients (SRTR) database with follow-up until February 6, 2008. Actively listed patients were evaluated for MELD score at listing, MELD score at removal, time waiting, post transplant survival, and waitlist events translated from the database. Overall survival was defined as the interval between listing date until death. Survival was assessed as "intention to treat" and included mortality with or without receiving a liver transplant as well as patients removed from the list or made inactive. Post transplant survival was assessed from the time of liver transplantation until death at the time of follow-up. Events after listing were assessed at 90-d intervals. Patients with missing MELD scores or donor age were excluded (< 1%). There were 24295 patients who met the study criteria. D-MELD was calculated as the product of donor age and MELD score excluding United Network for Organ Sharing exception points, and capped at 40. Patients were sub-stratified into 5 MELD categories (6-14, 15-22, 23-29, ≥ 30 and Status 1) based on initial and end state as recorded in the database. Final end state measured was death or survival. Patients who were made inactive on the list were given the same survival as patients removed from the list; however, survivors after 90 d were allowed to re-enter at the same MELD as they had at time of removal. Analysis of events for the years 2002-2004 did not allow assessment of changes in MELD category for patients remaining waitlisted (*e.g.*, movement from MELD 6-14 to MELD 15-22). To estimate change in MELD for each 90-d period for patients

who did not die or receive a transplant, we analyzed the most recent complete 90-d time period between September 31, 2007 and December 31, 2007. Rates of change between MELD subgroups were then used to predict MELD progression for the study period 2002-2004. A boot strap cohort was then modeled with initial MELD and donor age distributions derived from the database.

To confirm accuracy of the model, we compared the model's predictions for both overall and post transplant survival with actual survival for all patients as a whole and then by MELD category. After accuracy of the model was confirmed, we analyzed the impact of proposed D-MELD risk caps with and without Rule 14 as compared to the model. Using the D-MELD risk cap, donor/recipient matches with a score above a 1200 or 1600 threshold remained on the waiting list until a suitable donor was found. Donor livers that were prevented from matching under the D-MELD cap were redistributed to patients with matches below the risk cap using the formula [D-MELD (1200 or 1600)]/(donor age in years) = maximum acceptable MELD at transplant. The liver was then allocated to the highest MELD subgroup allowable. For example, allocating a 61-year-old liver with a D-MELD 1600 filter would give a maximum tolerated MELD of 1600/61 = 26.2. This liver then would be allocated to the subgroup MELD 23-29. Survival for the donor/recipient match was predicted from the D-MELD stratification curves for each MELD subgroup derived from SRTR data.

To analyze the impact of Rule 14, matches were not allowed for patients with a MELD score of 14 or below. The livers from this group were re-distributed as before without surpassing the proposed 1200 or 1600 D-MELD cap. Because status 1 listed patients might suffer from increased waiting times under a D-MELD risk cap, allocation to this subgroup was not limited by D-MELD. Redistributed livers from Rule 14 and D-MELD risk caps were allocated to Status 1 listed patients in the same proportion as actual data from 2002-2004 SRTR data.

RESULTS

A Markov model encompassing all events (transitions)
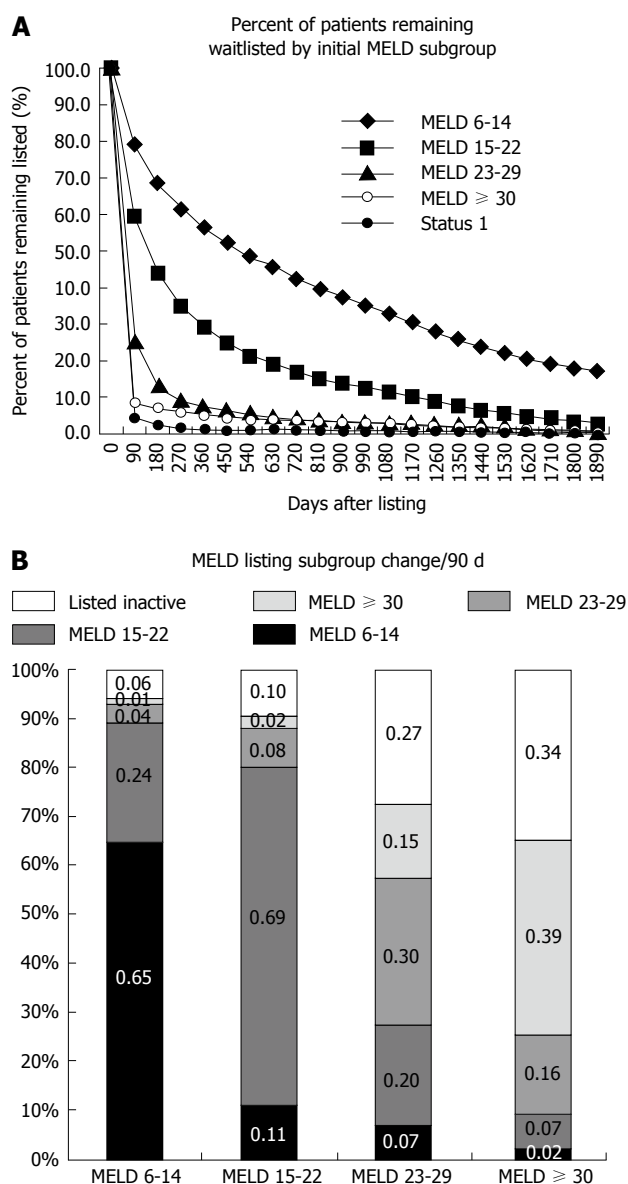
A

B

Figure 2 Model for End-Stage Liver Disease subgroups. A: Percentage of patients remaining waitlisted at initial Model for End-Stage Liver Disease over time; B: Analysis of patient movement between subgroups for those remaining waitlisted at 90 d. MELD: Model for End-Stage Liver Disease.

potentially occurring after listing (initial state) was created (Figure 1). Events (list removal or inactivation, transplantation or active listing) by MELD subgroup were calculated directly from actual data. Rate to first event and list removal was dependent on MELD subgroup. As expected, the MELD 6-14 subgroup was slowest to leave the list, with 55% of patients remaining waitlisted at one year. The percentage of patients remaining waitlisted over time decreased dramatically as MELD increased. In the sickest subgroups, MELD $\geq$ 30 and Status 1, less than 10% of patients remained listed after the first 90-d period (Figure 2A). Analysis of movement between MELD subgroups for patients remaining waitlisted demonstrated the lower MELD subgroups (6-14, 65% and 15-22, 69%) to be most likely to remain waitlisted at the same MELD after 90 d (Figure 2B). Patients at moderate

MELD (MELD 15-22 and MELD 23-29) who remained waitlisted were slightly more likely to move to a lower rather than a higher MELD subgroup per 90-d period if still waiting. The small percentage of patients who remained waitlisted in the subgroup MELD $\geq$ 30 were most likely to wait at the same MELD followed by inactive listing.

The Markov microsimulation correlated well with post transplant and overall survival when compared to actual data (Figure 3). Comparison of predicted overall survival by individual MELD subgroup demonstrated no greater than 1.8% and 2.3% variation between model and actual data for the years 2002 at 1 year and 4 years, respectively (Figure 4A). Comparison of predicted post transplant survival by MELD subgroup to actual data demonstrated no greater than 2.3% variation between model and actual at 1 year and no greater than 2.4% variation at 4 years (Figure 4B).

The impact of D-MELD risk caps was analyzed (Figure 5). A D-MELD risk cap of 1600 demonstrated no overall improvement in post transplant survival. A D-MELD risk cap of 1200 improved post transplant survival by 1.6% at one year and by 2.0% at 4 years (Figure 5E). Examination of post transplant survival by MELD subgroup at transplant demonstrated a similar benefit for both D-MELD risk caps for the MELD $\geq$ 30 subgroup, with an approximately 4% improvement in one-year patient survival for both the 1200 and 1600 risk caps and approximately 2.9% improvement for both groups at 4 years (Figure 5D).

The MELD distribution at transplant was examined for both the 1200 and 1600 risk caps $\pm$ Rule 14 (Figure 6). Institution of the 1600 risk cap shifted allocation slightly in favor of the MELD 15-22 and MELD 23-29 subgroups. The 1200 risk cap had an expectedly greater effect, shifting greater than 90% of transplants to patients with MELD scores of $\leq$ 29.

Addition of Rule 14 to either of the risk caps resulted in an increased percentage of transplants for the MELD $\geq$ 30 subgroup with a greater number of livers redistributing to high MELD under the D-MELD 1600 risk cap compared to the 1200 risk cap (Figure 6). For example, the D-MELD risk cap in combination with Rule 14 resulted in 26.5% of transplants performed at MELD $\geq$ 30% *vs* 12.8% for the model. Addition of Rule 14 to the D-MELD risk cap of 1200 resulted in 47% of transplants performed at MELD 15-22 compared to 19.4% in the model.

Likelihood of transplant by MELD subgroup at listing was examined for each model. For the proposals including Rule 14, patients initially listed in the group 6-14 required progression to a higher MELD to obtain a match. A D-MELD risk cap of 1200 in combination with Rule 14 resulted in the highest likelihood of receiving a transplant for all MELD sub-groups (Figure 7).

Analysis of overall survival from the time of listing was examined for all patients together as well as by initial MELD subgroup at listing. All proposals resulted in im-
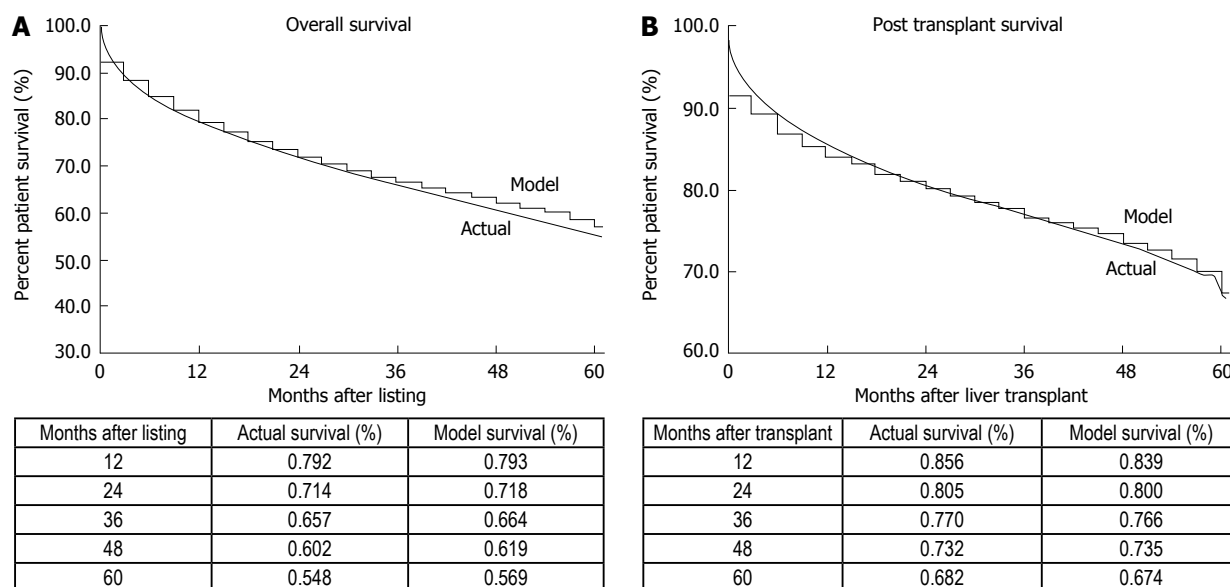
Figure 3 Comparison of actual data to model, overall and post transplant survival for all patients. A: Overall; B: Post transplant.

Months after listing	Actual survival (%)	Model survival (%)
12	0.792	0.793
24	0.714	0.718
36	0.657	0.664
48	0.602	0.619
60	0.548	0.569

Months after transplant	Actual survival (%)	Model survival (%)
12	0.856	0.839
24	0.805	0.800
36	0.770	0.766
48	0.732	0.735
60	0.682	0.674
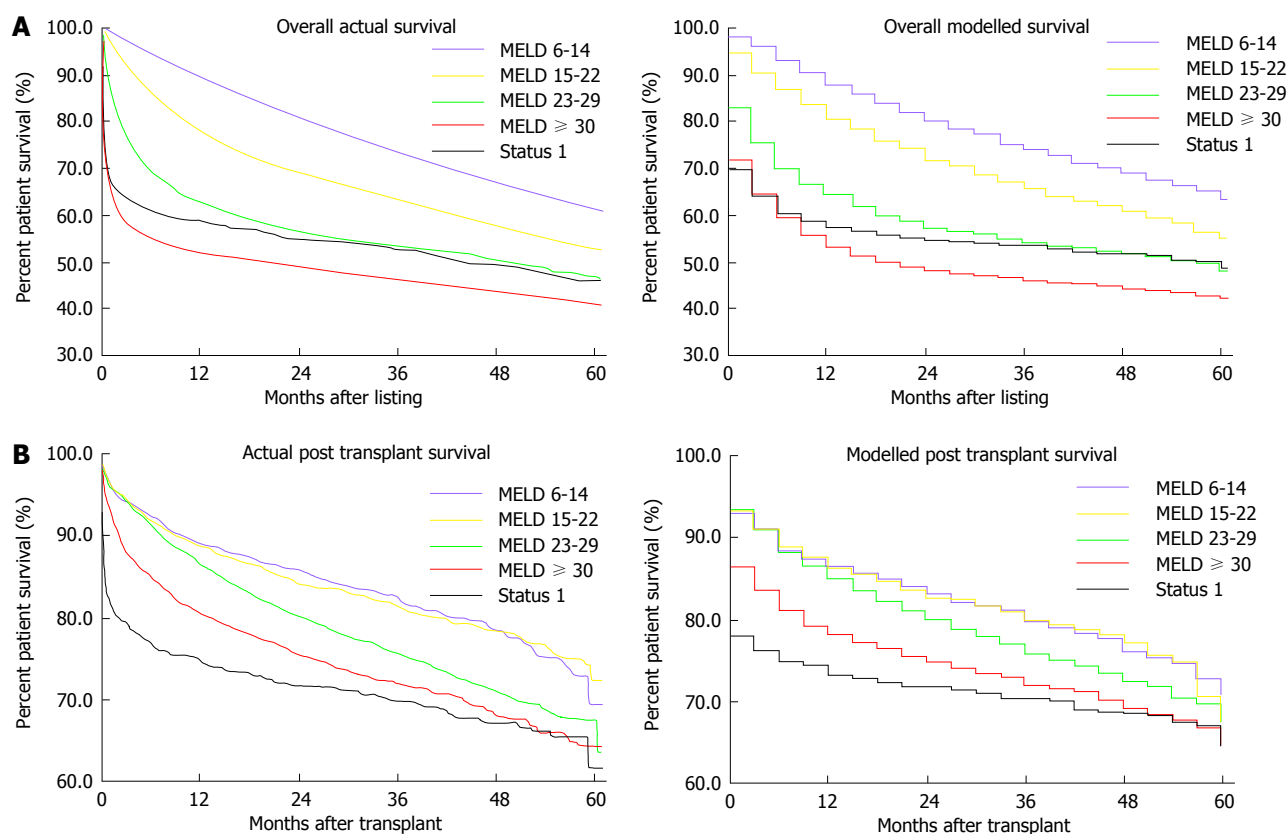
Figure 4 Comparison curve. A: Overall survival is subgrouped by MELD at listing. Comparison of actual data *vs* model; B: Post transplant survival subgrouped by MELD at transplantation. Comparison of actual data *vs* model. MELD: Model for End-Stage Liver Disease.

proved survival from the time of listing when compared to the modeled current survival (Figure 8). The proposed D-MELD 1200/Rule 14 rule change provided the greatest improvement for the whole waitlisted population, increasing overall survival by 3.6% at one year and by 8.5% at 4 years (Figure 8E). Examination by MELD subgroup at listing demonstrated a similar pattern, with the most significant improvement in overall survival for the most

restrictive risk cap of 1200 in combination with Rule 14. The subgroups gaining the greatest survival benefit were patients listed at MELD 23-29 and MELD ≥ 30 (Figure 8C and D).

DISCUSSION

Currently, liver allocation based on the MELD system is
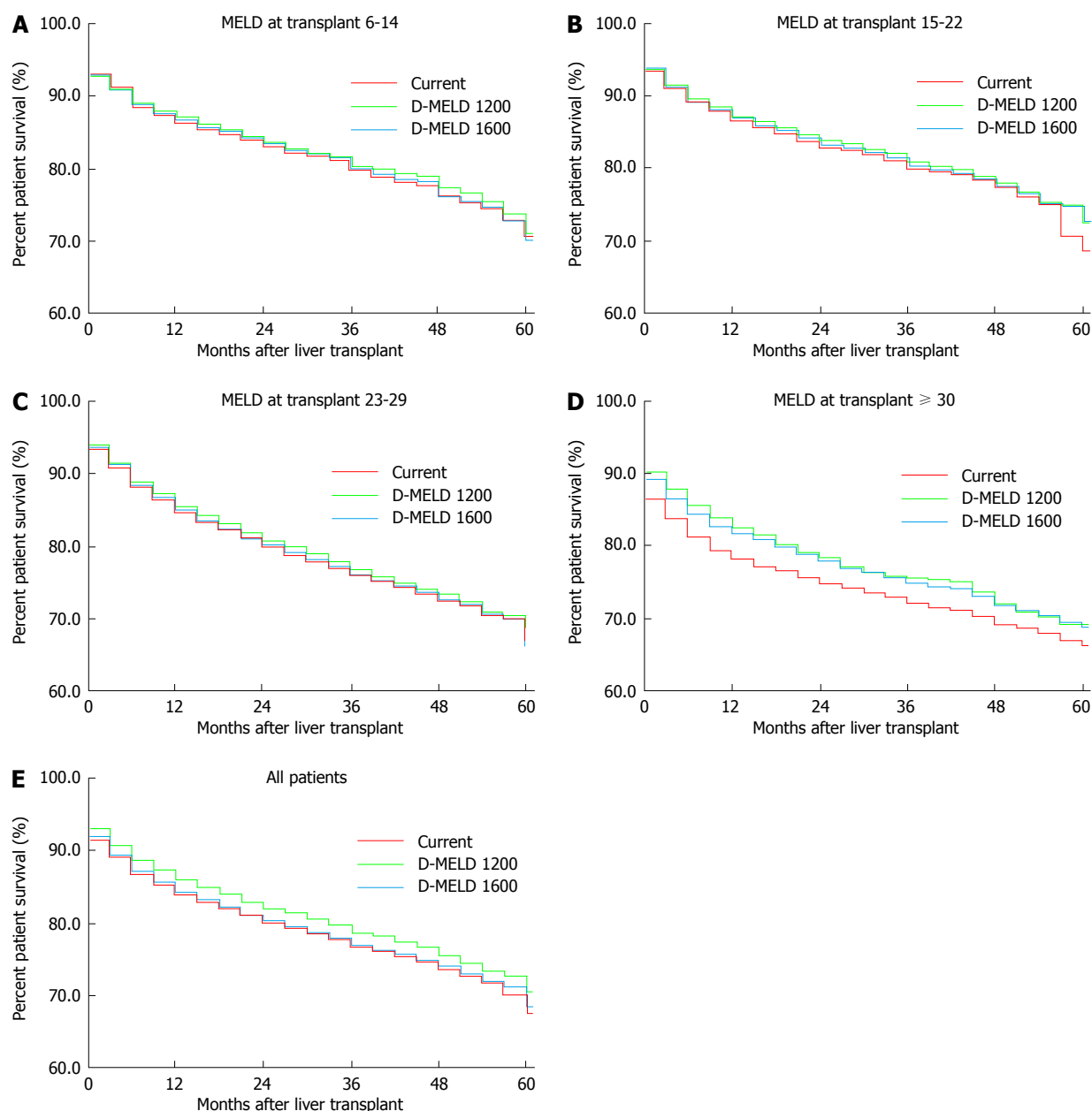
Figure 5 **Comparison of post transplant survival between current modeled survival and Model for End-Stage Liver Disease score excluding exception points and donor age risk caps of 1200 *vs* 1600.** Comparison is demonstrated for MELD subgroups at transplant and for all patients. MELD: Model for End-Stage Liver Disease; D-MELD: MELD score excluding exception points and donor age.
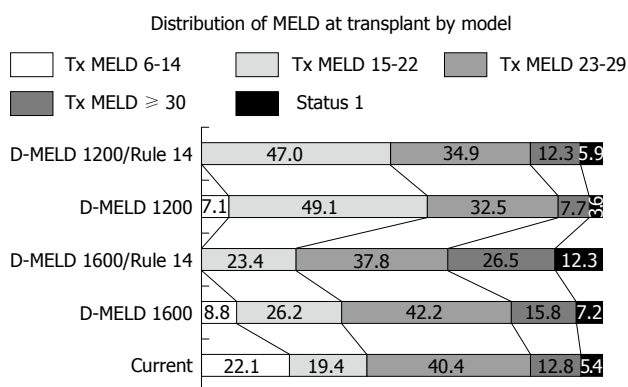
Figure 6 **Model for End-Stage Liver Disease distribution at transplantation comparing modeled (current) *vs* proposed changes.** MELD: Model for End-Stage Liver Disease; D-MELD: MELD score excluding exception points and donor age.

urgency-based without regard for post transplant survival or consideration of the potential benefit of transplantation at a less advanced disease state. The merits of the current system are simplicity, objectivity, and accuracy predicting waiting list mortality, as well as equity since disease severity is the only prioritizing factor[16,17]. Disadvantages of the current system include a continued pressure toward poorer outcomes since the sickest (highest MELD) recipients often match with higher risk donors. This pressure towards transplantation at higher MELD worsens as transplant centers "compete" for scarce donor livers under a matching system solely prioritizing disease severity (MELD)[18]. The desire for a better balance between urgency and utility in organ allocation has been an elusive goal[10].

In order to better balance the risk for individual do-
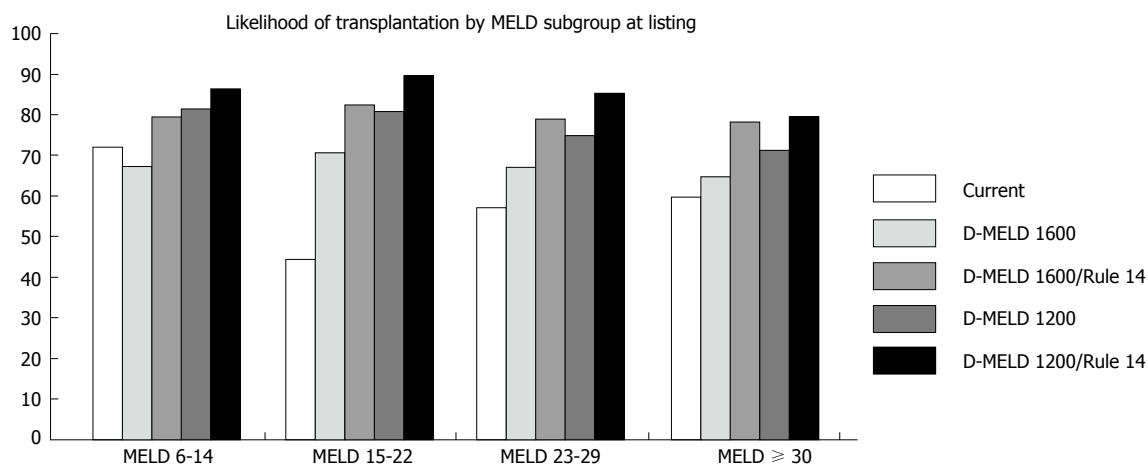
Figure 7 Likelihood of transplantation by Model for End-Stage Liver Disease subgroup at listing with comparison between current (modeled) *vs* proposed changes. MELD: Model for End-Stage Liver Disease; D-MELD: MELD score excluding exception points and donor age.
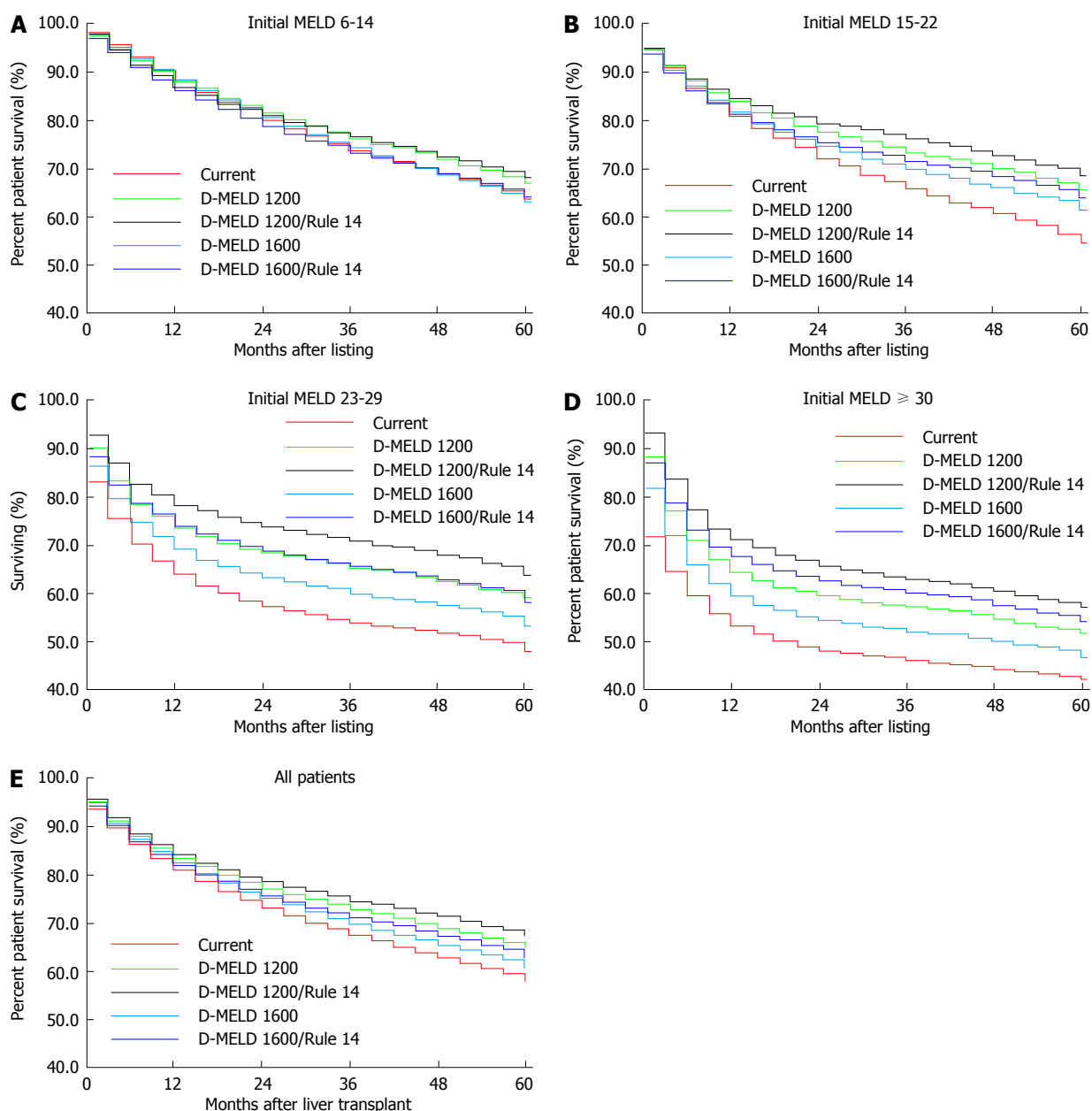
Figure 8 Overall survival for waitlisted patients subgrouped by initial Model for End-Stage Liver Disease at listing and all patients. MELD: Model for End-Stage Liver Disease; D-MELD: MELD score excluding exception points and donor age.

nor recipient combinations in a manner ethically acceptable for allocation, we developed a statistic, D-MELD, designed to account for the two dominant variables in donor/recipient matching, patient physiologic condition at transplantation as measured by MELD score and donor quality which is most significantly related to donor age[13,14]. As the product of two continuous variables, donor age and MELD (excluding exception points), D-MELD calculation produces a continuous gradient of risk for increased post-operative mortality and length of stay. Strata from the gradient can be defined and risk caps can be assigned to optimize distribution of organs for the population.

While the impact of risk capping has an intuitive benefit for improving post-transplant survival, the impact on overall survival for all waitlisted patients is less intuitive since the D-MELD risk cap would prohibit transplants for some high MELD recipients who might incur higher waitlist mortality. Conversely, re-distribution of livers to recipients within the moderate MELD subgroup would also decrease the number of patients progressing to high MELD prior to transplant. Whether the redistribution would have an overall positive or negative effect likely depends on the relative availability of donor livers, the rate of disease progression and the ability of patients to wait for a higher quality donor offer. An additional concern is whether redistribution of older donors to moderate MELD recipients would worsen post-transplant outcome for this subgroup and negate the benefit of greater liver availability.

Using a model derived from SRTR data, D-MELD risk capping in combination with Rule 14 provided a survival benefit for the waitlisted population as a whole without harming any subpopulation of listed patients. The more restrictive risk cap of 1200 had a greater impact on MELD at transplant, shifting a greater proportion of transplants to an earlier stage of disease thereby preventing progression to high MELD prior to transplant for many recipients. As the high MELD subgroup decreased in size, the D-MELD risk capping actually increased the likelihood of transplantation for patients with MELD $\geqslant$ 30, with the added potential post transplant survival advantage that the highest quality young donors were reserved for the sickest patients. Addition of Rule 14 further redistributed livers disproportionally towards higher MELD recipients, providing additional insurance against prolonged waiting times at high MELD and improving overall survival by approximately 2%-3% for both 1200 and 1600 risk caps. This improvement in survival for the Rule 14 is consistent with prior modeling experiments[11].

Our model suffers from a number of limitations that require further study prior to any recommendation to modify allocation policy. We chose a Markov microsimulation paradigm to model survival given the data available from the SRTR database. We considered initial MELD, end MELD, time on the list, deaths while waiting and survival after transplant to be the most robust information available from the database. Certain information was not directly obtainable from analysis of the database for the time period 2002-2004, and modifications were required to attain accuracy of the model.

The first modification was necessary to understand the expected changes in MELD over each 90-d period. In order to correct this deficit, we analyzed data from the most recent complete 90-d time period available, September-December 2007, comparing initial MELD to final MELD for patients remaining waitlisted. These probabilities were then used to estimate 90-d changes in MELD for the period 2002-2004. The second modification was required to estimate survival for patients listed inactive. Patients in this group were given the same survival probability as those removed from the list for other reasons by MELD subgroup. Similar model calibrations have been required for accuracy in described modeling experiments[19-22].

Using the Markov microsimulation based on probabilities alone also limited the range of output data, and we were not able to assess, for example, changes in waiting time by MELD subgroup. Although our model is simpler than other described simulations, it was able to achieve accuracy predicting overall and post transplant survival for all subgroups for the time period studied.

A second limitation of our model is the assumption of minimal friction in organ redistribution across the whole waitlisted population. Allocation practice during the time period of the study were quite limited by geographic and political considerations, so that other than for status 1 recipients, sharing of organs outside of local service areas was only practiced for livers that did not match locally. The current environment, in which regional sharing is now also mandated for recipients with MELD > 35, now matches our model more closely. Nevertheless, as survival in our model improved comparing D-MELD risk caps of 1200 and 1600, the beneficial effect of the model cannot result from sharing alone. Certainly, any negative effect from increased cold ischemia times that would result if livers were re-distributed over long distances might counter benefits gained from better matching and would need to be assessed as part of any increased sharing paradigm.

Given the real world limitations inherent to the current allocation system, implementation of a D-MELD risk cap at the regional level may require optimization to achieve optimal impact on survival[13,23]. For example, in regions 4, 6 and 10, which have a higher donor/recipient ratio and higher likelihood of transplantation, a D-MELD score of 1200 may be optimal. In contrast, in regions 5 and 9, which have a higher average MELD at transplantation and higher average D-MELD, a higher cap might produce optimal survival. Further modeling is required before any final conclusions should be drawn.

Instituting risk caps would require a change in the ethics of liver allocation by introducing societal limitations on the amount of acceptable risk for an individual patient. Given the knowledge that matches between high risk donors and high risk recipients still provide a

survival benefit for the recipient, some individuals who would otherwise have survival benefit from a transplant would remain waitlisted losing preference to a match with a better predicted outcome. This introduces a utilitarian ethic into the current urgency only liver allocation policy. Utilitarian considerations are only appropriate when medical resources are limited. According to the American Medical Association Code of Medical Ethics, Opinion 2.03-Allocation of Limited Medical Resources, only "ethically appropriate", criteria may be used for allocation decisions pertaining to "likelihood of benefit, urgency of need, change in quality of life, duration of benefit and resources required for treatment". Furthermore, the Code states that physician must "remain the patient advocate" and "should not make these allocation decisions"[24]. Policy favoring utility such as D-MELD risk caps and Rule 14 would help remove the caregiving physician from the potentially conflicted task of patient advocate and resource manager by displacing bypass decisions for unfavorable donor/recipient matches to the allocation mechanism.

Currently a risk cap paradigm is already in practiced in liver allocation in the form of an exception point system for hepatocellular carcinoma[25]. While patients with cirrhosis alone are prioritized solely by their risk for waiting list mortality (calculated MELD score), those with hepatocellular carcinoma are prioritized by a combination of their risk of death from cancer and expected survival after transplantation[26,27]. Since the risk for post transplant mortality from tumor recurrence can be predicted pre-operatively in an ethically neutral manner by measuring tumor size and number, the risk cap allows MELD exception points only for patients falling within an acceptable risk threshold based on the Milan criteria[28]. Risk capping for donor/recipient matching could be based follow a similar paradigm to better balance risk of death and optimal utility.

In summary, modeling suggests that adoption of D-MELD risk caps in combination with Rule 14 would improve both post transplant and overall survival for the population of patients with liver disease awaiting transplantation. This modification works by redistributing donor livers toward patients with MELD scores between 15-29 when transplantation is most effective thereby decreasing the number of futile transplants and reducing the severity of disease progression prior to a liver offer. Institution of risk caps could improve outcomes for the whole population of patients waiting for transplantation, but would require consensus among stakeholders given the potential negative consequences for individual patients.

ACKNOWLEDGMENTS

COMMENTS

Background

As a response to waitlist mortality, liver transplantation has witnessed an increasing use of marginal organs previously thought to have excessive risk for poor post-operative outcome (older donors, fatty liver donors, donors after cardiac death, donors with history of malignancy, *etc.*); the predominant risk assumed being the increased use of older donors. While this effort to increase the size of the donor pool was successful, donor risk has increased and is now a dominant risk factor when predicting post transplant survival. As patient survival after liver transplantation is dependent on the combination of both donor and recipient risk, poor donor/recipient matches in which total risk is excessive result in futile transplantations, an inefficient use of the critically limited resource of donor livers. The Model for End-Stage Liver Disease score excluding exception points and donor age (D-MELD) statistic was developed as a donor/recipient "risk capping" strategy with an eye to improve donor/recipient matching and reduce futile transplantations.

Innovations and breakthroughs

Markov decision analysis can be used to estimate the cumulative impact of decisions involving many potential outcome states in a complex system. In order to understand the potential impact of proposed allocation changes utilizing D-MELD risk capping, a Markov microsimulation was designed and validated based on outcomes from all patients listed for liver transplantation between 2002-2004 and followed until 2008. The impact of both more (D-MELD 1200) and less (D-MELD 1600) restrictive risk caps with and without Rule 14 was examined for its potential impact on post transplant survival, overall survival and Model for End-Stage Liver Disease (MELD) subgroup at transplantation.

Applications

This study demonstrates that allocation rule changes designed to cap combined donor/recipient risk may improve utility in liver transplantation by decreasing the frequency of futile transplantations and by decreasing average disease severity at transplantation.

Terminology

This study examines the potential impact of universal adoption of D-MELD risk caps. The D-MELD statistic is calculated as the product of donor age and the laboratory MELD score excluding United Network for Organ Sharing (UNOS) exception points. Importantly, the laboratory-based MELD score (excluding UNOS exception points) is utilized for the calculation as a measure of recipient physiologic condition. As the product of two continuous variables and laboratory MELD produces a risk gradient predicting postoperative mortality and length of stay. This gradient can then be used to identify strata where donor and recipient risk combine to result in inferior outcomes.

Peer review

Markov models are increasingly used in economic evaluations of population impacts of medical treatments. In this article described and validate is an original Markov microsimulation model designed to accurately assess the potential impact of risk limitation based allocation of donor livers.

REFERENCES

1   **Waki K**. UNOS Liver Registry: ten year survivals. *Clin Transpl* 2006; 29-39 [PMID: 18368704]
2   **Renz JF**, Kin C, Kinkhabwala M, Jan D, Varadarajan R, Goldstein M, Brown R, Emond JC. Utilization of extended donor criteria liver allografts maximizes donor use and patient access to liver transplantation. *Ann Surg* 2005; **242**: 556-563; discussion 563-565 [PMID: 16192816]
3   **Volk ML**, Lok AS, Pelletier SJ, Ubel PA, Hayward RA. Impact of the model for end-stage liver disease allocation policy on the use of high-risk organs for liver transplantation. *Gastroenterology* 2008; **135**: 1568-1574 [PMID: 19009713 DOI: 10.1053/j.gastro.2008.08.003]
4   **Mutimer DJ**, Gunson B, Chen J, Berenguer J, Neuhaus P, Castaing D, Garcia-Valdecasas JC, Salizzoni M, Moreno GE, Mirza D. Impact of donor age and year of transplantation on graft and patient survival following liver transplantation for hepatitis C virus. *Transplantation* 2006; **81**: 7-14 [PMID: 16421468 DOI: 10.1097/01.tp.0000188619.30677.84]
5   **Lake JR**, Shorr JS, Steffen BJ, Chu AH, Gordon RD, Wiesner

RH. Differential effects of donor age in liver transplant recipients infected with hepatitis B, hepatitis C and without viral hepatitis. *Am J Transplant* 2005; **5**: 549-557 [PMID: 15707410 DOI: 10.1111/j.1600-6143.2005.00741.x]

6   **Schaubel DE**, Sima CS, Goodrich NP, Feng S, Merion RM. The survival benefit of deceased donor liver transplantation as a function of candidate disease severity and donor quality. *Am J Transplant* 2008; **8**: 419-425 [PMID: 18190658 DOI: 10.1111/j.1600-6143.2007.02086.x]

7   **Segev DL**, Maley WR, Simpkins CE, Locke JE, Nguyen GC, Montgomery RA, Thuluvath PJ. Minimizing risk associated with elderly liver donors by matching to preferred recipients. *Hepatology* 2007; **46**: 1907-1918 [PMID: 17918247 DOI: 10.1002/hep.21888]

8   **Kim WR**, Kremers WK. Benefits of "the benefit model" in liver transplantation. *Hepatology* 2008; **48**: 697-698 [PMID: 18752329 DOI: 10.1002/hep.22497]

9   **Dawwas MF**, Gimson AE. Candidate selection and organ allocation in liver transplantation. *Semin Liver Dis* 2009; **29**: 40-52 [PMID: 19235658 DOI: 10.1055/s-0029-1192054]

10 **Avolio AW**, Halldorson JB, Burra P, Dutkowski P, Agnes S, Clavien PA. Balancing utility and need by means of donor-to-recipient matching: a challenging problem. *Am J Transplant* 2013; **13**: 522-523 [PMID: 23282243 DOI: 10.1111/ajt.12031]

11 **Perkins JD**, Halldorson JB, Bakthavatsalam R, Fix OK, Carithers RL, Reyes JD. Should liver transplantation in patients with model for end-stage liver disease scores < or = 14 be avoided? A decision analysis approach. *Liver Transpl* 2009; **15**: 242-254 [PMID: 19177441 DOI: 10.1002/lt.21703]

12 **Rana A**, Hardy MA, Halazun KJ, Woodland DC, Ratner LE, Samstein B, Guarrera JV, Brown RS, Emond JC. Survival outcomes following liver transplantation (SOFT) score: a novel method to predict patient survival following liver transplantation. *Am J Transplant* 2008; **8**: 2537-2546 [PMID: 18945283 DOI: 10.1111/j.1600-6143.2008.02400.x]

13 **Halldorson JB**, Bakthavatsalam R, Fix O, Reyes JD, Perkins JD. D-MELD, a simple predictor of post liver transplant mortality for optimization of donor/recipient matching. *Am J Transplant* 2009; **9**: 318-326 [PMID: 19120079 DOI: 10.1111/j.1600-6143.2008.02491.x]

14 **Feng S**, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, Greenstein SM, Merion RM. Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant* 2006; **6**: 783-790 [PMID: 16539636 DOI: 10.1111/j.1600-6143.2006.01242.x]

15 **Andersen PK**, Keiding N. Multi-state models for event history analysis. *Stat Methods Med Res* 2002; **11**: 91-115 [PMID: 12040698 DOI: 10.1191/0962280202SM276ra]

16 **Coombes JM**, Trotter JF. Development of the allocation system for deceased donor liver transplantation. *Clin Med Res* 2005; **3**: 87-92 [PMID: 16012126 DOI: 10.3121/cmr.3.2.87]

17 **Lake JR**. MELD--an imperfect, but thus far the best, solution to the problem of organ allocation. *J Gastrointestin Liver Dis* 2008; **17**: 5-7 [PMID: 18392236]

18 **Halldorson JB**, Paarsch HJ, Dodge JL, Segre AM, Lai J, Roberts JP. Center competition and outcomes following liver transplantation. *Liver Transpl* 2013; **19**: 96-104 [PMID: 23086897 DOI: 10.1002/lt.23561]

19 **Shechter SM**, Bryce CL, Alagoz O, Kreke JE, Stahl JE, Schaefer AJ, Angus DC, Roberts MS. A clinically based discrete-event simulation of end-stage liver disease and the organ allocation process. *Med Decis Making* 2005; **25**: 199-209 [PMID: 15800304 DOI: 10.1177/0272989X04268956]

20 **Freeman RB**, Harper AM, Edwards EB. Redrawing organ distribution boundaries: results of a computer-simulated analysis for liver transplantation. *Liver Transpl* 2002; **8**: 659-666 [PMID: 12149756 DOI: 10.1053/jlts.2002.34385]

21 **Thompson D**, Waisanen L, Wolfe R, Merion RM, McCullough K, Rodgers A. Simulating the allocation of organs for transplantation. *Health Care Manag Sci* 2004; **7**: 331-338 [PMID: 15717817 DOI: 10.1007/s10729-004-7541-3]

22 **Ratcliffe J**, Young T, Buxton M, Eldabi T, Paul R, Burroughs A, Papatheodoridis G, Rolles K. A simulation modelling approach to evaluating alternative policies for the management of the waiting list for liver transplantation. *Health Care Manag Sci* 2001; **4**: 117-124 [PMID: 11393740 DOI: 10.1023/A:1011405610919]

23 **Roberts JP**, Dykstra DM, Goodrich NP, Rush SH, Merion RM, Port FK. Geographic differences in event rates by model for end-stage liver disease score. *Am J Transplant* 2006; **6**: 2470-2475 [PMID: 16939519 DOI: 10.1111/j.1600-6143.2006.01508.x]

24 **American Medical Association**. Opinion 2.03-Allocation of Limited Medical Resources. 2009. Available from: URL: http;//www.ama-assn.org/ama/pub/physician-resources/medical-ethics/code-medical-ethics/opinion203.shtml

25 **United Network for Organ Sharing**. Policy 3.6.4.4: Liver transplant candidates with hepatocellular carcinoma (HCC). 2009. Available from: URL: http: //www.unos.org/PoliciesandBylaws2/policies/pdfs/po9licy_8.pdf.

26 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]

27 **Yao FY**. Liver transplantation for hepatocellular carcinoma: beyond the Milan criteria. *Am J Transplant* 2008; **8**: 1982-1989 [PMID: 18727702 DOI: 10.1111/j.1600-6143.2008.02351.x]

28 **Mazzaferro V**, Rondinara GF, Rossi G, Regalia E, De Carlis L, Caccamo L, Doci R, Sansalone CV, Belli LS, Armiraglio E. Milan multicenter experience in liver transplantation for hepatocellular carcinoma. *Transplant Proc* 1994; **26**: 3557-3560 [PMID: 7998274]

World Journal of

Transplantation

World Journal of Transplantation

Contents

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Renato Ferreira da Silva, MD, PhD, Professor, Department of Surgery and Postgraduate Program, Faculty of Medicine of São José do Rio Preto-(FAMERP), Director of the Liver Transplant Unit of Hospital de Base (FUNFARME). Av Brigadeiro Faria Lima, N 5416, São José do Rio Preto- Sao Paulo , Brazil

AIM AND SCOPE

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

AIM AND SCOPE

World Journal of Transplantation is now indexed in PubMed Central, PubMed, Digital Object Identifier.

EDITORS FOR THIS ISSUE

TOPIC HIGHLIGHT

Maurizio Salvadori, Professor, *Series Editor*

Pancreas transplantation in type Ⅱ diabetes mellitus

Phillip Weems, Matthew Cooper

Phillip Weems, Matthew Cooper, MedStar Georgetown Transplant Institute, Washington, DC 20007, United States
Author contributions: Weems P drafted the manuscript; Cooper M contributed with final review and editing as well as submission for peer review.
Correspondence to: Phillip Weems, MD, MedStar Georgetown Transplant Institute, 3800 Reservoir Road, NW 2 Main, Washington, DC 20007,
United States. weems.s.phillip@gunet.georgetown.edu
Telephone: +1-202-4440032 Fax: +1-202-4440899
Received: April 12, 2014   Revised: June 4, 2014
Accepted: July 15, 2014
Published online: December 24, 2014

Abstract

Although the diagnosis of type 2 diabetes mellitus was once considered a contraindication to simultaneous pancreas-kidney transplantation, a growing body of evidence has revealed that similar graft and patient survival can be achieved when compared to type 1 diabetes mellitus recipients. A cautious strategy regarding candidate selection may limit appropriate candidates from additional benefits in terms of quality of life and potential amelioration of secondary side effects of the disease process. Although our current understanding of the disease has changed, uniform listing characteristics to better define and study this population have limited available data and must be established.

Key words: Pancreas transplantation; Type 2 diabetes mellitus; Simultaneous pancreas-kidney transplantation

Core tip: Comparable outcomes have been achieved in simultaneous-pancreas kidney transplant among both type 1 diabetes mellitus and type 2 diabetes mellitus (DM2) recipients. Our current understanding of the pathogenesis of DM2 is in evolution and denial of simultaneous pancreas-kidney transplantation to appropriately screened DM2 recipients may limit access to a potential life-saving measure with beneficial quality of life improvements. Cautious utilization of DM2 listing criteria should be employed among all pancreas transplant centers in order to ensure optimum patient and graft survivals are achieved.

INTRODUCTION

In October 1920, Dr. Frederick Banting approached Professor John Macleod with an idea that would result in one of the most significant discoveries of twentieth century medicine. Dr. Banting correctly theorized the presence of an "antidiabetic secretion" isolated from a surgically ligated pancreas. His proposed method for isolation and extraction was reluctantly rewarded with skepticism, an inadequate work space, ten canines to form an animal model, and the assistance of a young medical student, Charles Best. Banting and Best named the initial product of their extraction technique "isletin" and would use this substance to prove the endocrine function of the pancreas. Their impressive results were furthered with the addition of a talented biochemist, Bertram Collip, who was tasked with the purification of the insulin extract for testing in human subjects. In January 1922, a 14-year-old diabetic boy, Leonard Thompson, was chosen to be the first human to receive the team's purified insulin[1]. This landmark experiment led to the reversal of the young man's near-death condition and the effort was quickly expanded to other volunteer test subjects with equally positive results. The brilliant results of this team were rewarded with the Nobel Prize in Physiology and

Medicine in 1923[2].

The end of the twentieth century was greeted with the emergence of a new worldwide pandemic. It has been estimated that more than 340 million people are afflicted with diabetes worldwide, with 90% of cases manifesting as type 2 diabetes mellitus (DM2)[3,4]. In the United States alone, diabetes mellitus is the leading cause of end-stage renal disease (ESRD), accounting for 48215 new cases (44%) of renal failure in 2006; an incidence increasing at twice the rate of all other causes of ESRD[5]. The current United States renal transplant waiting list is compromised of > 40% of patients suffering from ESRD complications secondary to diabetes mellitus (DM).

With the discovery of insulin, diabetes was transformed from a rapidly fatal disease to a chronic condition with the emergence of noteworthy secondary conditions related to the primary disease process. Diabetes has been shown to vastly increase the risk of heart disease and stroke and is among the leading causes of chronic renal disease[6]. Diabetic retinopathy, a result of long-term accumulated damage to the small blood vessels of the retina, has been estimated to contribute to one percent of cases of blindness worldwide[7]. Diabetic neuropathy increases the risk of foot ulceration and, when found in conjunction with peripheral vascular disease, may lead to infectious limb complications and accelerated limb loss[6]. Since its proposal in the mid-twentieth century, the goals of pancreas transplantation have remained universal: to establish insulin independence and prevent/ameliorate the damaging secondary complications of the disease process.

PHENOTYPICAL ANALYSIS AND GENETICS OF DIABETES MELLITUS

Diabetes mellitus as a global disorder is characterized by hyperglycemia resulting from either an inadequate production or a decreased sensitivity to circulating insulin. Clinically, diabetes is broadly categorized as either type 1 (DM1) or DM2, depending on the genetic preponderance, age of onset, body habitus, inciting origin, and associated symptoms[8]. Traditionally, the DM2 phenotype is that of an older age and a larger body habitus with a lack of underlying autoimmunity prior to disease onset. In contrast, DM1 patients tend to present with an abrupt onset at an early age, possess a lean body habitus, and require immediate insulin therapy to reverse the consequences of the disease (Table 1).

As our knowledge regarding the pathophysiology of diabetes has further expanded, the distinction between these two seemingly separate disease processes has become decidedly less clear. The accelerator hypothesis of DM proposes a unique pathogenetic origin whereby excess body mass contributes to hyperglycemia resulting in increased insulin production to meet physiologic demands, the acceleration of β-cell apoptosis, and the induction of β-cell "immunogens" in a subset genetically predisposed to islet autoimmunity[9]. The accelerator hypothesis proposes an overlay rather than an overlap exists between the clinical

manifestations of diabetes types with excess body mass central to the rising incidence of the disease worldwide[10].

Although the exact etiology of DM2 remains elusive, a series of common genetic variants, most of which (CDKAL1, CDKN2A, CDKN2, MTNR1B, TCF7L2, KCNJ11B) are associated with either reduced islet cell mass or reduced β-cell function, have been identified[11,12]. Recent studies have shown a similar frequency of DM2 risk genotypes for the transcription factor TCF7L2 in latent autoimmune (DM1) diabetic adults when compared to DM2[12]. The genomic identity of a similar pathologic predisposition further suggests that DM1 and DM2 are representative of the same disorder of insulin resistance, set against different phenotypic backgrounds.

EFFICACY OF PANCREAS TRANSPLANTATION IN DM2

Since the first reported successful pancreas transplant in 1966[13], more than 35000 pancreas transplantations have been reported to the International Pancreas Transplant Registry (IPTR). Of those, more than 24000 were reported from United States centers[14]. Traditionally, pancreas transplantation has been reserved for medically and surgically suitable candidates with DM1 suffering with ESRD (simultaneous kidney and pancreas, SPK), DM1 patients that have previously received a functioning renal graft (pancreas after kidney transplantation, PAK), or patients with brittle diabetes and hypoglycemic unawareness (pancreas transplant alone, PTA).

Although the diagnosis of DM2 was once considered a contraindication to pancreas transplantation, a growing body of evidence has revealed that favorable results can be achieved in selected candidates. Reluctance among some physician groups has favored denial to DM2 candidates secondary to a poorly understood mechanism by which transplanted pancreata may overcome the underlying pathophysiology of insulin resistance. In addition, elevated cardiovascular risks, an enlarged body habitus, an associated older age, and advanced secondary diabetic complications have been suggested as listing deterrents. This cautious judiciary strategy may account for the limited number of DM2 pancreas transplant recipients and small yet encouraging results reported for SPK transplants in DM2[15].

Light has reported a large retrospective series of SPK recipients with 20-year follow-up stratified according to detectable (> 0.8 ng/mL) *vs* undetectable (< 0.8 ng/mL) C-peptide values[16]. The patients with detectable C-peptide values were found to be older in age at the time of clinical diagnosis [24.2 years *vs* 15.4 years ($P < 0001$)], age of transplant [42.8 years *vs* 38.5 years ($P < 0001$)], and had a shorter duration of insulin dependence [19.1 years *vs* 23.1 years ($P < 0.012$)]. Study findings revealed increased graft survival with similar rates of glycemic control in detectable C-peptide patients when compared to non-detectable patients ($P = 0.064$). This finding was contrasted by increased

Table 1 Epidemiologic features differentiating type 1 from type 2 diabetes mellitus		
Characteristic	Type 1 DM	Type 2 DM
Age (yr, at diagnosis)	< 25	> 25
Onset	Abrupt	Gradual
Body Habitus	Lean (weight < 105% of IBW)	Overweight/Obese (weight > 115% of IBW)
HLA-association	Yes	No
C-peptide	Undetectable	Detectable
Ketoacidosis	Yes	No
Immediate need for insulin	Yes	No

DM: Diabetes mellitus; IBW: Ideal body weight; HLA: Human leukocyte antigen.

patient survival discovered in the non-detectable C-peptide group ($P = 0.019$), hypothesized secondary to a younger age and fewer long-term secondary side effects associated within the undetectable C-peptide group. Light's findings caution the use of C-peptide to determine candidacy for pancreas transplantation and adds further controversy to the observed clinical overlap of the two disease phenotypes. In fact, of the study population, 17% of patients who were considered to have DM1 based upon standard clinical criteria (Table 1) were found to have elevated c-peptide values (≥ 0.8 ng/mL) while nearly 40% of patients considered having DM2 (where c-peptide should have been positive) had undetectable values[16].

Margreiter *et al*[17] conducted a single-center retrospective review analyzing twenty-one DM2 SPK recipients with comparisons to historical DM1 SPK and DM2 kidney transplant alone (KTA) controls. Actuarial pancreas graft survival for SPK recipients at 1- and 5-years post-transplant were calculated to be 92.6% and 80.7% respectively for the DM1 SPK group *vs* 81% and 75.9% respectively for the DM2 SPK group ($P = 0.19$). Kidney allograft survival at 5 years post-transplant was found to be 83.6% for DM1 SPK recipients, 80.4% for DM2 SPK recipients, and 52.7% for DM2 KTA recipients ($P < 0.001$). A multivariate analysis adjusting for potential confounders (donor/recipient age, presence of diabetic secondary complications, body mass index (BMI), wait list time, cold ischemic time, delayed graft function, and coronary risk factors) revealed no findings of statistical significance[17].

Several noteworthy registry-based studies have been conducted in order to further analyze clinical outcomes of SPK recipients among DM2 recipients. Sampaio *et al*[18] utilized the United Network for Organ Sharing (UNOS) database to compare outcomes of SPK transplants based upon recipient diabetes type. Of the 6756 SPK recipients transplanted between 2000 and 2007, 586 (8.6%) were reported as having type 2 diabetes. Rates of delayed graft function (11.7% *vs* 7.8%, $P < 0.001$) and kidney primary non-function (0.47% *vs* 1.03%, $P < 0.03$) were significantly more frequent in DM2 patients. Pancreas transplant complications were similar between groups and not statistically significant. Initial findings revealed inferior

five-year overall and death-censored kidney graft survival in type 2 diabetics. However, after adjustment for recipient (age, race, body weight, dialysis time, and cardiovascular comorbidities), donor, and transplant immune characteristics, DM2 was not associated with increased risk of death or kidney or pancreas allograft failure when compared to DM1.

Wiseman utilized Scientific Registry of Transplant Recipients (SRTR) data to conduct a review of DM2 pancreas transplant recipients while utilizing a historical control population of selected DM2 transplant recipients (18-59 years of age, BMI from 18-30 kg/m^2) having received either a live donor kidney alone (LDKA) *vs* deceased donor kidney alone (DDKA)[19]. On adjusted analysis, patient and kidney graft survival rates were superior for LDKA *vs* SPK and DDKA. After 1-year post-transplant, patient and graft survival began to favor SPK when compared to DDKA (82.0% *vs* 75.5%; $P = 0.04$); a finding on multivariable analysis related to younger recipient and donor ages within this cohort. Surprisingly, 40% (269 out of 424 patients) of the SPK cohort were aged 50-59 years of age, and a significant percentage of these were older than age 55 years. Unadjusted pancreas allograft survival rates were 83.7% and 71% at 1- and 5-years, respectively, whereas death-censored pancreas graft survival rates were 87.7% at 1-year and 83.6% at 5-years[20]. These numbers are markedly similar to reported pancreas allograft survival rates within DM1 recipients and further reiterate the premise that excellent outcomes of SPK transplantation can be achieved regardless of recipient diabetes type.

CURRENT CONTROVERSIES IN PANCREAS TRANSPLANTATION AMONG TYPE 2 DIABETICS

In a review of > 35000 pancreas transplants reported to the International Pancreas Transplant Registry (IPTR), Gruessner *et al*[14] revealed an upward trend in the rate of pancreas transplantation performed upon DM2 candidates. Since 1994, diabetic type has been consistently reported within the registry with an overall rate of DM2 recipients increasing from 2% in 1995 to 7% in 2010 ($P < 0.0001$)[14]. Despite this upward trend, the rate of DM2 may in fact be lower (or higher) secondary to the absence of a unified and defined criteria by which transplant centers select DM2 candidates.

Although many defined criteria (age at diagnosis, BMI, family history, HLA association, detectable C-peptide) have been proposed to differentiate DM1 from DM2, no reliable and objective test(s) exist. In fact, as noted prior, several patients are found to categorically overlap. Fasting or stimulated C-peptide levels have long been used as a primary differentiating criterion to define DM1 *vs* DM2 transplant candidates[20-22]. As C-peptide is primarily metabolized in the kidney, levels in patients with ESRD can be disproportionately high and not representative of

Table 2 Proposed simultaneous pancreas-kidney type 2 diabetic selection criteria
Age < 55 yr
BMI < 30 kg/m^2
Insulin dependence
Total insulin requirements < 1 U/kg of IBW/d
Presence of renal failure (dialysis dependent or pre-dialysis advanced diabetic nephropathy with GFR ≤ 20 mL/min per 1.73 m^2
Fasting c-peptide < 10 ng/mL
Low cardiac and vascular disease risk
History of medical and dietary compliance

IBW: Ideal body weight; GFR: Glomerular filtration rate.

the actual functioning β-cell mass. Wang *et al*[22] furthered this controversy by demonstrating that C-peptide levels, using ultrasensitive methods, may be detected in 10% of DM1 patients up to 30-years after disease onset. In addition, Singh confirmed that pre-transplant C-peptide levels had no influence on death-censored SPK survival rates for up to 3-years post-transplant. In this study, the selection criteria utilized to define their DM2 group included minimum insulin requirements of more than 5-years duration with daily requirements less than 1 U/kg per day, C-peptide levels ≥ 1.8 ng/mL, BMI ≤ 32 kg/m^2, and absence of advanced cardiovascular disease[23].

In order to properly evaluate and define selected DM2 candidates for SPK transplantation, universal listing criteria should be adopted. The definition of DM2 has been left to the discretion of the individual reporting centers and often does not account for variations in diabetes phenotype. Until recently, neither the UNOS database nor the SRTR required data regarding patient medication use, C-peptide values, or any other feature which may further confirm categorization of diabetes type. Others have proposed listing criteria to define the DM2 SPK populations. These have often been selected according to younger age, a relatively lean body habitus, and a limited advanced diabetic cardiovascular disease[16,23]. We propose the adoption of a defined list of selection criteria to better define potential DM2 recipients that may benefit from SPK transplantation and allow for closer population-based longitudinal studies (Table 2).

Contemporary management of DM2 patients has been profoundly influenced by the results of the United Kingdom Prospective Diabetes Study (UKPDS)[24-27]. The authors demonstrated a continuous relationship between euglycemia and microvascular complications, with a 35% reduction in risk for each 1% decrement in HbA1c. In most patients with DM2, a multimodal management scheme is employed to address the issue of euglycemia as well the long-term secondary influences on the disease. Central to this approach are dietary and lifestyle modifications, management of dyslipidemia and hypertension, and pharmacologic therapy with a goal of improved glycemic control.

Current available pharmacologic treatments are vast and include medications in the following drug classes:

biguanides, sulfonylureas, meglitinide derivates, alpha-glucosidase inhibitors, thiazolidinediones, glucagon-like peptide-1 (GLP-1) agonists, dipeptidy-l peptidase Ⅳ (DPP-4), selective sodium-glucose transporter-2 (SGLT-2) inhibitors, amylinomimetics, and insulin. With demonstrated treatment failure from any of the aforementioned combination of medical and/or lifestyle modifications, pancreas transplantation may provide the positive effects of normoglycemia in insulin requiring DM2 patients with end-stage renal disease.

In DM2 patients, peripheral insulin resistance, which is associated with relative insulin deficiency and insulin secretory defects, plays a central role[19]. It was once hypothesized that β-cells within the transplant would be subjected to overstimulation leading to "islet exhaustion" in a damaging cascade resulting in allograft failure. This has been disproved in a large, often cited longitudinal case series by Chakkera *et al*[21] and Light *et al*[28,29]. In fact, insulin secretion and sensitivity have been shown to improve long term after SPK in DM2 recipients[30].

Although a greater survival advantage at 5 years post-transplant has been reported for LDKA *vs* both SPK and DDKA in DM2 recipients[19], the quality of life benefits of euglycemia or the possible effects that euglycemia might have on the secondary complications of DM cannot be underestimated[31-33]. These added benefits have been shown to result in improved mental and physical health, disease perception, mobility, vitality, and patient satisfaction[31,32]. Whether the euglycemic effects of the added pancreas ultimately may lead to a survival advantage when compared to LDKA cannot be ruled out, as large retrospective analyses of DM1 SPK recipients have shown the added benefits of the additional pancreas over a kidney transplant alone become more evident over time[34,35].

Importantly, however, expansion of this transplantable cohort may decrease the number of donor pancreata available, further affecting a larger pool of DM1 SPK, PAK, and PTA recipients; a population whose survival benefits have been better defined[19,36]. In addition, the current UNOS algorithm awards priority to SPK recipients over all other forms of DDKA transplants within a given region. Coupled with judicious donor selection criteria at most centers and a relatively short simultaneous kidney-pancreas compared to deceased donor kidney waitlist, listing selected DM2 candidates for SPK may improve an individual's chance to obtain a quality organ transplant with less waiting time. In order to address this potential, UNOS policy has employed a 6-mo review process with proposed reduction in BMI eligibility criteria 2 kg/m^2 if more than 10% of the SPK waiting less is composed with DM2 candidates[19]. Cautious utilization of DM2 listing criteria should be employed among all pancreas transplant centers in order to ensure optimum patient and graft survivals are achieved. As the long-term outcomes of pancreas transplantation in DM2 candidates is not entirely known, SPK transplantation in this cohort should be limited to specialized and well experienced transplant centers to ensure the possibility of continued positive outcomes.

REFERENCES

1   **Banting FG**, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. 1922. *CMAJ* 1991; **145**: 1281-1286 [PMID: 1933711]

2   The Discovery of Insulin. Nobelprize.org. Nobel Media AB 2013, Web. 4 Mar 2014. Available from:URL: http://www.nobelprize.org/educational/medicine/insulin/discovery-insulin.html

3   **Danaei G**, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* 2011; **378**: 31-40 [PMID: 21705069 DOI: 10.1016/S0140-6736(11)60679-X]

4   Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: World Health Organization, 1999 (WHO/NCD/NCS/99.2)

5   **Burrows NR**, Li Y, Geiss LS. Incidence of treatment for end-stage renal disease among individuals with diabetes in the U.S. continues to decline. *Diabetes Care* 2010; **33**: 73-77 [PMID: 20040673 DOI: 10.2337/dc09-0343]

6   **Morrish NJ**, Wang SL, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 2001; **44** Suppl 2: S14-S21 [PMID: 11587045 DOI: 10.1007/PL00002934]

7   Global data on visual impairments 2010. Geneva: World Health Organization, 2012

8   **Sener A**, Cooper M, Bartlett ST. Is there a role for pancreas transplantation in type 2 diabetes mellitus? *Transplantation* 2010; **90**: 121-123 [PMID: 20562677 DOI: 10.1097/TP.0b013e3181e5b7e6]

9   **Fourlanos S**, Harrison LC, Colman PG. The accelerator hypothesis and increasing incidence of type 1 diabetes. *Curr Opin Endocrinol Diabetes Obes* 2008; **15**: 321-325 [PMID: 18594271 DOI: 10.1097/MED.0b013e3283073a5a]

10   **Wilkin TJ**. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. *Diabetologia* 2001; **44**: 914-922 [PMID: 11508279 DOI: 10.1007/s001250100548]

11   **Scalea JR**, Cooper M. Surgical strategies for type II diabetes. *Transplant Rev* (Orlando) 2012; **26**: 177-182 [PMID: 22115951 DOI: 10.1016/j.trre.2011.07.002]

12   **McCarthy MI**. Genomics, type 2 diabetes, and obesity. *N Engl J Med* 2010; **363**: 2339-2350 [PMID: 21142536 DOI: 10.1056/NEJMra0906948]

13   **Kelly WD**, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery* 1967; **61**: 827-837 [PMID: 5338113]

14   **Gruessner AC**. 2011 update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the International Pancreas Transplant Registry (IPTR). *Rev Diabet Stud* 2011; **8**: 6-16 [PMID: 21720668 DOI: 10.1900/RDS.2011.8.6]

15   **Orlando G**, Stratta RJ, Light J. Pancreas transplantation for type 2 diabetes mellitus. *Curr Opin Organ Transplant* 2011; **16**: 110-115 [PMID: 21150617 DOI: 10.1097/MOT.0b013e3283424d1f]

16   **Light J**, Tucker M. Simultaneous pancreas kidney transplants in diabetic patients with end-stage renal disease: the 20-yr experience. *Clin Transplant* 2013; **27**: E256-E263 [PMID: 23480129]

17   **Margreiter C**, Resch T, Oberhuber R, Aigner F, Maier H, Sucher R, Schneeberger S, Ulmer H, Bösmüller C, Margreiter R, Pratschke J, Öllinger R. Combined pancreas-kidney transplantation for patients with end-stage nephropathy caused by type-2 diabetes mellitus. *Transplantation* 2013; **95**: 1030-1036 [PMID: 23407544 DOI: 10.1097/TP.0b013e3182861945]

18   **Sampaio MS**, Kuo HT, Bunnapradist S. Outcomes of simultaneous pancreas-kidney transplantation in type 2 diabetic recipients. *Clin J Am Soc Nephrol* 2011; **6**: 1198-1206 [PMID: 21441123 DOI: 10.2215/CJN.06860810]

19   **Wiseman AC**, Gralla J. Simultaneous pancreas kidney transplant versus other kidney transplant options in patients with type 2 diabetes. *Clin J Am Soc Nephrol* 2012; **7**: 656-664 [PMID: 22344508 DOI: 10.2215/CJN.08310811]

20   **Esmatjes E**, Fernández C, Rueda S, Nicolau J, Chiganer G, Ricart MJ, Junca E, Fernández-Cruz L. The utility of the C-peptide in the phenotyping of patients candidates for pancreas transplantation. *Clin Transplant* 2007; **21**: 358-362 [PMID: 17488385 DOI: 10.1111/j.1399-0012.2007.00649.x]

21   **Chakkera HA**, Bodner JK, Heilman RL, Mulligan DC, Moss AA, Mekeel KL, Mazur MJ, Hamawi K, Ray RM, Beck GL, Reddy KS. Outcomes after simultaneous pancreas and kidney transplantation and the discriminative ability of the C-peptide measurement pretransplant among type 1 and type 2 diabetes mellitus. *Transplant Proc* 2010; **42**: 2650-2652 [PMID: 20832562 DOI: 10.1016/j.transproceed.2010.04.065]

22   **Wang L**, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. *Diabetes Care* 2012; **35**: 465-470 [PMID: 22355018 DOI: 10.2337/dc11-1236]

23   **Singh RP**, Rogers J, Farney AC, Hartmann EL, Reeves-Daniel A, Doares W, Ashcraft E, Adams PL, Stratta RJ. Do pretransplant C-peptide levels influence outcomes in simultaneous kidney-pancreas transplantation? *Transplant Proc* 2008; **40**: 510-512 [PMID: 18374116 DOI: 10.1016/j.transproceed.2008.01.048]

24   Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]

25   Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977 DOI: 10.1016/S0140-6736(98)07037-80]

26   **United Kingdom Prospective Diabetes Study (UKPDS)**. 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *BMJ* 1995; **310**: 83-88 [PMID: 7833731 DOI: 10.1136/bmj.310.6972.83]

27   **Turner RC**, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA* 1999; **281**: 2005-2012 [PMID: 10359389 DOI: 10.1001/jama.281.21.2005]

28   **Light JA**, Sasaki TM, Currier CB, Barhyte DY. Successful long-term kidney-pancreas transplants regardless of C-peptide status or race. *Transplantation* 2001; **71**: 152-154 [PMID: 11211183]

29   **Light JA**, Barhyte DY. Simultaneous pancreas-kidney transplants in type I and type II diabetic patients with end-stage renal disease: similar 10-year outcomes. *Transplant Proc* 2005; **37**: 1283-1284 [PMID: 15848696 DOI: 10.1016/j.transproceed.2004.12.215]

30   **Pox C**, Ritzel R, Büsing M, Meier JJ, Klempnauer J, Schmiegel W, Nauck MA. Combined pancreas and kidney transplantation in a lean type 2 diabetic patient. Effects on insulin secretion and sensitivity. *Exp Clin Endocrinol Diabetes* 2002; **110**: 420-424 [PMID: 12518254 DOI: 10.1055/s-2002-36429]

31   **Smith GC**, Trauer T, Kerr PG, Chadban SJ. Prospective quality-of-life monitoring of simultaneous pancreas and kidney transplant recipients using the 36-item short form health survey. *Am J Kidney Dis* 2010; **55**: 698-707 [PMID: 20176426 DOI: 10.1053/j.ajkd.2009.12.025]

32   **Isla Pera P**, Moncho Vasallo J, Torras Rabasa A, Oppenheimer Salinas F, Fernández Cruz Pérez L, Ricart Brulles MJ. Quality of life in simultaneous pancreas-kidney transplant recipients. *Clin Transplant* 2009; **23**: 600-605 [PMID: 19674015 DOI: 10.1111/j.1399-0012.2009.01054.x]

33   **Gremizzi C**, Vergani A, Paloschi V, Secchi A. Impact of pancreas transplantation on type 1 diabetes-related complications. *Curr Opin Organ Transplant* 2010; **15**: 119-123 [PMID: 20010104 DOI: 10.1097/MOT.0b013e32833552bc]

34   **Morath C**, Zeier M, Döhler B, Schmidt J, Nawroth PP, Opelz G. Metabolic control improves long-term renal allograft and patient survival in type 1 diabetes. *J Am Soc Nephrol* 2008; **19**: 1557-1563 [PMID: 18495965 DOI: 10.1681/ASN.2007070804]

35   **Morath C**, Zeier M, Döhler B, Schmidt J, Nawroth PP, Schwenger V, Opelz G. Transplantation of the type 1 diabetic patient: the long-term benefit of a functioning pancreas allograft. *Clin J Am Soc Nephrol* 2010; **5**: 549-552 [PMID: 20093348 DOI: 10.2215/CJN.03720609]

36   **Sollinger HW**, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. *Ann Surg* 2009; **250**: 618-630 [PMID: 19730242 DOI: 10.1097/SLA.0b013e3181b76d2b]

World Journal of Transplantation

TOPIC HIGHLIGHT

Maurizio Salvadori, Professor, *Series Editor*

Challenges in pediatric renal transplantation

Licia Peruzzi, Alessandro Amore, Rosanna Coppo

Licia Peruzzi, Alessandro Amore, Rosanna Coppo, University-Hospital Health Agency Città della Salute e della Scienza di Torino, Nephrology, Dialysis and Transplantation Unit, Regina Margherita University Children's Hospital, 10126 Turin, Italy
Author contributions: Peruzzi L, Amore A and Coppo R equally reviewed the references, discussed the topic and wrote the paper.
Correspondence to: Licia Peruzzi, MD, PhD, University-Hospital Health Agency Città della Salute e della Scienza di Torino, Nephrology, Dialysis and Transplantation Unit, Regina Margherita University Children's Hospital, Piazza Polonia 94, 10126 Turin, Italy. licia.peruzzi@unito.it
Telephone: +39-1-13131761   Fax: +39-1-16635543
Received: September 4, 2014   Revised: November 20, 2014
Accepted: December 3, 2014
Published online: December 24, 2014

Abstract

Transplantation in children is the best option to treat renal failure. Over the last 25 years the improvements in therapy have dramatically reduced the risk of early acute rejection and graft loss, however the long term results in terms of graft survival and morbidity still require search for new immunosuppressive regimens. Tolerance of the graft and minimization of side effects are the challenges for improving the outcome of children with a grafted kidney. Notwithstanding the difficulties in settling in children large multicenter trials to derive statistically useful data, many important contributions in the last years brought important modifications in the immunosuppressive therapy, including minimization protocols of steroids and calcineurin inhibitors and new induction drugs. New methods for diagnosis of anti HLA antibodies and some new protocols to improve both chance and outcome of transplantation in immunized subjects represent area of ongoing research of extreme interest for children.

Key words: Kidney transplantation; Children; Immunosuppressive therapy; Acute humoral rejection; Desensitization

Core tip: Several novelties in the immunosuppressive treatment regimens in kidney transplantation in children are becoming available, with the aim of reducing the long terms side effects, particularly growth retardation, infections and malignancies, as well as improving the long term survival of the graft through a better treatment of chronic rejection. Moreover new induction drugs and specific protocols addressed to sensitized subjects may widen the possibility to receive a graft even for highly immunized children. These innovative aspects of therapy in kidney transplantation in children are reviewed.

Peruzzi L, Amore A, Coppo R. Challenges in pediatric renal transplantation. *World J Transplant* 2014; 4(4): 222-228 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/i4/222.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i4.222

INTRODUCTION

In children and adolescents affected by chronic renal failure the treatment of choice is kidney transplant. Transplantation indeed, is advocated even before dialysis as the best option to treat the metabolic, psychological and familiar derangement induced by renal failure.

Over the last 25 years remarkable improvements have been reached not only in terms of graft and patient survival[1,2] but also for comorbidities and full rehabilitation[3]. However the optimal immunosuppressive and supportive treatments assuring long term and high quality survival have not been standardized yet.

The immunosuppressive regimens adopted in the last 25 years have dramatically reduced the risk of acute rejection and graft loss within the first months after transplant but concerning the long term results the rate of graft loss is still high, particularly in patients receiving a transplant as small children and facing adolescence with an aged graft. They are bearing the cumulative risks of prolonged therapies,

malignancies, infections and cardio vascular diseases. Cardiovascular risk is one of the most important aspects clarified in recent years as conditioning patient survival and requiring a proactive and systemic preventive approach since the early phases of renal failure[4].

While primary non function and delayed graft function reduction have allowed progressive improvements of short term allograft survival, data on the long run are still not fully satisfactory. Optimal management of chronic allograft nephropathy remains one of the critical challenges to improve long-term kidney transplant outcomes in children. Both immunologic and non immunologic factors are involved in the pathogenesis of chronic allograft nephropathy, often in a subclinical way, and great efforts are frequently required for prompt diagnosis and appropriate treatment. The search for non invasive markers of immunological damage has not produced so far predictive and satisfactory tools to avoid the graft biopsy and protocol biopsies often are advocated also in children for better follow up[2].

The utopistic search for an ideal immunosuppressive regimen able to allow tolerance of the graft and the minimization of the side effects due to over-or under-immunosuppression in children match with the difficulties in settling multicenter trials with sufficiently large number of enrolled patients to derive statistically useful data.

However, several pivotal studies have consistently improved the perspective outcome of children with a grafted kidney, assessing new challenging frontiers in this delicate area.

STEROID MINIMIZATION, EARLY INTERRUPTION AND AVOIDANCE IN PAEDIATRIC KIDNEY TRANSPLANTATION

For more than 40 years steroid therapy has been a cornerstone of immunosuppressive therapy in renal transplantation. Despite their effectiveness, steroids are associated with severe well known side effects including glucose intolerance, diabetes, hypertension, hyperlipidemia, cataract formation, osteoporosis, fractures, mood and cosmetic changes. In children, steroid therapy has the additional very important drawback of marked growth retardation. Because of these side effects, many efforts have been made on trying to withdraw, minimize or avoid steroid therapy in paediatric renal transplantation.

The early attempts of steroid withdrawal after kidney transplantation in children were performed in the late eighties (1987-1990). However the high rate of acute rejections observed suggested the need of steroids for maintenance therapy in paediatric patients.

The introduction of new powerful immunosuppressive agents and new effective induction therapy led to the development in the last years of new trials aimed at steroid early withdrawal or avoidance in children.

One of the first report was the randomized controlled trial (RCT) from Benfield *et al*[5], who used anti CD25

monoclonal Ab (basiliximab), sirolimus, calcineurin inhibitors (CNIs) and steroids for 6 mo. Before randomization a renal biopsy was performed in each case. Fifty nine of the 132 enrolled children were randomized to maintain 0.15 mg/kg per day of prednisone while the remaining 73 children to steroid withdrawal. There was a trend ($P < 0.06$) of increased frequency of acute rejection in the steroid-free group, and moreover, after three years follow-up, frequency of graft loss or death in the steroid-free group became statistically significant ($P < 0.002$). The study started in 2001 but was discontinued in 2004 because of an unanticipated high risk of post-transplant lymphoproliferative disorders (PTLD). In the steroid-free group, 106/107 children treated for > 6 mo had at least one adverse event during the first 6 mo and most worrying, 10 children developed PTLD. From this study it was concluded that in children it is possible to withdraw or avoid steroids if other immunosuppressive agents are given in large doses; however high immunosuppression carries an increased risk of PTLD, which was considered unacceptable.

More satisfying data came from the TWIST RCT led by Grenda *et al*[6] in Europe aimed at investigating the effect of steroid withdrawal on children's growth. All 220 children were treated with daclizumab 1 mg/kg at transplantation and at day 14, tacrolimus (TAC) 0.3 mg/kg per day (target through levels 10-20 ng/mL on days 0-21; 5-15 ng/mL on days 22-186) in combination with mycofenolate mofetil (MMF) 1200 mg/m^2 per day for 2 wk, followed by 600 mg/m^2 per day. In addition to these drugs, children were randomized to (1) arm with steroid withdrawal, assuming methylprednisolone (MP) 300-600 mg/m^2, with daily reduction (60, 40, 30, 20 mg/m^2) and discontinuation at day 5; and (2) arm with steroids: MP 300-600 mg/m^2 and 40 mg/m^2 days 2-7, reduced from day 43 to 183 at discretion of investigators.

The primary end point was fully achieved in pre-pubertal children, who showed a significant benefit from steroid early discontinuation in modification of height standard deviation score. In the latter group, the absolute change in mean height at 6 mo was significantly better. The estimated rate of children free from biopsy proven acute rejection at protocol biopsy performed after 6 mo was 89% *vs* 92%, thus not proving any statistical difference between children with or without steroid discontinuation. Outcome of rejection, as well as graft and patients' survival were similar in the two groups. However, the follow-up was very short, being six months only.

There was a need for longer follow-up, provided by the Stanford University group, which has been the leader in trying the steroid minimization strategy. Sarwal *et al*[7] addressed to complete steroid avoidance in a multicenter RCT with three years of follow-up. The protocol was based on a common treatment with TAC 0.15 mg/kg per day (12-14 ng/mL day 0-7; 10-12 ng/mL from 2nd wk; 4-6 ng/mL at 1 year and 3-5 ng/mL after 1th year) in association with MMF: 1200 mg/m^2 per day for 2 d, than 600-900 mg/m^2 per day. Children were randomized

in two arms, including: (1) Steroid free arm, daclizumab 2 mg/kg pre transplant, at weeks 2, 4, 6, 8, 11 and months 4, 5, 6; (2) Steroid based arm, daclizumab 1 mg/kg pre transplantation, at weeks 2, 4, 6, 8. Moreover, prednisone was given, MP 10 mg/kg perioperatively, followed by 2 mg/kg and 0.5, 0.3, 0.2, 0.1, 0.15, 0.1 mg/kg per day at the end of weeks 1, 2, 4, 6, 16. The dose of 0.1 mg/kg was achieved no later than six months post transplantation.

After three years of follow-up no significant difference in estimated glomerular filtration rate was found between the two groups as well as in protocol biopsies at 6, 12 and 24 mo, despite some borderline changes were slightly more frequent in the steroid-free group. This observation induced further subanalysis on subclinical inflammation and chronic renal graft injury in children who underwent this NIH organized RCT[8]. No difference between steroid and steroid free regimens was found as far as T mediated rejection or T mediated borderline changes were concerned. There was a significant increase in blood pressure in children on steroids in comparison to those without it as well as an increase in cholesterol. Changes in height-Z score from baseline tended to be different in the two groups over the first months after transplantation (as observed in TWIST RCT) but this effect was lost after one year of transplantation. From this RCT it was concluded that three year follow up of steroid free regimen in unsensitized recipients at first transplantation with double dose of daclizumab in comparison to children on steroids was safe and did not increase the frequency of PTLD. However, no significant difference was observed in linear growth at three years even though at 6 mo there was a better growth in the steroid free group. In this study 13% of children had a failure to maintain steroid-free regimen and had a worse prognosis compared to those who maintained the steroid-free protocol, mostly due to difficulty to control acute rejection or to recurrence of original glomerulonephritis.

A recent systematic review by Pascual *et al*[9] including children and adults, concluded that the issue of steroid withdrawal is still controversial. After analysis of 9 RCT and 1934 subjects investigated, death and graft loss were similar in steroid avoidance and control patients, with no differences between CsA and TAC studies. After steroid avoidance, acute rejection was more frequent than conventional steroid use in CsA trials but not when TAC was used. Steroid avoidance was associated with less frequent new-onset diabetes mellitus, but this decrease was only evident with CsA, whereas this difference was not significant analyzing TAC studies. Despite this trend, the corresponding interaction tests were not statistically significant for acute rejection and new-onset diabetes mellitus, respectively.

The conclusions from this meta-analysis were that steroid avoidance or early withdrawal within the first two weeks is safe in kidney transplant recipients receiving induction with anti-interleukin-2 receptor antibodies or thymoglobulin and a drug regimen based on calcineurin inhibitor and MMF. However, the real benefits remain unclear.

CALCINEURIN INHIBITORS−FREE PROTOCOLS IN PAEDIATRIC RENAL TRANSPLANTATION

CNI carry relevant side effects, including hirsutism, hypertension, diabetes, seizures and renal toxicity which contributes to long term graft loss. Hence the search for CNI free protocols is one of the frontiers for renal transplantation in children. The Renal transplantation Center in Atlanta reported a five-year experience using sirolimus (SRL)-based, CNI-free immunosuppression in pediatric renal transplantation[10]. A cohort of low-risk renal pediatric transplant recipients was switched from TAC to SRL. All children received basiliximab induction and TAC, MMF, and prednisone. Conversion was pursued in cases at first transplant without history of nephrotic syndrome and without histologic evidence for acute rejection at three months after transplantation. Fifty-one children were converted from TAC to SRL. SRL was discontinued in 11 cases over the first year because of adverse events, particularly in 20% of the cases for aphtous ulcers. The remaining 40 children had 91% graft survival at five years. Acute rejection was detected in 13% of patients during the first year after conversion. BK viremia was detected in 20% and proteinuria in 7%. This study concluded that SRL-based immunosuppression associated with a CNI-free regimen can be successful in selected lower-risk patients, though the side effects are relevant.

A very relevant issue in children transplantation is growth since height is compromised by previous long term-uremia, dialysis treatment, and children undergoing renal transplantation have to face the need of steroids after transplant, which further limits the possibility of attaining a satisfactory final height. A report from Heidelberg Group has recently investigated the growth in pediatric kidney transplant recipients on an everolimus *vs* an MMF-based steroid-free immunosuppressive regimen[11]. Indeed some concerns were raised about the possible interference of mammalian target of rapamycin inhibitors (mTORi) in pediatric transplant recipients with bone growth by inhibition of growth factor signaling and growth plate chondrocyte proliferation. The study focused on longitudinal growth over 2 years in steroid-free pediatric kidney transplant recipients. Fourteen children on a steroid-free maintenance immunosuppressive regimen with low-dose everolimus (EVR) associated with low-dose CsA were compared to 14 children on steroid-free protocol and standard MMF regimen in conjunction with a standard CNI dose. No difference in change in height standard deviation score was detected between EVR and MMF groups. Similarly, the percentage of prepubertal patients experiencing catch-up growth, was similar in children in the two protocols. The Authors concluded that low-dose EVR does not have a negative impact on growth in pediatric renal transplant recipients.

A recently proposed drug for CNI free protocol is belatacept (which differs from abatacept only for two amino

acids), a fusion protein constituted by the Fc fragment of human IgG1 linked to the extracellular domain of CTLA-4, which is crucial for T-cell costimulation. In pediatric kidney transplantation belatacept is a promising agent for allowing steroid-free and CNI free immunosuppression. In a recent report[12] in living donor kidney transplant belatacept was used monthly in association with daily sirolimus. Belatacept and sirolimus effectively prevented kidney allograft rejection without CNIs or steroids when used following alemtuzumab induction. The effect of a similar protocol in children is under investigation.

NEW INDUCTION PROTOCOLS FOR RENAL TRANSPLANTATION IN CHILDREN

Alemtuzumab (Campath-1H) a humanized monoclonal antibody directed against CD52, is a new interesting option for induction with good results also in children[13,14]. Alemtuzumab recognizes CD52, a glycoprotein expressed on T and B lymphocytes, monocytes and natural killer cells[15,16]. This drug is the most efficient presently available lymphocyte-depleting agents, inducing, after a single administration, a prompt and prolonged depletion of circulating lymphocytes. Alemtuzumab was used since 1998[17] with the interesting result of allowing a low-dose CsA monotherapy. Recent RCT in adults have shown lower frequency of acute rejection in comparison to basiliximab in patients non at high immunological risk[18,19]. In children the first relevant experience was from Kidney Transplantation Center in Moskow, as Kaabak *et al*[20] reported, in living related pediatric renal transplants. The rationale was to eradicate peripheral lymphomonocytes and induce donor- specific tolerance, by infusing two doses of 30 mg alemtuzumab, one 12-29 d prior to transplantation and the other at surgery. They reported a large experience on 101 living-donor kidney transplantations in pediatric recipients. The maintenance immunosuppression included low doses CNI and MMF. The mean follow-up was 3 years. Graft survival was 96% at one year and 89% at three years. Acute rejection was detected at protocol biopsies in 26% of children at one year and in 35% at two years, while no rejection was detected thereafter. The conclusion from this study were that alemtuzumab pretreatment before living related kidney transplantation is a good option allowing a reduction in usual doses of CNI and obtaining satisfactory middle-term results.

A subsequent study performed by the Portland Group of pediatric kidney transplantation[21] investigated the effects of alemtuzumab, 0.5 mg/kg for a maximum of 30 mg, in 25 children undergoing cadaveric kidney transplantation, in whom the drug was given after anesthesia, before kidney transplantation. MP was given 10 mg/kg peri-operatively and before revascularization. Children received steroid therapy for other four days. TAC as monotherapy was initiated at day 1 (target through levels of 8-10 ng/mL over 6 mo, then 6-8 ng/mL). MMF

was added only in cases of high immunological risk or prolonged delayed graft function. Over a mean follow-up of two years, TAC monotherapy was maintained in 48% of children, and steroids were avoided in 80%. The actuarial survival rate at 3 years was 100%. Acute rejection rate was 12% within the first year and 16% in the following two years. The frequency of BK or CMV infection was 16%. The Authors concluded that alemtuzumab induction with TAC monotherapy is a good option for children with low immunological risk ensuring excellent short and medium-term follow-up outcome.

A recent report provided interesting results of 7 years follow-up in children treated with alemtuzumab and corticosteroid minimization after cadaveric renal transplantation[22]. The maintenance therapy was a steroid-free regimen with TAC and MMF immunosuppression. All children had immediate graft function and graft survival was excellent (95%). No patient had cytomegalovirus infection, PTLD or polyoma BK nephropathy. The conclusion of this study was that steroid avoidance provided a good outcome with adequate immunosuppression after single-dose alemtuzumab with maintenance therapy with TAC and low-dose MMF.

DESENSITIZATION PROTOCOLS IN CHILDREN

Over the last years a growing interest has been focused on donor-specific antibodies (DSA Ab) for a previously unsuspected role in graft function and survival[23]. Acute antibody-mediated graft rejection is a problem involving children as well as adults, but even more relevant is becoming the role of DSA Ab as one of the mayor causes of graft loss[24]. Children candidates to a kidney transplant, particularly after a first failed graft, more often than in the past present with antibodies against HLA antigens, often at high titres, raising the problem of the risk of hyperacute or acute humoral rejections and reducing the chances of being transplanted[25,26]. The new flow cytometry based techniques used to investigate the presence of anti HLA antibodies have a much higher sensitivity than complement dependent cytotoxicity assays and are able to reveal panels of antibodies whose capacity to bind complement and induce antibody mediated lysis of target cells is not ascertained. For some years the true role of these low titres antibodies has not been clearly defined: hyperacute rejection is not common but either acute rejection and a chronic damage induced by these antibodies has been demonstrated[23,24].

Sensitization may occur after blood transfusion with red blood cells not appropriately washed or filtered, however the main origin of sensitization is a previous transplant. Proteins as well as stem cells of donor origin have been demonstrated to be persistently present even after removal of the graft, being able to maintain the persistence of immunological stimulus[25]. De novo antibodies, mostly directed against HLA, have been detected in a United States multicenter report in up to 24% of children with renal transplant. Six percent of these antibodies were DSA

Ab and 6% anti MHC class 1 related chain A (MICA), and were equally found either on steroid-free or steroid-based regimens[25]. The presence of anti HLA and anti MICA Ab was significantly associated with acute and chronic rejection with faster graft loss. Similar results were reported by a single center Italian study[26] in 82 children who underwent kidney transplantation, without prior DSA Ab: 23% of this cohort developed after 4 years of follow-up de novo DSA Ab, mostly directed against HLA-DQ antigens. A significant correlation was found between DSA Ab and chronic antibody-mediated rejection. The conclusion of both studies[25,26] were that children developing DSA Ab are at risk of graft dysfunction and that there is the need of developing new strategies to prevent antibody mediated graft damage and progression to graft failure.

In candidates to a kidney transplant persistent large panel of antibodies against HLA and PRA > 50% require a desensitization approach for increasing the chance of receiving a graft. Several protocols have been proposed also in children aiming at reducing the antibody titres. The desensitizing protocols include removal of DSA by high-dose *i.v.* immunoglobulins administration (IVIg), plasmapheresis, immunoadsorption, or a combination of the two approaches. In the attempt of reducing recurrence of DSA Ab, rituximab has been introduced in the last years. In some cases immunosuppression with alkylating agents is also considered[23]. The major drawbacks of these protocols are the risk of infections and the rebound of antibodies allowing a short window interval time for receiving a transplant, requiring repeated desensitization if a suitable donor is not found. In pediatric age, due to low numbers of desensitized patients there is a lack of large studies.

Most protocols are based on intravenous immunoglobulins which in children have been reported to be effective even when used alone in significantly reducing PRA. Al-Uzri *et al*[27] showed that weekly infusion for three consecutive weeks every 12 wk of high-dose (500 mg/kg) Immunoglobulins reduced PRA to zero, and the effect lasted for over three years. Tyan reported a case where IVIG were successfully used to reduce PRA from 95% to 15% and allow retransplant in a 13 years old boy[28].

In adults Immunoglobulin infusion alone have not produced satisfactory results, hence different protocols of combination treatment with other drugs or procedures have been tried and adopted also in children. The combination of rituximab with plasmapheresis was able to maintain over longer time the immunoglobulin depleting effect of plasmapheresis maintaining the lowering effect so as to allow the use of this protocol also in deceased-donor transplant. Rituximab cannot by itself reduce anti HLA antibody level, but can prevent clonal B cells expansion and consequently DSA production. The advantage of rituximab (1 g/1.73 m^2) for children is the wide experience in pediatric nephrotic syndrome which reported low incidence of infections and of major complications and effects lasting sometimes even one year, avoiding the need for vascular access and repeated procedures, like in the case of plasmapheresis. Rituximab was given in some protocols after plasmapheresis[29].

Billing *et al*[30] treated children with active chronic DSA Ab rejection with 4 weekly doses of 1 g/kg IVIg followed by one single dose of rituximab (375 mg/m^2). They reported a significantly lower loss of GFR over 6 mo of treatment in 4/6 cases. These results were confirmed in a larger trial enrolling 20 children followed over 2 years, with a response rate (evaluated as reduction of GFR loss) in 70% of the patients. Meanwhile, there was a reduction of 60% of antibodies against both HLA class Ⅰ and Class Ⅱ[31].

Another drug used to successfully prevent or reduce DSA Ab is MMF (390 to 500 mg/m^2 per day), which gave satisfactory results in a 4-year-old child[32].

New treatments, like Eculizumab which is a complement inhibitor directed against terminal complement protein C5, and the proteasome inhibitor Bortezomib, are theoretically useful to block the final effects of preformed anti HLA antibodies and their noxious effect, but still not yet experienced in sensitized children. A recent retrospective study reported 4 cases of children with grafted kidneys who were treated with bortezomib for high levels of DSA and acute antibody mediated rejection[33]. Children received four doses of bortezomib 1.3 mg/m^2 at day 1, 4, 8 and 11. All of them were treated with various drug combinations, including rituximab, methylprednisolone, plasmapheresis or IVIg. The conclusion from this limited series were that bortezomib therapy is an effective and safe methods for a rapid reduction in DSA levels, although its effectiveness from the clinical point of view was not clearly defined in this preliminary experience in children.

CONCLUSION

In agreement with a recent systematic review performed by the Cochrane group[34] to highlight the current trends in immunosuppression in pediatric renal transplantation, when we focus on challenging new frontiers for these children, we still face an uncertain horizon. Newly proposed drugs, including belatacept and alemtuzumab, carry serious side-effects, and interleukin-2 receptor antagonists remain the safest and effective agents for pediatric kidney transplantation. The new steroid-free regimens can improve growth and not hamper graft survival over a short follow-up, however, long-term outcome remains to be determined. mTOR inhibitors, sirolimus and everolimus, are a promising option for primary immunosuppression as CNI sparing agents, however beneficial results on long term graft survival are still to be proven. Desensitization protocols are being performed, but benefits and harms are still to be analyzed and long-term graft survival analysis studies are needed.

In spite of these apparently non optimistic considerations, the improvement of the short and long term results of kidney transplantation in children have been so impressive over the last decades, that we optimistically think that the new frontiers presently representing a challenge will be achieved in a few years as consistent point for further improving the

outcome of kidney transplanted children.

REFERENCES

1   **Patel UD**. Outcomes after pediatric kidney transplantation improving: how can we do even better? *Pediatrics* 2014; **133**: 734-735 [PMID: 24616364 DOI: 10.1542/peds.2014-0124]
2   **Van Arendonk KJ**, Boyarsky BJ, Orandi BJ, James NT, Smith JM, Colombani PM, Segev DL. National trends over 25 years in pediatric kidney transplant outcomes. *Pediatrics* 2014; **133**: 594-601 [PMID: 24616363 DOI: 10.1542/peds.2013-2775]
3   **Ellis EN**, Martz K, Talley L, Ilyas M, Pennington KL, Blaszak RT. Factors related to long-term renal transplant function in children. *Pediatr Nephrol* 2008; **23**: 1149-1155 [PMID: 18301925 DOI: 10.1007/s00467-008-0779-0]
4   **Kaidar M**, Berant M, Krauze I, Cleper R, Mor E, Bar-Nathan N, Davidovits M. Cardiovascular risk factors in children after kidney transplantation--from short-term to long-term follow-up. *Pediatr Transplant* 2014; **18**: 23-28 [PMID: 24134654 DOI: 10.1111/petr.12174]
5   **Benfield MR**, Bartosh S, Ikle D, Warshaw B, Bridges N, Morrison Y, Harmon W. A randomized double-blind, placebo controlled trial of steroid withdrawal after pediatric renal transplantation. *Am J Transplant* 2010; **10**: 81-88 [PMID: 19663893 DOI: 10.1111/j.1600-6143.2009.02767.x]
6   **Grenda R**, Webb NJ. Steroid minimization in pediatric renal transplantation: Early withdrawal or avoidance? *Pediatr Transplant* 2010; **14**: 961-967 [PMID: 20874824 DOI: 10.1111/j.1399-3046.2010.01403.x]
7   **Sarwal MM**, Ettenger RB, Dharnidharka V, Benfield M, Mathias R, Portale A, McDonald R, Harmon W, Kershaw D, Vehaskari VM, Kamil E, Baluarte HJ, Warady B, Tang L, Liu J, Li L, Naesens M, Sigdel T, Waskerwitz J, Salvatierra O. Complete steroid avoidance is effective and safe in children with renal transplants: a multicenter randomized trial with three-year follow-up. *Am J Transplant* 2012; **12**: 2719-2729 [PMID: 22694755 DOI: 10.1111/j.1600-6143.2012.04145.x]
8   **Naesens M**, Salvatierra O, Benfield M, Ettenger RB, Dharnidharka V, Harmon W, Mathias R, Sarwal MM. Subclinical inflammation and chronic renal allograft injury in a randomized trial on steroid avoidance in pediatric kidney transplantation. *Am J Transplant* 2012; **12**: 2730-2743 [PMID: 22694733 DOI: 10.1111/j.1600-6143.2012.04144.x]
9   **Pascual J**, Royuela A, Galeano C, Crespo M, Zamora J. Very early steroid withdrawal or complete avoidance for kidney transplant recipients: a systematic review. *Nephrol Dial Transplant* 2012; **27**: 825-832 [PMID: 21785040 DOI: 10.1093/ndt/gfr374]
10   **Hymes LC**, Warshaw BL. Five-year experience using sirolimus-based, calcineurin inhibitor-free immunosuppression in pediatric renal transplantation. *Pediatr Transplant* 2011; **15**: 437-441 [PMID: 21338459 DOI: 10.1111/j.1399-3046.2011.01477.x]
11   **Billing H**, Burmeister G, Plotnicki L, Ahlenstiel T, Fichtner A, Sander A, Höcker B, Tönshoff B, Pape L. Longitudinal growth on an everolimus- versus an MMF-based steroid-free immunosuppressive regimen in paediatric renal transplant recipients. *Transpl Int* 2013; **26**: 903-909 [PMID: 23865768 DOI: 10.1111/tri.12148]
12   **Kirk AD**, Guasch A, Xu H, Cheeseman J, Mead SI, Ghali A, Mehta AK, Wu D, Gebel H, Bray R, Horan J, Kean LS, Larsen CP, Pearson TC. Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *Am J Transplant* 2014; **14**: 1142-1151 [PMID: 24684552 DOI: 10.1111/ajt.12712]
13   **Li L**, Chaudhuri A, Chen A, Zhao X, Bezchinsky M, Concepcion W, Salvatierra O, Sarwal MM. Efficacy and safety of thymoglobulin induction as an alternative approach for steroid-free maintenance immunosuppression in pediatric

renal transplantation. *Transplantation* 2010; **90**: 1516-1520 [PMID: 20935596 DOI: 10.1097/TP.0b013e3181fc8937]
14   **De Serres SA**, Mfarrej BG, Magee CN, Benitez F, Ashoor I, Sayegh MH, Harmon WE, Najafian N. Immune profile of pediatric renal transplant recipients following alemtuzumab induction. *J Am Soc Nephrol* 2012; **23**: 174-182 [PMID: 22052056 DOI: 10.1681/ASN.2011040360]
15   **Ratzinger G**, Reagan JL, Heller G, Busam KJ, Young JW. Differential CD52 expression by distinct myeloid dendritic cell subsets: implications for alemtuzumab activity at the level of antigen presentation in allogeneic graft-host interactions in transplantation. *Blood* 2003; **101**: 1422-1429 [PMID: 12393688 DOI: 10.1182/blood-2002-04-1093]
16   **Kirk AD**, Hale DA, Mannon RB, Kleiner DE, Hoffmann SC, Kampen RL, Cendales LK, Tadaki DK, Harlan DM, Swanson SJ. Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). *Transplantation* 2003; **76**: 120-129 [PMID: 12865797 DOI: 10.1097/01.TP.0000071362.99021.D9]
17   **Calne R**, Friend P, Moffatt S, Bradley A, Hale G, Firth J, Bradley J, Smith K, Waldmann H. Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. *Lancet* 1998; **351**: 1701-1702 [PMID: 9734890 DOI: 10.1016/S0140-6736(05)77739-4]
18   **Study T**, Group C. Alemtuzumab-based induction treatment versus basiliximab-based induction treatment in kidney transplantation (the 3C Study): a randomised trial. *Lancet* 2014; **6736**: 1-7 [PMID: 25078310 DOI: 10.1016/S0140-6736(14)61095-3]
19   **Kuypers DRJ.** Alemtuzumab induction therapy in kidney transplantation. *Lancet* 2014; **6736**: 10-11 [PMID: 25078307 DOI: 10.1016/S0140-6736(14)61174-0]
20   **Kaabak MM**, Babenko NN, Samsonov DV, Sandrikov VA, Maschan AA, Zokoev AK. Alemtuzumab induction in pediatric kidney transplantation. *Pediatr Transplant* 2013; **17**: 168-178 [PMID: 23442101 DOI: 10.1111/petr.12048]
21   **Sung J**, Barry JM, Jenkins R, Rozansky D, Iragorri S, Conlin M, Al-Uzri A. Alemtuzumab induction with tacrolimus monotherapy in 25 pediatric renal transplant recipients. *Pediatr Transplant* 2013; **17**: 718-725 [PMID: 24164824 DOI: 10.1111/petr.12159]
22   **Supe-Markovina K**, Melquist JJ, Connolly D, DiCarlo HN, Waltzer WC, Fine RN, Darras FS. Alemtuzumab with corticosteroid minimization for pediatric deceased donor renal transplantation: a seven-yr experience. *Pediatr Transplant* 2014; **18**: 363-368 [PMID: 24712738 DOI: 10.1111/petr.12253]
23   **Salvadori M**, Bertoni E. Impact of donor-specific antibodies on the outcomes of kidney graft: Pathophysiology, clinical, therapy. *World J Transplant* 2014; **4**: 1-17 [PMID: 24669363 DOI: 10.5500/wjt.v4.i1.1]
24   **Pape L,** Becker JU, Immenschuh S, Ahlenstiel T. Acute and chronic antibody-mediated rejection in pediatric kidney transplantation. *Pediatr Nephrol* 2014 [PMID: 24865478 DOI: 10.1007/s00467-014-2851-2]
25   **Chaudhuri A**, Ozawa M, Everly MJ, Ettenger R, Dharnidharka V, Benfield M, Mathias R, Portale A, McDonald R, Harmon W, Kershaw D, Vehaskari VM, Kamil E, Baluarte HJ, Warady B, Li L, Sigdel TK, Hsieh SC, Dai H, Naesens M, Waskerwitz J, Salvatierra O, Terasaki PI, Sarwal MM. The clinical impact of humoral immunity in pediatric renal transplantation. *J Am Soc Nephrol* 2013; **24**: 655-664 [PMID: 23449533 DOI: 10.1681/ASN.2012070663]
26   **Ginevri F**, Nocera A, Comoli P, Innocente A, Cioni M, Parodi A, Fontana I, Magnasco A, Nocco A, Tagliamacco A, Sementa A, Ceriolo P, Ghio L, Zecca M, Cardillo M, Garibotto G, Ghiggeri GM, Poli F. Posttransplant de novo donor-specific hla antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant* 2012; **12**: 3355-3362 [PMID: 22959074 DOI: 10.1111/j.1600-6143.2012.04251.x]
27   **Al-Uzri AY**, Seltz B, Yorgin PD, Spier CM, Andreoni K.

Successful renal transplant outcome after intravenous gamma-globulin treatment of a highly sensitized pediatric recipient. *Pediatr Transplant* 2002; **6**: 161-165 [PMID: 12000474 DOI: 10.1034/j.1399-3046.2002.01055.x]

28   **Tyan DB**, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation* 1994; **57**: 553-562 [PMID: 8116041]

29   **Jackson AM**, Kraus ES, Orandi BJ, Segev DL, Montgomery RA, Zachary AA. A closer look at rituximab induction on HLA antibody rebound following HLA-incompatible kidney transplantation. *Kidney Int* 2014 Jul 23; Epub ahead of print [PMID: 25054778 DOI: 10.1038/ki.2014.261]

30   **Billing H**, Rieger S, Ovens J, Süsal C, Melk A, Waldherr R, Opelz G, Tönshoff B. Successful treatment of chronic antibody-mediated rejection with IVIG and rituximab in pediatric renal transplant recipients. *Transplantation* 2008; **86**: 1214-1221 [PMID:

19005402 DOI: 10.1097/TP.0b013e3181880b35]

31   **Billing H**, Rieger S, Süsal C, Waldherr R, Opelz G, Wühl E, Tönshoff B. IVIG and rituximab for treatment of chronic antibody-mediated rejection: a prospective study in paediatric renal transplantation with a 2-year follow-up. *Transpl Int* 2012; **25**: 1165-1173 [PMID: 22897111 DOI: 10.1111/j.1432-2277.2012.01544.x]

32   **Wong H**, Laberge R, Harvey E, Filler G. Preventing sensitization with mycophenolate mofetil in a pediatric kidney recipient. *Pediatr Transplant* 2006; **10**: 367-370 [PMID: 16677363 DOI: 10.1111/j.1399-3046.2005.00469.x]

33   **Nguyen S**, Gallay B, Butani L. Efficacy of bortezomib for reducing donor-specific antibodies in children and adolescents on a steroid minimization regimen. *Pediatr Transplant* 2014; **18**: 463-468 [PMID: 24814755 DOI: 10.1111/petr.12274]

34   **Kim S**, Webster AC, Craig JC. Current trends in immunosuppression following organ transplantation in children. *Curr Opin Organ Transplant* 2013 [PMID: 23995377 DOI: 10.1097/MOT.0b013e3283651b35]

TOPIC HIGHLIGHT

Maurizio Salvadori, Professor, *Series Editor*

Female gender in the setting of liver transplantation

Kryssia Isabel Rodríguez-Castro, Eleonora De Martin, Martina Gambato, Silvia Lazzaro, Erica Villa,
Patrizia Burra

Kryssia Isabel Rodríguez-Castro, Eleonora De Martin, Martina Gambato, Silvia Lazzaro, Patrizia Burra, Multivisceral Transplant Unit, Deparment of Surgery, Oncology and Gastroenterology, Padua University Hospital, 35128 Padua, Italy
Erica Villa, Department of Gastroenterology, Azienda Ospedaliero-Universitaria and University of Modena and Reggio Emilia, 41124 Modena, Italy
Author contributions: Rodríguez-Castro KI contributed to the conception, design, and performance of the research, drafting of the manuscript, approval of the final version; De Martin E contributed to the conception, design, and performance of the research, drafting of the manuscript, approval of the final version; Gambato M contributed to the critical revision of the manuscript, approval of the final version; Lazzaro S critically revised the manuscript, approval of the final version; Villa E contributed to the conception and design of the research, critical revision of the manuscript, approval of the final version; Burra P contributed to the conception and design of the research, critical revision of the manuscript, approval of the final version.
Correspondence to: Patrizia Burra, Professor, MD, PhD, Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Via Giustiniani 2, 35128 Padua, Italy. burra@unipd.it
Telephone: +39-49-8212892 Fax: +39-49-8218727
Received: April 8, 2014   Revised: May 27, 2014
Accepted: July 15, 2014
Published online: December 24, 2014

Abstract

The evolution of liver diseases to end-stage liver disease or to acute hepatic failure, the evaluation process for liver transplantation, the organ allocation decision-making, as well as the post-transplant outcomes are different between female and male genders. Women's access to liver transplantation is hampered by the use of model for end-stage liver disease (MELD) score, in which creatinine values exert a systematic bias against women due to their lower values even in the presence of variable degrees of renal dysfunction. Furthermore, even when correcting MELD score for gender-appropriate creatinine determination, a quantifiable uneven access to transplant prevails, demonstrating that other factors are also involved. While some of the differences can be explained from the epidemiological point of view, hormonal status plays an important role. Moreover, the pre-menopausal and post-menopausal stages imply profound differences in a woman's physiology, including not only the passage from the fertile age to the non-fertile stage, but also the loss of estrogens and their potentially protective role in delaying liver fibrosis progression, amongst others. With menopause, the tendency to gain weight may contribute to the development of or worsening of pre-existing metabolic syndrome. As an increasing number of patients are transplanted for non-alcoholic steatohepatitis, and as the average age at transplant increases, clinicians must be prepared for the management of this particular condition, especially in post-menopausal women, who are at particular risk of developing metabolic complications after menopause.

Key words: Liver transplantation; Female gender; Estrogens; Model for end-stage liver disease score; Creatinine; Gender donor-recipient match

Core tip: Gender differences play an important role in liver diseases, their evolution and outcome, and in liver transplantation, not only in terms of access to this resource, but also in terms of graft survival, metabolic aspects, and quality of life after liver transplantation. Not only gender differences, are important, however, but clearly the different hormonal status throughout a woman's lifetime determines many aspects not only regarding fertility and sexual issues such as pregnancy, but also metabolic complications. Notwithstanding this, decision-making algorithms regarding indications, risk factors, and outcomes after transplant do not yet incorporate many of these concepts that affect the clinical practice.

INTRODUCTION

It is ever clearer that gender differences play an important role in liver diseases, their evolution and outcome, and in liver transplantation (LT), not only in terms of access to this resource, but also in terms of graft survival, metabolic aspects, and quality of life after LT. Nevertheless, proposed measures for correcting the systematic bias disadvantaging women's access to LT, and the gender variable itself, are not yet fully incorporated into decision-making algorithms regarding evaluation of indications, risk factors, and outcomes in LT. The present review, therefore aims at highlighting gender differences in diseases that lead to LT, access to LT, and outcomes after transplant.

GENDER DISPARITY IN ACCESS TO LT

Sociodemographic determinants

Access to a life-saving resource such as LT has unfortunately been hampered for ethnic minorities, women, and patients of low socioeconomic status or inadequate insurance coverage; in a study analyzing health care inequities that prevent patients with end-stage liver disease from being evaluated and waitlisted for LT, patients were less likely to undergo evaluation, waitlisting and transplantation if they were women, black and lacked commercial insurance ($P < 0.001$ each)[1].

This disparity of access to LT probably owes to several factors, including body and organ size considerations, differences in the etiology of the underlying liver disease, and limits of the model for end-stage liver disease (MELD) score, especially regarding creatinine levels[2].

MELD, MELD-related issues and non-MELD determinants of access to LT

In the pre-MELD era, a study from the Organ Procurement and Transplantation Network (OPNT) showed that female sex was significantly correlated with longer stay on the liver transplant waiting list and also with the risk of dying before LT[3]. Unequal access to LT for women was unfortunately perpetuated upon implementation of the MELD score for organ allocation, however. In a study based on UNOS data comparing pre- and post-MELD cohorts, women were more likely than men to die or become too sick for LT post-MELD [23.7% *vs* 21.4%; odds ratio (OR) = 1.30; $P = 0.003$] *vs* pre-MELD (22.4% *vs* 21.9%; OR = 1.08; $P = 0.37$). Similarly, women were less likely than men to receive a liver transplant within 3 years both pre-MELD (64.8% *vs* 67.6%; OR = 0.80; $P = 0.002$) and post-MELD (39.9% *vs* 48.7%; OR = 0.70; $P < 0.001$)[4]. Actually, organ allocation based on MELD sore has further increased gender disparity, as waiting list mortality risk has risen, particularly for MELD

scores > 15[5]. In fact, female gender, together with primary non-function, fulminant hepatic failure, blood group O, CTP ≥ 11 and MELD score ≥ 20 have been found to be predictors of waiting list mortality[6].

A systematic bias against women, resulting in part from the use of creatinine as a measure of renal function, has been identified in MELD-based liver allocation. Women's lesser body (and muscular) mass determines lower creatinine levels, one of the most important determinants of MELD score; due to the employment of creatinine instead of weight-adjusted glomerular filtration rate (GFR), the degree of renal dysfunction is likely in women is likely underestimated. Thus, MELD scores will be lower in women than in men with the same degree of renal compromise, which inevitably leads to a decreased access for women to LT[2]. Moreover, attempts at correcting creatinine-induced MELD bias against women by including estimated GFR have not improved discrimination for 3-mo mortality after enrolment for LT[7]. Likewise, the accuracy of MELD score in predicting 3- and 6-mo mortality in female LT candidates did not improve with the employment of the Modification of Diet in Renal Disease formula[8]. Providing that renal function assessment was adequately corrected for gender, a negative bias against women would still remain, since women are more likely than men to suffer from autoimmune liver diseases, including primary biliary cirrhosis, which are less likely than hepatitis C (HCV) to lead to kidney dysfunction and higher MELD scores[2].

Moreover, aside from the inaccuracy of MELD score in terms of renal dysfunction assessment in female patients, it is well known that patients with certain pathological conditions are poorly served by this score, including refractory ascites, refractory encephalopathy, recurrent cholangitis, and intractable pruritus in cholestatic diseases[9], the latter of which encompass mainly women[10-12]. Nevertheless, some of these conditions constitute symptom-based MELD exceptions and are awarded extra MELD points[13].

On the other hand, standard exclusions to MELD, which are more regularly applied, include the presence of hepatocellular carcinoma (HCC)[14-18], which is more common in men, further increasing the disparity of access to LT. After the implementation of the Milan Criteria[14], the number of LTs for HCC has increased worldwide and currently in Europe about 27% of all LT patients have HCC with countries peaking over 40%[19]. While exception points have greatly improved access to transplantation for HCC patients[20], recent studies suggest that the current point scheme inadvertently prioritizes HCC over patients without HCC diagnosis (non-HCC) by overestimating the presumed risk of tumor progression[21,22]. Even more vexing is the observation that even for equal MELD scores, women are at a disadvantage with respect to men in terms to LT access, suggesting that other factors must play a role in the gender disparity documented for LT rates[7,23].

GENDER DIFFERENCES IN INDICATIONS FOR LT

According to the OPTN records for LT performed in the US

between January 1, 1988 until December 31, 2013[24], a search for gender by diagnosis outlines several significant gender differences: significantly more women than men underwent LT for Wilson disease (410/47608 *vs* 326/78534, *P* < 0.0001), primary biliary cirrhosis (4796/47608 *vs* 809/78534, *P* < 0.0001), drug-induced acute hepatic necrosis (748/47608 *vs* 295/78534, *P* < 0.0001), Budd Chiari syndrome 441/47608 *vs* 233/78534), autoimmune cirrhosis 3025/47608 *vs* 959/78534, *P* < 0.0001), cryptogenic cirrhosis (4245/47608 *vs* 5009/78534, *P* < 0.0001), and non-alcoholic steatohepatitis (NASH) (1673/47608 *vs* 1875/78534, *P* < 0.0001).

On the contrary, significantly more men underwent LT for alcoholic cirrhosis (11195/78534 *vs* 3227/47608, *P* < 0.0001), alcoholic cirrhosis with HCV (4938/78534 *vs* 888/47608, *P* < 0.0001), HCC (2768/78534 *vs* 899/47608, *P* < 0.0001), HCC and cirrhosis (77555/78534 *vs* 2099/47608, *P* < 0.0001), HBsAg + Hepatitis B (2778/78534 *vs* 651/47608, *P* < 0.0001), and HCV (18187/78534 *vs* 8135/47608, *P* < 0.0001)[24].

Viral hepatitis

Several studies have demonstrated a differential effect of gender on the outcomes of patients infected with HCV, showing that in female patients, the natural history of HCV virus infection tends to be characterized by slower rates of progression to advanced liver disease, with better response rates to antiviral therapy[25-28]. Moreover, overall lower death rates for HCV-related liver disease as well as lower rates of HCC are observed in female patients[29].

Regarding menopausal course of HCV-related liver disease, however, recent studies have reported that the reduced estrogen levels that characterize this state may determine the accelerated progression to fibrosis and higher rates of no response to antiviral therapy observed in this subpopulation, especially in genotype 1 HCV-infected patients[30-32]; a statistically significant increase of tumor necrosis factor-α and interleukin-6 occur in menopause, and these proinflammatory cytokines have been associated to increased resistance to interferon-based therapy[33]. A higher SVR rate with Peg-IFNα-2b plus ribavirin *vs* IFNα-2a plus ribavirin has been documented in menopausal women, which likely corresponds the former's pharmacokinetic properties that allow the drug to reach visceral fat and oppose the increased cytokine production and enhanced inflammatory status in menopause[34].

Regarding hepatitis B (HBV), although significantly more men than women are transplanted for chronic HBV, LT for fulminant HBV is significantly more frequent in women[35]. As well, hepatitis E virus (HEV) is unfortunately associated with disproportionately high rates of fulminant hepatitis in pregnant women, particularly during the third trimester, with case-fatality rates in epidemics ranging from 0.2%-4% in the general population, *vs* 10%-25% in the pregnant population[36-38], possibly reflecting hormonal changes that increase susceptibility to a more aggressive course[39].

Non-alcoholic steatohepatitis

NASH has increased in frequency as indication for LT[40-42], and is bound to become one of the principal indications in many Western countries, with the increasing worldwide prevalence of this entity[43], and with the advent of new-acting direct antiviral agents, which will probably contribute to decreasing the percentage of HCV patients who necessitate LT.

In a study analyzing characteristics of patients referred for LT evaluation due to NASH (*n* = 71) from 1998 to 2008, and compared to the non-NASH possible candidates (*n* = 472)[44], it was found that patients with NASH were older (58.7 years *vs* 52.5 years, *P* < 0.0001) and more likely of female gender (50.7% *vs* 32.1%, *P* = 0.003). As expected, NASH patients were more likely to suffer from diabetes, hypertension, obesity, and cardiac disease (*P* < 0.05). Moreover, for paired MELD scores, NASH was associated with similar bilirubin levels (2.34 mg/dL *vs* 3.16 mg/dL; *P* = 0.11), but significantly increased creatinine values (1.26 mg/dL *vs* 0.98 mg/dL; *P* = 0.0018) and lower international normalized ratio (INR) values (1.14 *vs* 1.27; *P* = 0.04), in contrast with LT candidates without NASH, respectively. This suggests that NASH is associated with renal dysfunction, which is translated into greater priority, as established by the MELD calculus.

Thus, MELD score in this setting might not truly reflect liver dysfunction, but could be more directly related to features of the metabolic syndrome, including microvascular renal damage associated with diabetes and hypertension. Therefore, the disadvantage posed to women by creatinine's weight in the MELD calculus formula might be outweighed in the future, with increasing number of patients being transplanted for NASH, most of them being of female gender. However, the present state of the matter is yet far from this scenario, as only 5%-8% of LT are currently performed for this indication, and the time needed for MELD's disparity to be counterbalanced by this theoretical female gender benefit is expectedly long[2].

Putting together all these data is especially concerning, since women are generally more likely to have GFR < 60 mL/min per 1.73 m^2 previous to LT with respect to men, and the presence of this factor (OR = 3.28, *P* $\leqslant$ 0.001), aside from female gender (OR = 2.96, *P* < 0.001) and age (OR = 1.09, *P* < 0.001), has been demonstrated to be independently predictive of stage $\geqslant$ 3 chronic kidney disease (CKD) at 1 year post-LT[45]. In addition, this same study demonstrated that female gender (OR = 2.52, *P* = 0.004), age (OR = 1.05, *P* = 0.003) and NASH (OR = 2.95, *P* = 0.039) were independently predictive of $\geqslant$ stage 3 CKD at 5 years post-LT.

Considering, that NASH LT recipients are more frequently women, that women's renal dysfunction is not adequately accounted for by creatinine measurement and thus not well served by MELD score, together with the fact that women are more likely to have compromised renal function prior to transplant, and that this variable predicts advanced CKD after LT, it becomes clear that this population stands a particular risk and should be addressed more carefully.

Autoimmune hepatitis

Differences in sex-hormone (estrogen and androgen) modulation of the immune system may be responsible

for gender variations observed in autoimmune disorders; women have a significantly higher number of CD4+T lymphocytes and a higher CD4+/CD8+ ratio than men[46], secretion of interferon-γ (IFN-γ) and interleukin 10 (IL-10) are enhanced after the addition of estrogen in T-cell clones isolated from women[47], while androgens have been demonstrated to inhibit the secretion of IFN-γ, IL-4, and IL-5 in murine T cells[48].

Autoimmune hepatitis, characterized by progressive inflammatory destruction of the liver parenchyma associated with the presence of circulating autoantibodies, hypergammaglobulinemia and interface hepatitis on liver biopsy, is strongly preponderant in females (female/male ratio is 3.6/1)[49]. Although corticosteroid treatment tends to achieve transaminase normalization more frequently in female patients[50], women appear to have worse long-term survival than men[51].

Primary biliary cirrhosis

Primary biliary cirrhosis, a chronic cholestatic liver disease characterized by immune-mediated inflammatory destruction of the small intrahepatic bile ducts and fibrosis, affects predominantly women with respect to men, with incidence rates ranging from 3:1 to 22:1, with an average incidence rate in women of 10:1[52]. Gender differences also characterize the evolution of the disease: diagnosis of PBC is usually established at a younger age in women (51 years in women *vs* 62 years in men)[53]. Women are more likely to be symptomatic, and experience pruritus as a single symptom more often than males, while jaundice, jaundice with pruritus, and upper gastrointestinal bleeding are more frequently manifested in men[54]. Some symptoms such as severe daytime somnolence and depressive symptoms seem to affect men and women in an equal proportion, while autonomic symptoms seem to be more severe in women[55,56]. The presence of concomitant autoimmune disorders such as Sicca syndrome, overlap syndrome, and autoimmune hepatitis, also determining a more aggressive course and generally poorer response to therapy, is more frequent in women, especially in those of Hispanic origin, as has been recently demonstrated in a US cross-sectional study[57]. Development of hepatocellular carcinoma, however, seems to be more frequent in men[58]. Although PBC entails a high risk of postmenopausal osteoporosis, it seems to be more associated with the severity of chronic liver disease, rather than specifically the PBC etiology[59], and a recent Cochrane database systematic review reported that in female patients with cirrhosis, hormone replacement had no effect on all-cause mortality, fractures, liver-related mortality, liver transplantation, liver-related morbidity, serum bilirubin concentration nor lumbar spine bone mineral density. On the contrary, hormone replacement significantly increased the frequency of adverse events[60].

Wilson disease

Although this autosomic recessive disorder characterized by a wide spectrum of clinical manifestations should theoretically be present in females and males in equal proportion[61], a slight female predominance has been reported[62] partly reflecting the variable penetrance of genetic mutations that cause this disease. More significantly, however, neurological symptoms have been more frequently associated with female gender ($P = 0.051$) and with an acute, often fulminant course upon presentation when there is hepatic involvement ($P = 0.046$)[63]. In a French study analyzing medical records of 121 patients who underwent LT for Wilson Disease, male gender, pre-transplant renal insufficiency, non-elective procedure, and neurological indication for LT were significantly associated with poorer survival rate ($P = 0.04$) at univariate analysis. However, none of these factors remained statistically significant on multivariate analysis[64].

Alcohol

Alcohol has been demonstrated to exert a more deleterious effect in women and female animal models with respect to males[65], which can partly be explained by lower levels of gastric alcohol dehydrogenase in females, resulting in lower alcohol threshold for women[66]. Moreover, acute liver injury develops more rapidly and more extensively in women than in men even for a smaller quantity consumed[67]. Ethanol has been demonstrated to increase TNF-α mRNA expression and cause more severe acute liver injury in females[68]. Interestingly, estrogens have a major influence on Kupffer cell reactivity and proinflammatory cytokine production, and this could constitute a major determinant of women's increased risk of alcohol-induced liver disease[69].

Drug-induced liver injury and gender

Different patterns of drug-induced liver damage between males and females have been recognized both in humans[70] as well as in animal models[71]. It has been reported that overall, women have a 1.5- to 1.7-fold greater risk of developing adverse drug reactions than men[72], and a prospective, multicenter study based on intensive pharmacovigilance confirmed a higher risk of acute adverse drug reactions in women *vs* men[73]. Excluding behavioural or dosing differences, there are three main hypotheses regarding the mechanisms behind these differences, including: (1) different pharmacokinetics between females and males; (2) gender-specific hormonal effects or interaction with signalling molecules that may affect drug safety; and (3) differences in aberrant immune response that targets the liver following drug exposure that can result in adverse drug reactions[70]. Gender-based differences that may have an impact on drug pharmacokinetics and subsequent toxicity include differences in gastrointestinal blood flow, gastric acid secretion, relative amount of circulating drug-binding proteins, relative proportions of muscular and adipose tissue, renal blood flow, gender-specific expression of cytochrome P450 (CYP450) isozymes, as well as physiologic and hormonal changes during the menstrual cycle, during pregnancy and after menopause[74].

A study based on World Health Organization-endorsed VigiBase™, the largest and most comprehensive database

on global "Individual Case Safety Reports", analyzed gender and age differences in reporting of drug-induced hepatic failure for a 10-year period (2000-2009). From a total of 6370 reports from 38 countries, and excluding missing gender data in 379 cases, females accounted for 54.03% of cases. The largest proportion of hepatic failure cases corresponded to patients younger than 55 years (42.57%), with a female predominance (56.81%), whereas gender was almost evenly distributed in the group above 55 years of age. Regarding drug types, there was a significant female preponderance in hepatic failure associated with analgesics, antiepileptics, anti-inflammatory and antirrheumatic agents, antidiabetics, and antibacterials for systemic use, whereas males were significantly overrepresented in hepatic failure cases associated with antivirals[75].

Female gender is more frequently associated with paracetamol overdose, which fortunately only in a fraction of patients leads to acute liver injury and acute liver failure; in a study from Iceland analyzing 1913 drug-related poisoning episodes, of which 352 involved paracetamol overdoses, the female/male ratio was 3.0, and the principal age group was 16-25 years. However, amongst those who required hospitalization, 16% were accidental overdoses and there were no gender differences[76].

HCC

In spite of the striking preponderance of male sex amongst patients with HCC, probably estrogens play a very important role in liver carcinogenesis[77] and wild-type *vs* variant estrogen receptors in the liver accurately predict survival in patients with HCC[78]. If transplant centers maintain the adopted trend of allocating nearly 17%-40% of organs to patients who have HCC[19,79], women, whom are listed for LT less frequently for this indication, will have a reduced access to LT with respect to men, since while men will have theoretically 100% of organs available, women will have to "compete" against men for the remaining organs allocated to non-HCC indications for LT.

Notwithstanding the fact that HCC affects men more frequently, and that previous database studies had found gender disparities favouring men in rates of LT in cohorts of HCC patients only, a recent retrospective US database analysis spanning 10 years and over 40000 patients[80] demonstrated that women with HCC present less often with decompensated liver disease (OR = 0.79, $P < 0.001$), and are more likely to receive invasive HCC treatment, with significantly higher rates of resection across different ethnicities and diagnoses (OR = 1.34 and 1.44, $P < 0.001$). In this study, univariate analysis showed that although women have lower unadjusted rates of LT, disparity resolves after controlling for other clinical and demographic factors[80].

ISSUES OF SIZE AND GENDER IN DONOR-RECIPIENT MATCHING

Liver donor size mismatch has been proposed as partially accountable for the disparity between LT rates between

male and female patients[2]. A large study based on the OPTN demonstrated that, controlling for region and blood type, women were 25% less likely to undergo LT in a given month in comparison with men ($P < 0.001$). Including gender within the model increased the OR for this variable to 0.84. Of this 25%, 9% was found to be attributable to MELD score. Stemming from this study, an additional 3% increase in the OR for gender (0.87, $P < 0.001$) is imputable to estimated liver volume (mean estimated liver volume was significantly lower for female patients than for male patients on the LT waiting list, $P < 0.001$), therefore partly explaining gender disparity in LT rates[81]. Henceforth, even after accounting for MELD score and estimated liver size, approximately half of the 25% gender disparity remains unexplained.

In fact, other relevant factors related to survival on the waiting list for LT, such as the metabolic and nutritional status, are not accounted for by the MELD score. Notwithstanding the fact that in general women are characterized by less muscle mass than men, this difference is furthermore often not evaluated nor compensated for with adequate formulas[82]. The standardized triceps skinfold thickness and mid-arm muscular circumference determinations, which are more adequate for evaluation of nutritional status than body mass index in patients with ascites, were found to be lower in female patients[83]. Moreover, in a recent study analyzing pretransplant muscle mass on more than 300 LT recipients, of whom 68% could have been defined as cachectic, in female patients, muscle mass predicted intensive care unit stay, total length of stay, and days of intubation, but did not predict survival after LT (mean follow-up of 2.8 years)[84].

The impact of gender mismatch between donors and recipients on the outcome of LT is still a matter of debate, and may differ amongst deceased-donor LT (DDLT), living-donor LT (LDLT), and pediatric LDLT. Lehner *et al*[85] reported that gender mismatch does not play a role in the outcome of LT. On the contrary, some studies have reported on the negative impact of gender mismatch on graft failure, specifically regarding male recipients who receive grafts from female donors in DDLT[86-89]. Furthermore, a recently published prospective study analyzing outcomes of 1042 LT recipients demonstrated that graft survival in patients who received an organ matched for their gender was better than those receiving a gender mismatch ($P = 0.047$), and the worst combination was female-to-male LT ($P < 0.001$)[90].

Regarding LDLT, a male recipient receiving a graft from a female donor was shown to be an independent risk factor for recipient mortality in adults[91], while in pediatric LDLT, an interesting finding has been that recipients of maternal grafts have reportedly lower rates of graft failure and refractory rejection in contrast with recipients of paternal grafts[92]. In the specific setting of HCV infection, no difference has been observed in terms of graft nor patient survival according to donor-recipient gender matching[93].

Being smaller, female patients have a limited access to the pool of available organs, and may have to wait longer for organs of an appropriate size, since livers from

pediatric donors are preferentially allocated to children awaiting LT. Further increasing this disparity is the fact that a small organ may be adequate for a large individual, but the contrary is not always possible[2].

Interestingly, a Japanese study analyzing 114 LDLT using parental grafts performed for recipients with biliary atresia demonstrated that gender mismatch alone was an independent risk factor for acute cellular rejection ($P = 0.012$), and paternal grafts with gender mismatch were associated with a higher incidence of acute cellular rejection with respect to maternal grafts with gender match ($P = 0.002$)[94]. The authors infer that maternal antigens may have an important clinical impact on graft tolerance in LDLT, which is in line with what was first hypothesized by Starzl *et al*[95] regarding induction of tolerance by microchimerism, and what has been demonstrated regarding non-inherited maternal antigens and maternal microchimerism in blood and various organs[96,97]. Exposure to maternal antigens, in fact, may have tolerogenic effects on offspring, resulting in acceptance or rejection of allografts expressing the maternal antigens[98], although a functional linkage between microchimerism and tolerance has been difficult to establish[99,100].

Another factor that might play a relevant role in gender-matching is the different hormonal array regarding estrogens (and their receptors). Female-to-female matched LT have been associated with a decreased risk of graft failure with respect to male-male matched transplants, but only for non-HCV female recipients[86]. In animal models, a greater degree of hepatic lactic acidosis during warm ischemia has been demonstrated to occur in females with respect to males[101], which may provide a potential metabolic explanation for the worse outcome in recipients of female donors. However, the matter entails complex aspects that have not yet been fully understood, and this is reflected by the disparity in reports on the role of estrogens in ischemia-reperfusion[102-105]. Apparently, females are more susceptible to hepatic reperfusion injury, but experimental data in the mouse model have shown that estrogens actually reduce ischemia/reperfusion damage[106]. The mechanisms for sex differences in the liver's metabolic response to ischemia do seem, however, to be estrogen-mediated, even in the presence of male hormones[107].

However, again, not all of these differences may be attributable to hormone status solely, but may actually represent an immunological basis. Late-presenting nonanastomotic biliary strictures after LT have been reported to occur more frequently in female-male gender donor-recipient matches, as well as in patients transplanted for primary sclerosing cholangitis, and in patients in whom Roux-en-Y bile duct reconstructions were performed[108], and while ischemia and preservation factors seem to play a preponderant role in early-presenting non-anastomotic biliary strictures, immunological factors are the predominant factor in late-presenting non-anastomotic biliary structures. Interestingly, the fact that immunological processes are implied, does not rule out the fact that still poorly understood linkages between hormones, hormonal receptor, and immunological mechanisms exist.

OUTCOMES AFTER LIVER TRANSPLANTATION IN FEMALE RECIPIENTS

Overall outcomes after LT, especially in the long-term, are reportedly better in women[24] with respect to men. A 20-year follow-up study of 313 LT recipients revealed that, together with primary indication ($P < 0.001$), age ($P < 0.001$), impaired renal function at 6 mo ($P < 0.001$) and retransplantation ($P = 0.034$), gender ($P = 0.017$) had a significant impact on patient survival[109]. The reported protective effect of female gender in the development of metabolic complications related to hyperglycemia[109] has been confirmed in other series as well; a study based on the OPTN/United Network Sharing (UNOS) database including 19582 DDLT non-diabetic recipients (in whom the incidence of new-onset diabetes after transplantation (NODAT) has been established to be greater with respect to LDLT recipients), demonstrated that male sex was a predictor for NODAT, while this was not the case for LDLT recipients[110].

After LT, de novo NASH or non-alcoholic fatty liver disease (NAFLD) reportedly develop in 20% and 10% of cases, respectively[92], while approximately 50% of patients transplanted for NASH will experience recurrence[90], with 5% to 10% of patients progressing to cirrhosis[91]. Importantly, menopausal status, which is associated with weight gain and increased central fat mass[111], constitutes a risk factor for developing NASH and metabolic syndrome; in a long-term observational study spanning 12 years, metabolic syndrome was a significant risk factor for mortality in postmenopausal women compared to men and premenopausal women[93].

Regarding renal function, as mentioned above, in a recent study, female gender was found to be an independent and significant predictor of advanced stages of CKD at 1 year post-LT (OR = 2.96, $P < 0.001$) and at 5 years post-LT (OR = 2.52, $P = 0.004$)[45], and results from the MOST study had revealed that 1-year GFR is significantly affected both by HCV infection and recipient female gender ($P < 0.01$ for both)[112].

The impact of gender on outcomes after LT varies according to the indication for LT. Along with recurrent HCC ($P < 0.001$) and retransplantation ($P = 0.01$), female gender ($P = 0.002$) has been significantly associated with worse survival after LT for Hepatitis B, as shown in a multicener US study pooling 738 LT recipients[113]. Concerning HCV, post-LT recurrence is nearly universal[114-116], and female gender has been described as a risk factor for severe HCV recurrence and graft lost after LT, and the risk increases with increasing donor age[86,117,118]. The important fibrosis suppression effect of estrogens demonstrated experimentally in animal models[119,120] is reflected in the clinically slower fibrosis progression observed in women with respect to men in chronic HCV[121,122]. However, most LT female recipients are post-menopausal, and the lower estrogenic levels associated with this state have been clinically associated with higher degrees of fibrosis[30,123]. Although in immune-

competent HCV-infected women menopause is per se frequently associated with steatosis, which is an important cofactor for disease progression[118,124], another hypothesis is that women who require LT are the ones with genetic, virological and immunological factors that determine a more severe course of HCV-related disease, leading to LT, which in turn progresses more rapidly after LT[117]. Moreover, female gender has been shown to be an independent negative prognostic factor for the outcome of HCV antiviral therapy after LT[125]. Although male and female patients did not differ in HCV viral load, histology, or rate of diabetes at baseline, SVR was significantly lower in females than in males (29.5% *vs* 42.1%; *P* = 0.03). Partly explaining this unfavorable response rate, the authors found that compliance to therapy was also significantly lower in women with respect to men (43.4% *vs* 23.8%; *P* = 0.001), and that anemia was the main reason for lower adherence. On multivariate analysis, female gender (*P* < 0.04), early virological response (*P* < 0.0001), and adherence to therapy (*P* < 0.0001) were independent predictors for SVR[126].

SPECIAL ISSUES REGARDING LIVER TRANSPLANTATION AND GENDER

Bone metabolism

Immunosuppressive medication is a major contributor to osteoporosis in the post transplant period[127,128], and post-menopausal women are at higher risk for developing osteoporosis compared to women in the fertile age, as a consequence of decreased serum estrogen levels[129]. The predominant deleterious effects of steroids on bone metabolism include reduced bone formation by decreasing osteoblast replication and differentiation, and increased apoptosis[130,131]. Among calcineurin inhibitors, cyclosporine has increase bone turnover[132], whereas tacrolimus may cause less bone loss[133,134]. A prospective study evaluated 23 women who underwent LT, of whom 13% were peri-menopausal and 56.5% were post-menopausal, finding that in peri- and post-menopausal women, an inferior bone mass was observed in 81.2% of patients: of whom 50% diagnosed with having low bone mass and 31.2% with osteoporosis. Moreover, the postmenopausal stage was significantly associated with a decreased bone mass (*P* < 0.0001)[135].

Risk of de novo malignancy

Aside from the risks concerning bone disease, immunosuppression increases the probability of de novo tumors[136-138]; in a multicentric Italian study showed that the risk for some types of tumors was particularly and significantly higher in women, specifically carcinomas of tongue, all tumors of the oral cavity, and head/neck cancers[139]. In contrast, a smaller study analyzing predictors of de novo malignancies in 534 LT recipients, did not find gender to play a role[140].

Sexual life, fertility and pregnancy

Reproductive function is often severely compromised in women with advanced liver disease, and is frequently characterized by menstrual irregularity, amenorrhea, and infertility in nearly half of patients[141,142]. Etiologies of chronic liver disease which more frequently affect female patients, such as autoimmune hepatitis, may worsen during the course of pregnancy, as most diseases of autoimmune origin, with flares of disease activity reported in 7%-21% and 11%-86% of women during the gestational period and during the post-partum period, respectively[141,143-146]. Although maternal outcomes are generally favorable, pregnancy has been reportedly the trigger for hepatic decompensation (leading to LT in some cases) and maternal death (including liver-related death), with fetal outcomes which are lower than those of the general population, but comparable to those of other autoimmune diseases[141,143-147]. In the study by Westbrook and collaborators[147], of 81 pregnancies in 53 autoimmune hepatitis patients, 41% took place in the context of cirrhosis, and live birth rate was significantly lower within this category. Furthermore, a serious maternal adverse event (death or need for LT) during or within 12-mo of delivery, or hepatic decompensation during or within 3-mo of delivery, occurred with 9 pregnancies (11%) and was more common in women with cirrhosis (*P* = 0.028), and patients who experienced a flare in association with pregnancy were more likely to develop hepatic decompensation (*P* = 0.01)[147]. As flares are more frequent in patients who are not on therapy or who have had a disease flare in the year prior to conception and, pre-conception counselling and adequate gestational management are paramount.

In general, an elevated percentage of women are sexually active after LT[148,149]. Approximately 70% of transplant recipients in a study from Brazil were reportedly sexually active after a median of 36 mo after successful LT[150], whereas decreased libido and difficulty to reach orgasm with intercourse has been described in 26% of female LT recipients[151]. Successful LT restores menstrual function in 97% of female patients, as well as childbearing potential[152-154]. In general, LT leads to partial or complete normalization of both levels of sex hormones and sexual function within several months of LT[155], with nearly 48% of women in their fertile age experiencing regular menses, 26% irregular bleeding, and 26% amenorrhea[153], while more than 60% of peri-menopausal women reportedly experience a higher frequency of menstrual pattern disorders[156]. In the United States only, approximately 14000 women of childbearing age are currently LT recipients, and another 500 women will undergo LT annually[24]. The optimal timing of conception is still a matter of debate, but waiting at least 1 year after LT is generally recommended[157]. Regarding immunosuppression, calcineurin inhibitors and steroids can be used safely, while azathioprine and mycophenolate mofetil have been associated with increased toxic effects[158]. Pregnancy outcomes after LT are acceptable in terms of the health of the mother and of the newborn[159], and reportedly better in comparison to those obtained after kidney transplantation, with significantly lower rates of hypertension, preeclampsia, preterm

Table 1 Key points

Several factors contribute to the unequal access to liver transplantation that penalizes women, including inadequacy of MELD score in accounting for renal dysfunction in females, the limitation of MELD score in reflecting the actual severity of liver disease and associated complications in certain clinical conditions that are more frequent in women, and the centers' increasing prevalence of policies that favor transplantation for hepatocellular carcinoma, which is more frequent in males

Different etiologies of liver disease follow a characteristic pattern of gender-related frequency, natural evolution, and response to treatment, partly owing to socioepidemiological factors as well as to phenotypical differences regarding enzymatic activity and hormonal status

Within the female population, a clear difference exists between the pre- and the post-menopausal stages, and after this turning point, the protective effect of estrogens on slowing fibrosis progression, amongst others, is lost, causing an acceleration of hepatic injury, a detrimental response to therapy, and the potential establishment of a new set of complications associated with altered fat and bone metabolism

Although long-term overall outcomes after liver transplantation are better in women, certain conditions such as renal dysfunction, hepatocellular carcinoma as an indication for transplant and recurrent hepatitis C infection are associated with worse prognosis in women with respect to men

In spite fertility and sexual activity may be curbed in advanced cirrhosis, there are numerous reports of unaffected pregnancies in this stage, while successful liver transplantation restores fertility and sexual activity in most patients, with pregnancy outcomes which are reportedly better in comparison to those obtained after kidney transplantation

MELD: Model for end-stage liver disease.

deliveries, and birth of neonates small for their gestational age[160].

In a study from Vienna assessing 39 deliveries and 40 live births[161], the mean time from organ transplantation to delivery was 67.6 ± 47.2 mo. A meta-analysis on 450 pregnancies in 306 LT recipients showed that although the rates of pre-eclampsia (21.9%), caesarean section delivery (44.6%), and preterm delivery (39.4%) were higher than the rates for the US general population (3.8%, 31.9%, and 12.5%, respectively), the post-LT live birth rate (76.9%) was higher than the live birth rate for the US general population (66.7%), and the post-LT miscarriage rate (15.6%) was lower than the miscarriage rate for the general population (17.1%)[162].

Quality of life after liver transplantation

In a German cross-sectional, single-center study evaluating the quality of life in 281 LT recipients[163], similar results were observed between male and female subjects, whereas in another study analyzing gender differences after HCV-related LT, however, it emerged that male subjects score significantly higher on physical role functioning and physical activity compared with females, whereas women had reportedly better quality of life compared to males with regard to the emotional state and mental health 1-year after LT[164].

CONCLUSION

Important gender differences exist regarding etiologies of liver disease, severity of the course of these diseases, and on outcomes after LT. Unfortunately, access to LT is still governed by an imperfect allocation system, currently based on MELD score, which includes systematic biases against women, and is also hampered by factors that are not adequately taken into account by MELD score, doubly penalizing female gender. A delayed access to LT wait-listing and subsequently to LT due to renal dysfunction underestimation, is a determinant factor that has an impact on post-transplant renal function as well. Being generally smaller than men, organ allocation decisions generally favor children as recipients of small organs, and men as recipients of large organs, conditioning a longer waiting time for an organ in adult women.

Throughout a women's life, profound hormonal changes also determine the natural course of diseases; while estrogens may protect against inflammation and fibrosis during the fertile age, the post status takes a high toll on disease progression both before and after LT, and may be further complicated by obesity, NASH, NAFLD, and other components of the metabolic syndrome. The above are summarized in Table 1 (Key points). It is therefore ever clearer that special attention should be paid to the integral management of women during the different life periods, and with respect to special situations regarding natural evolution and risk factors for liver disease, as well as to those affecting post-transplant outcome.

Kryssia I Rodríguez-Castro, Padua, Italy; Caterina Sagnelli, Salerno, Italy; Teresa Santantonio, Foggia, Italy; Giada Sebastiani, Venice, Italy; Antonella Smedile, Turin, Italy; Gloria Taliani, Rome, Italy; Carmen Vandelli, Modena, Italy; Ranka Vukotic, Modena, Italy; Anna Linda Zignego, Florence, Italy.

REFERENCES

1   **Bryce CL**, Angus DC, Arnold RM, Chang CC, Farrell MH, Manzarbeitia C, Marino IR, Roberts MS. Sociodemographic differences in early access to liver transplantation services. *Am J Transplant* 2009; **9**: 2092-2101 [PMID: 19645706 DOI: 10.1111/j.1600-6143.2009.02737.x]

2   **Axelrod DA**, Pomfret EA. Race and sex disparities in liver transplantation: progress toward achieving equal access? *JAMA* 2008; **300**: 2425-2426 [PMID: 19033595 DOI: 10.1001/jama.2008.732]

3   **Klassen AC**, Klassen DK, Brookmeyer R, Frank RG, Marconi K. Factors influencing waiting time and successful receipt of cadaveric liver transplant in the United States, 1990 to 1992. *Med Care* 1998; **36**: 281-294 [PMID: 9520954]

4   **Moylan CA**, Brady CW, Johnson JL, Smith AD, Tuttle-Newhall JE, Muir AJ. Disparities in liver transplantation before and after introduction of the MELD score. *JAMA* 2008; **300**: 2371-2378 [PMID: 19033587 DOI: 10.1001/jama.2008.720]

5   **Mathur AK**, Schaubel DE, Gong Q, Guidinger MK, Merion RM. Sex-based disparities in liver transplant rates in the United States. *Am J Transplant* 2011; **11**: 1435-1443 [PMID: 21718440 DOI: 10.1111/j.1600-6143.2011.03498.x]

6   **Fink MA**, Berry SR, Gow PJ, Angus PW, Wang BZ, Muralidharan V, Christophi C, Jones RM. Risk factors for liver transplantation waiting list mortality. *J Gastroenterol Hepatol* 2007; **22**: 119-124 [PMID: 17201891 DOI: 10.1111/j.1440-1746.2006.04422.x]

7   **Myers RP**, Shaheen AA, Aspinall AI, Quinn RR, Burak KW. Gender, renal function, and outcomes on the liver transplant waiting list: assessment of revised MELD including estimated glomerular filtration rate. *J Hepatol* 2011; **54**: 462-470 [PMID: 21109324 DOI: 10.1016/j.jhep.2010.07.015]

8   **Mariante-Neto G**, Marroni CP, Fleck Junior AM, Marroni CA, Zanotelli ML, Cantisani G, Brandão AB. Impact of creatinine values on MELD scores in male and female candidates for liver transplantation. *Ann Hepatol* 2013; **12**: 434-439 [PMID: 23619260]

9   **Wiesner RH**. Patient selection in an era of donor liver shortage: current US policy. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 24-30 [PMID: 16265097 DOI: 10.1038/ncpgasthep0070]

10 **Leuschner U**. Primary biliary cirrhosis--presentation and diagnosis. *Clin Liver Dis* 2003; **7**: 741-758 [PMID: 14594129]

11 **Mela M**, Mancuso A, Burroughs AK. Review article: pruritus in cholestatic and other liver diseases. *Aliment Pharmacol Ther* 2003; **17**: 857-870 [PMID: 12656688]

12 **Levy C**, Lindor KD. Management of Primary Biliary Cirrhosis. *Curr Treat Options Gastroenterol* 2003; **6**: 493-498 [PMID: 14585238]

13 **Freeman RB**, Wiesner RH, Harper A, McDiarmid SV, Lake J, Edwards E, Merion R, Wolfe R, Turcotte J, Teperman L. The new liver allocation system: moving toward evidence-based transplantation policy. *Liver Transpl* 2002; **8**: 851-858 [PMID: 12200791 DOI: 10.1053/jlts.2002.35927]

14 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

15 **Figueras J**, Jaurrieta E, Valls C, Ramos E, Serrano T, Rafecas A, Fabregat J, Torras J. Resection or transplantation for hepatocellular carcinoma in cirrhotic patients: outcomes based on indicated treatment strategy. *J Am Coll Surg* 2000; **190**: 580-587 [PMID: 10801025]

16 **Pomfret EA**, Washburn K, Wald C, Nalesnik MA, Douglas D, Russo M, Roberts J, Reich DJ, Schwartz ME, Mieles L, Lee FT, Florman S, Yao F, Harper A, Edwards E, Freeman R, Lake J. Report of a national conference on liver allocation in patients with hepatocellular carcinoma in the United States. *Liver Transpl* 2010; **16**: 262-278 [PMID: 20209641 DOI: 10.1002/lt.21999]

17 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]

18 **Liver allocation policy**. Last accessed March 2014. Available from: URL: http: //optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/ policy_8.pdf. 2012

19 **Adam R**, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]

20 **Sharma P**, Balan V, Hernandez JL, Harper AM, Edwards EB, Rodriguez-Luna H, Byrne T, Vargas HE, Mulligan D, Rakela J, Wiesner RH. Liver transplantation for hepatocellular carcinoma: the MELD impact. *Liver Transpl* 2004; **10**: 36-41 [PMID: 14755775 DOI: 10.1002/lt.20012]

21 **Washburn K**, Edwards E, Harper A, Freeman R. Hepatocellular carcinoma patients are advantaged in the current liver transplant allocation system. *Am J Transplant* 2010; **10**: 1643-1648 [PMID: 20486906 DOI: 10.1111/j.1600-6143.2010.03127.x]

22 **Volk ML**. Unfair priority for HCC: A problem whose ideal solution remains unsolved. *Am J Transplant* 2010; **10**: 1507-1508 [PMID: 20642675 DOI: 10.1111/j.1600-6143.2010.03154.x]

23 **Mindikoglu AL**, Regev A, Seliger SL, Magder LS. Gender disparity in liver transplant waiting-list mortality: the importance of kidney function. *Liver Transpl* 2010; **16**: 1147-1157 [PMID: 20879013 DOI: 10.1002/lt.22121]

24 **Liver transplantation between 1988 and 2013 in the United States.** Last accessed February 2014. Available from: URL: http: //optn.transplan.hrsa.gov/latestData/rptData.asp

25 **Barrett S**, Goh J, Coughlan B, Ryan E, Stewart S, Cockram A, O'Keane JC, Crowe J. The natural course of hepatitis C virus infection after 22 years in a unique homogenous cohort: spontaneous viral clearance and chronic HCV infection. *Gut* 2001; **49**: 423-430 [PMID: 11511566]

26 **Pagliaro L**, Peri V, Linea C, Cammà C, Giunta M, Magrin S. Natural history of chronic hepatitis C. *Ital J Gastroenterol Hepatol* 1999; **31**: 28-44 [PMID: 10091101]

27 **Burra P**, De Martin E, Gitto S, Villa E. Influence of age and gender before and after liver transplantation. *Liver Transpl* 2013; **19**: 122-134 [PMID: 23172830 DOI: 10.1002/lt.23574]

28 **De Martin E**, Rodriguez-Castro K, Gambato M, Villa E, Burra P. (2014) Women after Liver Transplantation. *Organs, Tissues and Cells* 2014; In press

29 **Leone N**, Rizzetto M. Natural history of hepatitis C virus infection: from chronic hepatitis to cirrhosis, to hepatocellular carcinoma. *Minerva Gastroenterol Dietol* 2005; **51**: 31-46 [PMID: 15756144]

30 **Di Martino V**, Lebray P, Myers RP, Pannier E, Paradis V, Charlotte F, Moussalli J, Thabut D, Buffet C, Poynard T. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. *Hepatology* 2004; **40**: 1426-1433 [PMID: 15565616 DOI: 10.1002/hep.20463]

31 **Villa E**, Karampatou A, Cammà C, Di Leo A, Luongo M, Ferrari A, Petta S, Losi L, Taliani G, Trande P, Lei B, Graziosi A, Bernabucci V, Critelli R, Pazienza P, Rendina M, Antonelli

A, Francavilla A. Early menopause is associated with lack of response to antiviral therapy in women with chronic hepatitis C. *Gastroenterology* 2011; **140**: 818-829 [PMID: 21167831 DOI: 10.1053/j.gastro.2010.12.027]

32 **Villa E**, Vukotic R, Cammà C, Petta S, Di Leo A, Gitto S, Turola E, Karampatou A, Losi L, Bernabucci V, Cenci A, Tagliavini S, Baraldi E, De Maria N, Gelmini R, Bertolini E, Rendina M, Francavilla A. Reproductive status is associated with the severity of fibrosis in women with hepatitis C. *PLoS One* 2012; **7**: e44624 [PMID: 22970270 DOI: 10.1371/journal.pone.0044624]

33 **Neuman MG**, Benhamou JP, Malkiewicz IM, Akremi R, Shear NH, Asselah T, Ibrahim A, Boyer N, Martinot-Peignoux M, Jacobson-Brown P, Katz GG, Le Breton V, Le Guludec G, Suneja A, Marcellin P. Cytokines as predictors for sustained response and as markers for immunomodulation in patients with chronic hepatitis C. *Clin Biochem* 2001; **34**: 173-182 [PMID: 11408014]

34 **Villa E**, Cammà C, Di Leo A, Karampatou A, Enea M, Gitto S, Bernabucci V, Losi L, De Maria N, Lei B, Ferrari A, Vukotic R, Vignoli P, Rendina M, Francavilla A. Peginterferon-A_2B plus ribavirin is more effective than peginterferon-A_2A plus ribavirin in menopausal women with chronic hepatitis C. *J Viral Hepat* 2012; **19**: 640-649 [PMID: 22863268 DOI: 10.1111/j.1365-2893.2012.01593.x]

35 **Rifai K**, Hadem J, Wiegand J, Potthoff A, Pischke S, Klempnauer J, Strassburg C, Wedemeyer H, Manns MP, Tillmann HL. Gender differences in patient receiving liver transplantation for viral hepatitis. *Z Gastroenterol* 2012; **50**: 760-765 [PMID: 22895904 DOI: 10.1055/s-0031-1281631]

36 **Khuroo MS**, Teli MR, Skidmore S, Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *Am J Med* 1981; **70**: 252-255 [PMID: 6781338]

37 **Ramalingaswami V**, Purcell RH. Waterborne non-A, non-B hepatitis. *Lancet* 1988; **1**: 571-573 [PMID: 2894501]

38 **Tsega E**, Krawczynski K, Hansson BG, Nordenfelt E. Hepatitis E virus infection in pregnancy in Ethiopia. *Ethiop Med J* 1993; **31**: 173-181 [PMID: 8404882]

39 **Mateos Lindemann ML**, Morales JG, Fernández-Barredo S, Domínguez MR, García de la Hoz F, Halfon P, Pérez Gracia MT. Fulminant hepatitis E in a woman taking oral contraceptive medication. *Am J Trop Med Hyg* 2010; **82**: 12-15 [PMID: 20064988 DOI: 10.4269/ajtmh.2010.09-0436]

40 **United Network for Organ Sharing**. Last accessed February 2014. Available from: URL: http://optn.transplant.hrsa.gov/latestData/rptData.asp.2014

41 **European Liver Transplant Registry.** Last accessed February 2014. Available from: URL: http://www.eltr.org/spip.php?article161

42 **Charlton M**. Nonalcoholic fatty liver disease: a review of current understanding and future impact. *Clin Gastroenterol Hepatol* 2004; **2**: 1048-1058 [PMID: 15625647]

43 **Loomba R**, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 686-690 [PMID: 24042449 DOI: 10.1038/nrgastro.2013.171]

44 **Park CW**, Tsai NT, Wong LL. Implications of worse renal dysfunction and medical comorbidities in patients with NASH undergoing liver transplant evaluation: impact on MELD and more. *Clin Transplant* 2011; **25**: E606-E611 [PMID: 21958082 DOI: 10.1111/j.1399-0012.2011.01497.x]

45 **Fussner LA,** Charlton MR, Heimbach JK, Fan C, Dierkhising R, Coss E, Watt KD. The impact of gender and NASH on chronic kidney disease before and after liver transplantation. *Liver Int* 2013; **34**: 1259-1266 [PMID: 24262002]

46 **Amadori A**, Zamarchi R, De Silvestro G, Forza G, Cavatton G, Danieli GA, Clementi M, Chieco-Bianchi L. Genetic control of the CD4/CD8 T-cell ratio in humans. *Nat Med* 1995; **1**: 1279-1283 [PMID: 7489409]

47 **Gilmore W**, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 1997; **158**: 446-451 [PMID: 8977221]

48 **Araneo BA**, Dowell T, Diegel M, Daynes RA. Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and gamma-interferon, but not IL-2 by activated murine T cells. *Blood* 1991; **78**: 688-699 [PMID: 1830499]

49 **Manns MP**, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]

50 **Miyake Y**, Iwasaki Y, Sakaguchi K, Shiratori Y. Clinical features of Japanese male patients with type 1 autoimmune hepatitis. *Aliment Pharmacol Ther* 2006; **24**: 519-523 [PMID: 16886918 DOI: 10.1111/j.1365-2036.2006.03013.x]

51 **Al-Chalabi T**, Underhill JA, Portmann BC, McFarlane IG, Heneghan MA. Impact of gender on the long-term outcome and survival of patients with autoimmune hepatitis. *J Hepatol* 2008; **48**: 140-147 [PMID: 18023911 DOI: 10.1016/j.jhep.2007.08.013]

52 **Nalbandian G**, Van de Water J, Gish R, Manns M, Coppel RL, Rudich SM, Prindiville T, Gershwin ME. Is there a serological difference between men and women with primary biliary cirrhosis? *Am J Gastroenterol* 1999; **94**: 2482-2486 [PMID: 10484012 DOI: 10.1111/j.1572-0241.1999.01380.x]

53 **Kim WR**, Lindor KD, Locke GR, Therneau TM, Homburger HA, Batts KP, Yawn BP, Petz JL, Melton LJ, Dickson ER. Epidemiology and natural history of primary biliary cirrhosis in a US community. *Gastroenterology* 2000; **119**: 1631-1636 [PMID: 11113084]

54 **Rubel LR**, Rabin L, Seeff LB, Licht H, Cuccherini BA. Does primary biliary cirrhosis in men differ from primary biliary cirrhosis in women? *Hepatology* 1984; **4**: 671-677 [PMID: 6745856]

55 **Newton JL**, Davidson A, Kerr S, Bhala N, Pairman J, Burt J, Jones DE. Autonomic dysfunction in primary biliary cirrhosis correlates with fatigue severity. *Eur J Gastroenterol Hepatol* 2007; **19**: 125-132 [PMID: 17272997 DOI: 10.1097/01.meg.0000252629.96043.67]

56 **Newton JL**, Hudson M, Tachtatzis P, Sutcliffe K, Pairman J, Burt JA, Jones DE. Population prevalence and symptom associations of autonomic dysfunction in primary biliary cirrhosis. *Hepatology* 2007; **45**: 1496-1505 [PMID: 17538969 DOI: 10.1002/hep.21609]

57 **Levy C**, Naik J, Giordano C, Mandalia A, O'Brien C, Bhamidimarri KR, Schiff ER, Martin P. Hispanics with primary biliary cirrhosis are more likely to have features of autoimmune hepatitis and reduced response to ursodeoxycholic acid than non-Hispanics. *Clin Gastroenterol Hepatol* 2014; **12**: 1398-1405 [PMID: 24361417 DOI: 10.1016/j.cgh.2013.12.010]

58 **Lucey MR**, Neuberger JM, Williams R. Primary biliary cirrhosis in men. *Gut* 1986; **27**: 1373-1376 [PMID: 3792920]

59 **Floreani A**, Mega A, Camozzi V, Baldo V, Plebani M, Burra P, Luisetto G. Is osteoporosis a peculiar association with primary biliary cirrhosis? *World J Gastroenterol* 2005; **11**: 5347-5350 [PMID: 16149144]

60 **Rudic JS**, Poropat G, Krstic MN, Bjelakovic G, Gluud C. Hormone replacement for osteoporosis in women with primary biliary cirrhosis. *Cochrane Database Syst Rev* 2011; (**12**): CD009146 [PMID: 22161447 DOI: 10.1002/14651858.CD009146.pub2]

61 **Scheinberg IH**. Wilson's disease. *J Rheumatol Suppl* 1981; **7**: 90-93 [PMID: 6939890]

62 **Rodrigo Agudo JL**, Valdés Mas M, Vargas Acosta AM, Ortiz Sánchez ML, Gil del Castillo ML, Carballo Alvarez LF, Pons Miñano JA. [Clinical presentation, diagnosis, and long-term outcome of 29 patients with Wilson's disease]. *Rev Esp Enferm Dig* 2008; **100**: 456-461 [PMID: 18942896]

63 **Bruha R**, Marecek Z, Pospisilova L, Nevsimalova S, Vitek L, Martasek P, Nevoral J, Petrtyl J, Urbanek P, Jiraskova A, Ferenci P. Long-term follow-up of Wilson disease: natural history, treatment, mutations analysis and phenotypic correlation. *Liver Int* 2011; **31**: 83-91 [PMID: 20958917 DOI: 10.1111/j.1478-3231.2010.02354.x]

64   **Guillaud O**, Dumortier J, Sobesky R, Debray D, Wolf P, Vanlemmens C, Durand F, Calmus Y, Duvoux C, Dharancy S, Kamar N, Boudjema K, Bernard PH, Pageaux GP, Salamé E, Gugenheim J, Lachaux A, Habes D, Radenne S, Hardwigsen J, Chazouillères O, Trocello JM, Woimant F, Ichai P, Branchereau S, Soubrane O, Castaing D, Jacquemin E, Samuel D, Duclos-Vallée JC. Long term results of liver transplantation for Wilson's disease: experience in France. *J Hepatol* 2014; **60**: 579-589 [PMID: 24211743 DOI: 10.1016/j.jhep.2013.10.025]

65   **Meier Y**, Cavallaro M, Roos M, Pauli-Magnus C, Folkers G, Meier PJ, Fattinger K. Incidence of drug-induced liver injury in medical inpatients. *Eur J Clin Pharmacol* 2005; **61**: 135-143 [PMID: 15726344 DOI: 10.1007/s00228-004-0888-z]

66   **Soldin OP**, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 2009; **48**: 143-157 [PMID: 19385708 DOI: 10.2165/00003088-200948030-00001]

67   **Thurman RG**. Sex-related liver injury due to alcohol involves activation of Kupffer cells by endotoxin. *Can J Gastroenterol* 2000; **14** Suppl D: 129D-135D [PMID: 11110625]

68   **Kono H**, Wheeler MD, Rusyn I, Lin M, Seabra V, Rivera CA, Bradford BU, Forman DT, Thurman RG. Gender differences in early alcohol-induced liver injury: role of CD14, NF-kappaB, and TNF-alpha. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G652-G661 [PMID: 10762620]

69   **Müller C**. Liver, alcohol and gender. *Wien Med Wochenschr* 2006; **156**: 523-526 [PMID: 17103288 DOI: 10.1007/s10354-006-0348-8]

70   **Amacher DE.** Female gender as a susceptibility factor for drug-induced liver injury. *Hum Exp Toxicol* 2013; **33**: 928-939 [PMID: 24299907 DOI: 10.1177/0960327113512860]

71   **Sheng Y**, Liang Q, Deng Z, Ji L, Wang Z. Acetaminophen induced gender-dependent liver injury and the involvement of GCL and GPx. *Drug Discov Ther* 2013; **7**: 78-83 [PMID: 23715506]

72   **Rademaker M**. Do women have more adverse drug reactions? *Am J Clin Dermatol* 2001; **2**: 349-351 [PMID: 11770389]

73   **Zopf Y**, Rabe C, Neubert A, Gassmann KG, Rascher W, Hahn EG, Brune K, Dormann H. Women encounter ADRs more often than do men. *Eur J Clin Pharmacol* 2008; **64**: 999-1004 [PMID: 18604529 DOI: 10.1007/s00228-008-0494-6]

74   **Fletcher CV**, Acosta EP, Strykowski JM. Gender differences in human pharmacokinetics and pharmacodynamics. *J Adolesc Health* 1994; **15**: 619-629 [PMID: 7696281]

75   **Petronijevic M**, Ilic K. Associations of gender and age with the reporting of drug-induced hepatic failure: data from the VigiBase™. *J Clin Pharmacol* 2013; **53**: 435-443 [PMID: 23440959 DOI: 10.1002/jcph.3]

76   **Kjartansdottir I**, Bergmann OM, Arnadottir RS, Björnsson ES. Paracetamol intoxications: a retrospective population-based study in Iceland. *Scand J Gastroenterol* 2012; **47**: 1344-1352 [PMID: 22827594 DOI: 10.3109/00365521.2012.703236]

77   **Villa E**. Role of estrogen in liver cancer. *Womens Health* (Lond Engl) 2008; **4**: 41-50 [PMID: 19072450 DOI: 10.2217/17455057.4.1.41]

78   **Villa E**, Moles A, Ferretti I, Buttafoco P, Grottola A, Del Buono M, De Santis M, Manenti F. Natural history of inoperable hepatocellular carcinoma: estrogen receptors' status in the tumor is the strongest prognostic factor for survival. *Hepatology* 2000; **32**: 233-238 [PMID: 10915729 DOI: 10.1053/jhep.2000.9603]

79   **Liver transplantation for hepatocellular carcinoma**. Last accessed March 2014. Available from: URL: http://optn.transplant.hrsa.gov/latestData/rptData.asp

80   **Cauble S**, Abbas A, Balart L, Bazzano L, Medvedev S, Shores N. United States women receive more curative treatment for hepatocellular carcinoma than men. *Dig Dis Sci* 2013; **58**: 2817-2825 [PMID: 23812858 DOI: 10.1007/s10620-013-2731-9]

81   **Mindikoglu AL**, Emre SH, Magder LS. Impact of estimated liver volume and liver weight on gender disparity in liver transplantation. *Liver Transpl* 2013; **19**: 89-95 [PMID: 23008117 DOI: 10.1002/lt.23553]

82   **Yoshizumi T**, Shirabe K, Nakagawara H, Ikegami T, Harimoto N, Toshima T, Yamashita Y, Ikeda T, Soejima Y, Maehara Y. Skeletal muscle area correlates with body surface area in healthy adults. *Hepatol Res* 2014; **44**: 313-318 [PMID: 23607375 DOI: 10.1111/hepr.12119]

83   **Campillo B**, Paillaud E, Uzan I, Merlier I, Abdellaoui M, Perennec J, Louarn F, Bories PN. Value of body mass index in the detection of severe malnutrition: influence of the pathology and changes in anthropometric parameters. *Clin Nutr* 2004; **23**: 551-559 [PMID: 15297091 DOI: 10.1016/j.clnu.2003.10.003]

84   **DiMartini A**, Cruz RJ, Dew MA, Myaskovsky L, Goodpaster B, Fox K, Kim KH, Fontes P. Muscle mass predicts outcomes following liver transplantation. *Liver Transpl* 2013; **19**: 1172-1180 [PMID: 23960026 DOI: 10.1002/lt.23724]

85   **Lehner F**, Becker T, Klempnauer J, Borlak J. Gender-incompatible liver transplantation is not a risk factor for patient survival. *Liver Int* 2009; **29**: 196-202 [PMID: 18673439 DOI: 10.1111/j.1478-3231.2008.01827.x]

86   **Lai JC**, Feng S, Roberts JP, Terrault NA. Gender differences in liver donor quality are predictive of graft loss. *Am J Transplant* 2011; **11**: 296-302 [PMID: 21219572 DOI: 10.1111/j.1600-6143.2010.03385.x]

87   **Rustgi VK**, Marino G, Halpern MT, Johnson LB, Umana WO, Tolleris C. Role of gender and race mismatch and graft failure in patients undergoing liver transplantation. *Liver Transpl* 2002; **8**: 514-518 [PMID: 12037781 DOI: 10.1053/jlts.2002.33457]

88   **Francavilla R**, Hadzic N, Heaton ND, Rela M, Baker AJ, Dhawan A, Mieli-Vergani G. Gender matching and outcome after pediatric liver transplantation. *Transplantation* 1998; **66**: 602-605 [PMID: 9753339]

89   **Brooks BK**, Levy MF, Jennings LW, Abbasoglu O, Vodapally M, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Influence of donor and recipient gender on the outcome of liver transplantation. *Transplantation* 1996; **62**: 1784-1787 [PMID: 8990363]

90   **Croome KP**, Segal D, Hernandez-Alejandro R, Adams PC, Thomson A, Chandok N. Female donor to male recipient gender discordance results in inferior graft survival: a prospective study of 1,042 liver transplants. *J Hepatobiliary Pancreat Sci* 2014; **21**: 269-274 [PMID: 24123790 DOI: 10.1002/jhbp.40]

91   **Yoshizumi T**, Shirabe K, Taketomi A, Uchiyama H, Harada N, Ijichi H, Yoshimatsu M, Ikegami T, Soejima Y, Maehara Y. Risk factors that increase mortality after living donor liver transplantation. *Transplantation* 2012; **93**: 93-98 [PMID: 22203391 DOI: 10.1097/TP.0b013e318238dacd]

92   **Nijagal A**, Fleck S, Hills NK, Feng S, Tang Q, Kang SM, Rosenthal P, MacKenzie TC. Decreased risk of graft failure with maternal liver transplantation in patients with biliary atresia. *Am J Transplant* 2012; **12**: 409-419 [PMID: 22221561 DOI: 10.1111/j.1600-6143.2011.03895.x]

93   **Grąt M**, Kornasiewicz O, Lewandowski Z, Ligocka J, Grąt K, Wronka KM, Zieniewicz K, Krawczyk M. Post-transplant outcomes of patients with and without hepatitis C virus infection according to donor age and gender matching. *Ann Transplant* 2013; **18**: 705-715 [PMID: 24361968 DOI: 10.12659/AOT.889537]

94   **Sanada Y**, Kawano Y, Miki A, Aida J, Nakamura K, Shimomura N, Ishikawa N, Arai T, Hirata Y, Yamada N, Okada N, Wakiya T, Ihara Y, Urahashi T, Yasuda Y, Takubo K, Mizuta K. Maternal grafts protect daughter recipients from acute cellular rejection after pediatric living donor liver transplantation for biliary atresia. *Transpl Int* 2014; **27**: 383-390 [PMID: 24472036 DOI: 10.1111/tri.12273]

95   **Starzl TE**, Demetris AJ, Trucco M, Ramos H, Zeevi A, Rudert WA, Kocova M, Ricordi C, Ildstad S, Murase N. Systemic chimerism in human female recipients of male livers. *Lancet* 1992; **340**: 876-877 [PMID: 1357298]

96   **Loubière LS**, Lambert NC, Flinn LJ, Erickson TD, Yan Z,

Guthrie KA, Vickers KT, Nelson JL. Maternal microchimerism in healthy adults in lymphocytes, monocyte/macrophages and NK cells. *Lab Invest* 2006; **86**: 1185-1192 [PMID: 16969370 DOI: 10.1038/labinvest.3700471]

97   **Maloney S**, Smith A, Furst DE, Myerson D, Rupert K, Evans PC, Nelson JL. Microchimerism of maternal origin persists into adult life. *J Clin Invest* 1999; **104**: 41-47 [PMID: 10393697 DOI: 10.1172/JCI6611]

98   **Dutta P**, Burlingham WJ. Microchimerism: tolerance *vs.* sensitization. *Curr Opin Organ Transplant* 2011; **16**: 359-365 [PMID: 21666480 DOI: 10.1097/MOT.0b013e3283484b57]

99   **Bonilla WV**, Geuking MB, Aichele P, Ludewig B, Hengartner H, Zinkernagel RM. Microchimerism maintains deletion of the donor cell-specific CD8+ T cell repertoire. *J Clin Invest* 2006; **116**: 156-162 [PMID: 16395404 DOI: 10.1172/JCI26565]

100   **Burlingham WJ**, Grailer AP, Fechner JH, Kusaka S, Trucco M, Kocova M, Belzer FO, Sollinger HW. Microchimerism linked to cytotoxic T lymphocyte functional unresponsiveness (clonal anergy) in a tolerant renal transplant recipient. *Transplantation* 1995; **59**: 1147-1155 [PMID: 7732562]

101   **Wittnich C**, Belanger MP, Askin N, Boscarino C, Wallen WJ. Lower liver transplant success in females: gender differences in metabolic response to global ischemia. *Transplant Proc* 2004; **36**: 1485-1488 [PMID: 15251365 DOI: 10.1016/j.transproceed.2004.05.055]

102   **Jin B**, Liu CZ, Hu SY, Wang TT, Wang L, Jiang XS, Frezza EE. Influence of estrogen and androgen on the outcome of liver transplantation. *Hepatogastroenterology* 2008; **55**: 207-211 [PMID: 18507108]

103   **Vilatoba M**, Eckstein C, Bilbao G, Frennete L, Eckhoff DE, Contreras JL. 17beta-estradiol differentially activates mitogen-activated protein-kinases and improves survival following reperfusion injury of reduced-size liver in mice. *Transplant Proc* 2005; **37**: 399-403 [PMID: 15808658 DOI: 10.1016/j.transproceed.2004.12.053]

104   **Shen SQ**, Zhang Y, Xiong CL. The protective effects of 17beta-estradiol on hepatic ischemia-reperfusion injury in rat model, associated with regulation of heat-shock protein expression. *J Surg Res* 2007; **140**: 67-76 [PMID: 17336333 DOI: 10.1016/j.jss.2006.10.022]

105   **Harada H**, Pavlick KP, Hines IN, Hoffman JM, Bharwani S, Gray L, Wolf RE, Grisham MB. Selected contribution: Effects of gender on reduced-size liver ischemia and reperfusion injury. *J Appl Physiol* (1985) 2001; **91**: 2816-2822 [PMID: 11717250]

106   **Eckhoff DE**, Bilbao G, Frenette L, Thompson JA, Contreras JL. 17-Beta-estradiol protects the liver against warm ischemia/reperfusion injury and is associated with increased serum nitric oxide and decreased tumor necrosis factor-alpha. *Surgery* 2002; **132**: 302-309 [PMID: 12219027]

107   **Soric S**, Belanger MP, Askin N, Wittnich C. Impact of female sex hormones on liver tissue lactic acidosis during ischemia. *Transplantation* 2007; **84**: 763-770 [PMID: 17893610 DOI: 10.1097/01.tp.0000280542.61123.ec]

108   **Buis CI**, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, Porte RJ. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early *vs.* late presentation. *Liver Transpl* 2007; **13**: 708-718 [PMID: 17457932 DOI: 10.1002/lt.21166]

109   **Schoening WN**, Buescher N, Rademacher S, Andreou A, Kuehn S, Neuhaus R, Guckelberger O, Puhl G, Seehofer D, Neuhaus P. Twenty-year longitudinal follow-up after orthotopic liver transplantation: a single-center experience of 313 consecutive cases. *Am J Transplant* 2013; **13**: 2384-2394 [PMID: 23915357 DOI: 10.1111/ajt.12384]

110   **Yadav AD**, Chang YH, Aqel BA, Byrne TJ, Chakkera HA, Douglas DD, Mulligan DC, Rakela J, Vargas HE, Carey EJ. New Onset Diabetes Mellitus in Living Donor *versus* Deceased Donor Liver Transplant Recipients: Analysis of

the UNOS/OPTN Database. *J Transplant* 2013; **2013**: 269096 [PMID: 24205434 DOI: 10.1155/2013/269096]

111   **Polotsky HN**, Polotsky AJ. Metabolic implications of menopause. *Semin Reprod Med* 2010; **28**: 426-434 [PMID: 20865657 DOI: 10.1055/s-0030-1262902]

112   **Burra P**, Senzolo M, Masier A, Prestele H, Jones R, Samuel D, Villamil F. Factors influencing renal function after liver transplantation. Results from the MOST, an international observational study. *Dig Liver Dis* 2009; **41**: 350-356 [PMID: 19046932 DOI: 10.1016/j.dld.2008.09.018]

113   **Campsen J**, Zimmerman M, Trotter J, Hong J, Freise C, Brown RS, Cameron A, Ghobrial M, Kam I, Busuttil R, Saab S, Holt C, Emond JC, Stiles JB, Lukose T, Chang MS, Klintmalm G. Multicenter review of liver transplant for hepatitis B-related liver disease: disparities in gender and ethnicity. *Clin Transplant* 2013; **27**: 829-837 [PMID: 24033475 DOI: 10.1111/ctr.12224]

114   **Ponziani FR**, Viganò R, Iemmolo RM, Donato MF, Rendina M, Toniutto P, Pasulo L, Morelli MC, Burra P, Miglioresi L, Merli M, Di Paolo D, Fagiuoli S, Gasbarrini A, Pompili M, Belli L, Gerunda GE, Marino M, Montalti R, Di Benedetto F, De Ruvo N, Rigamonti C, Colombo M, Rossi G, Di Leo A, Lupo L, Memeo V, Bringiotti R, Zappimbulso M, Bitetto D, Vero V, Colpani M, Fornasiere E, Pinna AD, Morelli MC, Bertuzzo V, De Martin E, Senzolo M, Ettorre GM, Visco-Comandini U, Antonucci G, Angelico M, Tisone G, Giannelli V, Giusto M. Long-term maintenance of sustained virological response in liver transplant recipients treated for recurrent hepatitis C. *Dig Liver Dis* 2014; **46**: 440-445 [PMID: 24635906 DOI: 10.1016/j.dld.2014.01.157]

115   **De Martin E**, Senzolo M, Gambato M, Germani G, Vitale A, Russo FR, Burra P. Fibrosis progression and the pros and cons of antiviral therapy for hepatitis C virus recurrence after liver transplantation: a review. *Transplant Proc* 2010; **42**: 2223-2225 [PMID: 20692449 DOI: 10.1016/j.transproceed.2010.05.035]

116   **De Martin E**, Senzolo M, Boninsegna S, Guido M, Masier A, Germani G, Tomat S, Brolese A, Neri D, Cillo U, Gambato M, Russo FP, Farinati F, Burra P. HCV histological recurrence and survival following liver transplantation in patients with and without hepatocellular carcinoma. *Transplant Proc* 2008; **40**: 1974-1975 [PMID: 18675104 DOI: 10.1016/j. transproceed.2008.05.040]

117   **Lai JC**, Verna EC, Brown RS, O'Leary JG, Trotter JF, Forman LM, Duman JD, Foster RG, Stravitz RT, Terrault NA. Hepatitis C virus-infected women have a higher risk of advanced fibrosis and graft loss after liver transplantation than men. *Hepatology* 2011; **54**: 418-424 [PMID: 21538434 DOI: 10.1002/hep.24390]

118   **Belli LS**, Burroughs AK, Burra P, Alberti AB, Samonakis D, Cammà C, De Carlis L, Minola E, Quaglia A, Zavaglia C, Vangeli M, Patch D, Dhillon A, Cillo U, Guido M, Fagiuoli S, Giacomoni A, Slim OA, Airoldi A, Boninsegna S, Davidson BR, Rolles K, Pinzello G. Liver transplantation for HCV cirrhosis: improved survival in recent years and increased severity of recurrent disease in female recipients: results of a long term retrospective study. *Liver Transpl* 2007; **13**: 733-740 [PMID: 17370330 DOI: 10.1002/lt.21093]

119   **Shimizu I**, Mizobuchi Y, Yasuda M, Shiba M, Ma YR, Horie T, Liu F, Ito S. Inhibitory effect of oestradiol on activation of rat hepatic stellate cells in vivo and in vitro. *Gut* 1999; **44**: 127-136 [PMID: 9862839]

120   **Yasuda M**, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology* 1999; **29**: 719-727 [PMID: 10051473 DOI: 10.1002/hep.510290307]

121   **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257]

122   **Deuffic-Burban S**, Poynard T, Valleron AJ. Quantification of fibrosis progression in patients with chronic hepatitis C using a Markov model. *J Viral Hepat* 2002; **9**: 114-122 [PMID:

11876793]

123 **Codes L**, Asselah T, Cazals-Hatem D, Tubach F, Vidaud D, Paraná R, Bedossa P, Valla D, Marcellin P. Liver fibrosis in women with chronic hepatitis C: evidence for the negative role of the menopause and steatosis and the potential benefit of hormone replacement therapy. *Gut* 2007; **56**: 390-395 [PMID: 17005762 DOI: 10.1136/gut.2006.101931]

124 **Burra P**. Liver abnormalities and endocrine diseases. *Best Pract Res Clin Gastroenterol* 2013; **27**: 553-563 [PMID: 24090942 DOI: 10.1016/j.bpg.2013.06.014]

125 **Giannelli V**, Giusto M, Farcomeni A, Ponziani FR, Pompili M, Viganò R, Iemmolo RM, Donato MF, Rendina M, Toniutto P, Pasulo L, Morelli MC, De Martin E, Miglioresi L, Di Paolo D, Fagiuoli S, Merli M. Treatment of hepatitis C recurrence is less successful in female than in male liver transplant recipients. *Transpl Int* 2012; **25**: 448-454 [PMID: 22353419 DOI: 10.1111/j.1432-2277.2012.01440.x]

126 Abstracts of the 2011 Joint International Congress of ILTS (International Liver Transplantation Society), ELITA (European Liver and Intestine Transplant Association), and LICAGE (Liver Intensive Care Group of Europe). June 22-25, 2011. Valencia, Spain. *Liver Transpl* 2011; **17** Suppl 1: S79-324 [PMID: 21618695]

127 **Ebeling PR**. Approach to the patient with transplantation-related bone loss. *J Clin Endocrinol Metab* 2009; **94**: 1483-1490 [PMID: 19420272 DOI: 10.1210/jc.2009-0205]

128 **Kulak CA**, Borba VZ, Kulak J, Custódio MR. Osteoporosis after transplantation. *Curr Osteoporos Rep* 2012; **10**: 48-55 [PMID: 22167576 DOI: 10.1007/s11914-011-0083-y]

129 **Melton LJ**. How many women have osteoporosis now? *J Bone Miner Res* 1995; **10**: 175-177 [PMID: 7754796 DOI: 10.1002/jbmr.5650100202]

130 **Defranco DJ**, Lian JB, Glowacki J. Differential effects of glucocorticoid on recruitment and activity of osteoclasts induced by normal and osteocalcin-deficient bone implanted in rats. *Endocrinology* 1992; **131**: 114-121 [PMID: 1611989 DOI: 10.1210/endo.131.1.1611989]

131 **Canalis E**, Delany AM. Mechanisms of glucocorticoid action in bone. *Ann N Y Acad Sci* 2002; **966**: 73-81 [PMID: 12114261]

132 **Epstein S**. Post-transplantation bone disease: the role of immunosuppressive agents and the skeleton. *J Bone Miner Res* 1996; **11**: 1-7 [PMID: 8770690 DOI: 10.1002/jbmr.5650110102]

133 **Goffin E**, Devogelaer JP, Depresseux G, Squifflet JP, Pirson Y. Osteoporosis after organ transplantation. *Lancet* 2001; **357**: 1623 [PMID: 11386322]

134 **Monegal A**, Navasa M, Guañabens N, Peris P, Pons F, Martínez de Osaba MJ, Rimola A, Rodés J, Muñoz-Gómez J. Bone mass and mineral metabolism in liver transplant patients treated with FK506 or cyclosporine A. *Calcif Tissue Int* 2001; **68**: 83-86 [PMID: 11310351]

135 **Baccaro LF**, Boin IF, Pedro AO, Costa-Paiva L, Leal AL, Ramos CD, Pinto-Neto AM. Decrease in bone mass in women after liver transplantation: associated factors. *Transplant Proc* 2011; **43**: 1351-1356 [PMID: 21620127 DOI: 10.1016/j.transproceed.2011.02.027]

136 **Baccarani U**, Piselli P, Serraino D, Adani GL, Lorenzin D, Gambato M, Buda A, Zanus G, Vitale A, De Paoli A, Cimaglia C, Bresadola V, Toniutto P, Risaliti A, Cillo U, Bresadola F, Burra P. Comparison of de novo tumours after liver transplantation with incidence rates from Italian cancer registries. *Dig Liver Dis* 2010; **42**: 55-60 [PMID: 19497797 DOI: 10.1016/j.dld.2009.04.017]

137 **Baccarani U**, Adani GL, Serraino D, Lorenzin D, Gambato M, Buda A, Zanus G, Vitale A, Piselli P, De Paoli A, Bresadola V, Risaliti A, Toniutto P, Cillo U, Bresadola F, Burra P. De novo tumors are a major cause of late mortality after orthotopic liver transplantation. *Transplant Proc* 2009; **41**: 1303-1305 [PMID: 19460546 DOI: 10.1016/j.transproceed.2009.03.079]

138 **Serraino D**, Piselli P, Busnach G, Burra P, Citterio F, Arbustini E, Baccarani U, De Juli E, Pozzetto U, Bellelli S, Polesel J, Pradier C, Dal Maso L, Angeletti C, Carrieri MP, Rezza G,

Franceschi S. Risk of cancer following immunosuppression in organ transplant recipients and in HIV-positive individuals in southern Europe. *Eur J Cancer* 2007; **43**: 2117-2123 [PMID: 17764927 DOI: 10.1016/j.ejca.2007.07.015]

139 **Ettorre GM**, Piselli P, Galatioto L, Rendina M, Nudo F, Sforza D, Miglioresi L, Fantola G, Cimaglia C, Vennarecci G, Vizzini GB, Di Leo A, Rossi M, Tisone G, Zamboni F, Santoro R, Agresta A, Puro V, Serraino D. De novo malignancies following liver transplantation: results from a multicentric study in central and southern Italy, 1990-2008. *Transplant Proc* 2013; **45**: 2729-2732 [PMID: 24034034 DOI: 10.1016/j.transproceed.2013.07.050]

140 **Chatrath H**, Berman K, Vuppalanchi R, Slaven J, Kwo P, Tector AJ, Chalasani N, Ghabril M. De novo malignancy post-liver transplantation: a single center, population controlled study. *Clin Transplant* 2013; **27**: 582-590 [PMID: 23808800 DOI: 10.1111/ctr.12171]

141 **Heneghan MA**, Selzner M, Yoshida EM, Mullhaupt B. Pregnancy and sexual function in liver transplantation. *J Hepatol* 2008; **49**: 507-519 [PMID: 18715668 DOI: 10.1016/j.jhep.2008.07.011]

142 **Christopher V**, Al-Chalabi T, Richardson PD, Muiesan P, Rela M, Heaton ND, O'Grady JG, Heneghan MA. Pregnancy outcome after liver transplantation: a single-center experience of 71 pregnancies in 45 recipients. *Liver Transpl* 2006; **12**: 1138-1143 [PMID: 16799943 DOI: 10.1002/lt.20810]

143 **Terrabuio DR**, Abrantes-Lemos CP, Carrilho FJ, Cançado EL. Follow-up of pregnant women with autoimmune hepatitis: the disease behavior along with maternal and fetal outcomes. *J Clin Gastroenterol* 2009; **43**: 350-356 [PMID: 19077726 DOI: 10.1097/MCG.0b013e318176b8c5]

144 **Penney GC**, Mair G, Pearson DW. Outcomes of pregnancies in women with type 1 diabetes in Scotland: a national population-based study. *BJOG* 2003; **110**: 315-318 [PMID: 12628275]

145 **Schramm C**, Herkel J, Beuers U, Kanzler S, Galle PR, Lohse AW. Pregnancy in autoimmune hepatitis: outcome and risk factors. *Am J Gastroenterol* 2006; **101**: 556-560 [PMID: 16464221 DOI: 10.1111/j.1572-0241.2006.00479.x]

146 **Buchel E**, Van Steenbergen W, Nevens F, Fevery J. Improvement of autoimmune hepatitis during pregnancy followed by flare-up after delivery. *Am J Gastroenterol* 2002; **97**: 3160-3165 [PMID: 12492204 DOI: 10.1111/j.1572-0241.2002.07124.x]

147 **Westbrook RH**, Yeoman AD, Kriese S, Heneghan MA. Outcomes of pregnancy in women with autoimmune hepatitis. *J Autoimmun* 2012; **38**: J239-J244 [PMID: 22261501 DOI: 10.1016/j.jaut.2011.12.002]

148 **Park ES**, Villanueva CA, Viers BR, Siref AB, Feloney MP. Assessment of sexual dysfunction and sexually related personal distress in patients who have undergone orthotopic liver transplantation for end-stage liver disease. *J Sex Med* 2011; **8**: 2292-2298 [PMID: 21492399 DOI: 10.1111/j.1743-6109.2011.02264.x]

149 **Burra P**, Germani G, Masier A, De Martin E, Gambato M, Salonia A, Bo P, Vitale A, Cillo U, Russo FP, Senzolo M. Sexual dysfunction in chronic liver disease: is liver transplantation an effective cure? *Transplantation* 2010; **89**: 1425-1429 [PMID: 20463637 DOI: 10.1097/TP.0b013e3181e1f1f6]

150 **Parolin MB**, Rabinovitch I, Urbanetz AA, Scheidemantel C, Cat ML, Coelho JC. Impact of successful liver transplantation on reproductive function and sexuality in women with advanced liver disease. *Transplant Proc* 2004; **36**: 943-944 [PMID: 15194326 DOI: 10.1016/j.transproceed.2004.03.124]

151 **Ho JK**, Ko HH, Schaeffer DF, Erb SR, Wong C, Buczkowski AK, Scudamore CH, Yoshida EM. Sexual health after orthotopic liver transplantation. *Liver Transpl* 2006; **12**: 1478-1484 [PMID: 16741905 DOI: 10.1002/lt.20831]

152 **Cundy TF**, O'Grady JG, Williams R. Recovery of menstruation and pregnancy after liver transplantation. *Gut* 1990; **31**: 337-338 [PMID: 2323601]

153 **Mass K**, Quint EH, Punch MR, Merion RM. Gynecological and

reproductive function after liver transplantation. *Transplantation* 1996; **62**: 476-479 [PMID: 8781613]

154 **Jabiry-Zieniewicz Z**, Cyganek A, Luterek K, Bobrowska K, Kamiński P, Ziółkowski J, Zieniewicz K, Krawczyk M. Pregnancy and delivery after liver transplantation. *Transplant Proc* 2005; **37**: 1197-1200 [PMID: 15848667 DOI: 10.1016/j.transproceed.2005.01.011]

155 **Burra P**. Sexual dysfunction after liver transplantation. *Liver Transpl* 2009; **15** Suppl 2: S50-S56 [PMID: 19876949 DOI: 10.1002/lt.21899]

156 **Jabiry-Zieniewicz Z**, Kaminski P, Bobrowska K, Pietrzak B, Wielgos M, Smoter P, Zieniewicz K, Krawczyk M. Menstrual function in female liver transplant recipients of reproductive age. *Transplant Proc* 2009; **41**: 1735-1739 [PMID: 19545718 DOI: 10.1016/j.transproceed.2009.03.073]

157 **Murthy SK**, Heathcote EJ, Nguyen GC. Impact of cirrhosis and liver transplant on maternal health during labor and delivery. *Clin Gastroenterol Hepatol* 2009; **7**: 1367-1372, 1372.e1 [PMID: 19686866 DOI: 10.1016/j.cgh.2009.08.008]

158 **Kim HW**, Seok HJ, Kim TH, Han DJ, Yang WS, Park SK. The experience of pregnancy after renal transplantation: pregnancies even within postoperative 1 year may be tolerable. *Transplantation* 2008; **85**: 1412-1419 [PMID: 18497680 DOI: 10.1097/TP.0b013e318170f8ed]

159 **Gerlei Z**, Wettstein D, Rigó J, Asztalos L, Langer RM. Childbirth after organ transplantation in Hungary. *Transplant Proc* 2011; **43**: 1223-1224 [PMID: 21620095 DOI: 10.1016/j.transproceed.2011.03.087]

160 **Wielgos M**, Szpotanska-Sikorska M, Mazanowska N, Bomba-Opon D, Kociszewska-Najman B, Jabiry-Zieniewicz Z, Cyganek A, Kaminski P, Pietrzak B. Pregnancy risk in female kidney and liver recipients: a retrospective comparative study. *J Matern Fetal Neonatal Med* 2012; **25**: 1090-1095 [PMID: 21919553 DOI: 10.3109/14767058.2011.622010]

161 **Jabiry-Zieniewicz Z**, Szpotanska-Sikorska M, Pietrzak B, Kociszewska-Najman B, Foroncewicz B, Mucha K, Zieniewicz K, Krawczyk M, Wielgos M. Pregnancy outcomes among female recipients after liver transplantation: further experience. *Transplant Proc* 2011; **43**: 3043-3047 [PMID: 21996220 DOI: 10.1016/j.transproceed.2011.08.070]

162 **Deshpande NA**, James NT, Kucirka LM, Boyarsky BJ, Garonzik-Wang JM, Cameron AM, Singer AL, Dagher NN, Segev DL. Pregnancy outcomes of liver transplant recipients: a systematic review and meta-analysis. *Liver Transpl* 2012; **18**: 621-629 [PMID: 22344967 DOI: 10.1002/lt.23416]

163 **Zahn A**, Seubert L, Jünger J, Schellberg D, Weiss KH, Schemmer P, Stremmel W, Sauer P, Gotthardt DN. Factors influencing long-term quality of life and depression in German liver transplant recipients: a single-centre cross-sectional study. *Ann Transplant* 2013; **18**: 327-335 [PMID: 23817439 DOI: 10.12659/AOT.883962]

164 **Bianco T**, Cillo U, Amodio P, Zanus G, Salari A, Neri D, Bombonato G, Schiff S, Baggio G, Ronco C, Brocca A, Soni S, Minazzato L. Gender differences in the quality of life of patients with liver cirrhosis related to hepatitis C after liver transplantation. *Blood Purif* 2013; **36**: 231-236 [PMID: 24496196 DOI: 10.1159/000356362]

TOPIC HIGHLIGHT

Maurizio Salvadori, Professor, *Series Editor*

What's new in clinical solid organ transplantation by 2013

Maurizio Salvadori, Elisabetta Bertoni

Maurizio Salvadori, Elisabetta Bertoni, Department of
Transplantation, Careggi University Hospital, 50139 Florence,
Italy
Author contributions: Salvadori M and Bertoni E contributed
equally to conceivement, finding materials, writing and reviewing
this paper.
Correspondence to: Maurizio Salvadori, MD, Professor of
Nephrology, Deparment of Transplantation, Careggi University
Hospital, viale Pieraccini 18, 50139 Florence,
Italy. maurizio.salvadori1@gmail.com
Telephone: +39-05-5597151 Fax: +39-05-5597151
Received: May 22, 2014   Revised: July 11, 2014
Accepted: July 27, 2014
Published online: December 24, 2014

Abstract

Innovative and exciting advances in the clinical science
in solid organ transplantation continuously realize
as the results of studies, clinical trials, international
conferences, consensus conferences, new technologies
and discoveries. This review will address to the full
spectrum of news in transplantation, that verified by
2013. The key areas covered are the transplantation
activity, with particular regards to the donors, the
news for solid organs such as kidney, pancreas, liver,
heart and lung, the news in immunosuppressive
therapies, the news in the field of tolerance and some
of the main complications following transplantation
as infections and cancers. The period of time covered
by the study starts from the international meetings
held in 2012, whose results were published in 2013,
up to the 2013 meetings, conferences and consensus
published in the first months of 2014. In particular for
every organ, the trends in numbers and survival have
been reviewed as well as the most relevant problems
such as organ preservation, ischemia reperfusion
injuries, and rejections with particular regards to the
antibody mediated rejection that involves all solid
organs. The new drugs and strategies applied in organ
transplantation have been divided into new way of
using old drugs or strategies and drugs new not yet on
the market, but on phase Ⅰ to Ⅲ of clinical studies and
trials.

Key words: News in transplantation, kidney transplantation,
pancreas transplantation, liver transplantation; Heart
transplantation; Lung transplantation; New immunosuppressant;
Tolerance

Core tip: Basic and clinical science in solid organ transplantation
are continuously evolving. In this review we outlined the
most important innovative findings recently discovered.
The period of time chosen was 2013, but attention has
been paid to the outstanding conferences held in 2012,
but published in 2013, as well as to the conferences
and meetings held in 2013 but published in 2014.
We are aware that when this study will be published,
new interesting and relevant findings will have been
discovered. The science is flowing continuously,
nevertheless analyzing in depth a short period of time can
give useful information to the readers.

INTRODUCTION

Innovative and exciting advances in the clinical science in
organ transplantation continuously realize as the results of
studies, clinical trials, international conferences, consensus
conferences, new technologies and discoveries. This
review will address to the full spectrum of the news in
transplantation, that verified by 2013 and the key areas
covered for every organ as the organ transplant activity,

the organ survival rates, the organ preservation and allocation, the new immunosuppressive regimens, the new immunological findings and the most important complications following organ transplantation.

The organ procurement transplant network/scientific report transplant recipients (OPTN/SRTR), the most wide and extensive registry on transplantation, by the end of 2013 published the complete data[1] concerning organ transplantation for 2012 and allowed for several considerations on the transplant activity. In particular, in the 2013 report, for the first time, OPTN/SRTR has undertaken to publish the worldwide transplant rates as part of its annual data report[2].

This report found that the transplant counts and rates vary among the countries around the world for different reasons: (1) Differences in the rates of end-organ disease. Country to country variability in the underlying incidence of end-organ disease can be expected to affect the organ transplant rate. However other factors undoubtedly play a role in determining the transplant rates. For example the incidence of end stage renal disease (ESRD) in Norway in 2009 was one third of the incidence in the United States. Nevertheless, in 2010, the rates of kidney transplant were similar in Norway and the United States, probably due to the very high activity related to living donor that characterizes the Norway; (2) Socioeconomic factors. There is a strong correlation between the Human Development Index (HDI) and the rate of deceased and living donor kidney transplants among the world health organization member states[3]. Similarly, the rates of liver transplant are lower in the countries with lower HDIs; (3) Cultural differences. An example is Japan that has a very high HDI, but lower rate of kidney transplants; and (4) Thoroughness of the transplant reporting, that varies by country.

Worldwide, use of living kidney donors varies widely, from less than 10% to more than 75% The rates of liver transplant have increased by more than 10% in several countries and declined in very few countries. In the past 5 years, the lung transplant rates have remained stable. The heart transplant rates changed little in the majority of countries.

NEW INSIGHTS FOR DONORS

In 2012 the number of deaths eligible for organ recovery for transplantation was lower than 2011 and 2010[4]. Similarly the mean number of organs transplanted per donor in United States in 2012 was 3.02, lower than in 2011 and 2010. Numbers of hearts and lungs procured for transplant but not used are smaller than the numbers of kidneys, pancreas and livers because the former organs are recovered only after the acceptance by the transplant center.

Data from OPTN/SRTR show that the number of Standard Criteria Donors (SCD) have remained about the same in United States and Europe, but there has been a dramatic increase in older donors and organs classified as donation after cardiac death. Overall, among deceased donors there is an organ donor shift[5]. Indeed, the percentage of all donors who are SCD is on the decline and there is an increase in Expanded Criteria Donors (ECD).

This shift could impact on the outcomes and more research is necessary to improve the quality of organ used for transplant and to optimize the use of a further expanded donor pool.

A wide, retrospective study from Heaphy *et al*[6] confirms this issue, as the donor quality has significant interactions by race, primary diagnosis and age. Another study[7] suggests that the judicious use of ECD kidneys may be an appropriate strategy to expand the donor pool minimizing the effects upon the outcomes.

Improving the organ cold storage by machine perfusion (MP) has been proposed to improve the solid organ outcomes. Especially in liver[8], heart and lung transplantation[9], the MP seems to be a promising tool to improve post-operative outcome, but a general evidence-based recommendation for or against on application of MP, cannot be given due to the lack of highest level of clinical evidence.

In addition to the above mentioned shift among deceased donors, recently, at least in United States, a decline in living kidney donation rate has been observed. This decline is about 13% per year and is more pronounced among blacks, men, younger adults, siblings and parents[10]. This fact warrants an action by transplant centers and national governments, also because another wide study[11] documented that the public is supportive of the living donation and in favor of protecting the health and safety of living donors.

A barrier to solid organ transplantation is often represented by the pre-transplant presence of donor specific antigens (DSAs) in the recipient sera. This fact is well known for the kidneys but has clinical relevance also for liver, heart and lung transplantation[12].

In such condition, for deceased donor kidney donation, the technique of acceptable human leukocyte antigen (HLA) mismatches has shown its efficacy. Two 2013 large studies proved its transnational efficacy[13,14].

In the case of the living kidney donation the presence of preformed antibodies may represent a relevant barrier to transplantation. In kidney transplantation, this barrier may be overcome by the network called kidney paired donation (KPD). Originally conceived as simple two-way reciprocal exchange between AB0 incompatible, KPD has evolved to include complex, multicenter, discontinuous chains, with transcontinental transport of kidneys. To date the majority of the researches performed on KPD has involved computer generated mathematical optimization algorithms. Several 2013 papers confirm the effectiveness of such network[15-17].

NEW INSIGHTS FOR KIDNEY

Main kidney related issues considered in 2013 publications have been: the kidney and recipient graft survivals, the impact and consequences of ischemia-reperfusion injury, the antibody mediated rejection (ABMR) and the new

techniques involved in rejection diagnosis.

Transplant activity and kidney graft survival

According OPTN/SRTR data, the shortage of kidneys for transplant remains a major problem for patients with ESRD. The number of candidates on the waiting list continues to increase, while the organ donation numbers remain flat[18]. Many kidneys recovered for transplant are then discarded for organ related problems and the discard rate is increasing. Living donation rates have been unchanged for the past decade. For both living and deceased donor recipients, the early post-transplant results have shown ongoing improvement.

For the first time, the graft survival rates have been systematically compared between Europe and United States. Utilizing data from OPTN/SRTR for United States and data from the Collaborative Transplant Study for Europe, the 1, 5 and 10-year graft survival rates have been compared among Europeans and White, African and Hispanic Americans[19]. While the 1-year graft survival rate was similar, the 5 and 10-year graft survival rates were considerably higher for Europe than for any of the three United States populations. Differences increased beyond three to four years after transplantation and these differences are not explained by differences in baseline patient characteristics. Studies are needed to identify factors contributing to the observed graft survival differences. Previous studies have documented that the limitations in access to immunosuppressive medications[20,21] and related compliance[22] are important determinants of long-term graft failure. Indeed, in the past the extension of immunosuppressive coverage in the US has shown to effectively reduce the income-related disparities in graft survival[23]. An United States study in 2013 examined the impact of Community risk factors on the kidney transplant outcomes[24]. The study documented that community risks are powerful factors associated with processes of care; and represent important considerations for developing effective interventions.

Ischemia-reperfusion injury

The Food and Drug Administration (FDA) held an open public workshop in September 2011 to discuss the current state of science related to the effects of ischemic reperfusion injury (IRI) on the outcomes in kidney transplantations. The summary of the workshop has been published in 2013[25]. The conclusions were that IRI impacts on graft survival and a better understanding of the underlying mechanisms is needed. Medical products to impact on IRI are urgently needed, but their development relies on both clinical and non-clinical researches. Also qualification of biomarkers is essential to elucidate the mechanisms[26].

Necroptosis in immunity and IRI have been principally studied in 2013[27-30]. Pathways of regulated necrosis (RN), an alternative to apoptosis have been recently described. The best studied RN pathway, the necroptosis, is triggered by perturbation of caspase-8-mediated apoptosis. In this condition the necroptosome is assembled and quickly leads to the necrotic-type cell death, release of the cell death-associated molecular patterns and severe organ damage. Interference with necroptosis (*e.g.*, by necrostatin) is more likely to be of clinical benefit in situations in which the reperfusion damage can be anticipated as solid organ transplantation.

Antibody-mediated rejection

Recent studies indicate that ABMR is among the most important barriers to improving long term outcomes principally in kidney transplantation, but in other solid organs as well[31].

Additionally new knowledge in ABMR pathophysiology, classification, diagnostic techniques and therapeutic approaches has merged. While the new therapeutic approaches will be described in the therapy chapter, the other issues will be treated in this paragraph.

A relevant and new finding is that not only the donor specific antibodies anti HLA (DSAs-HLA) are involved in ABMR. The antibodies against other molecules[32,33] and also polyreactive antibodies directed against apoptotic cells may cause ABMR[34].

The antibodies cause graft damage by endothelial cell injury mediated by the activation of complement. C4d is a split product of C4 activation and is often present on endothelial cells in ABMR. Sis *et al*[35] described that 60% of kidneys with high endothelial activation and injury transcripts (ENDATs) and chronic ABMR were C4d negative. A recent microarray study from Sellarés *et al*[36] concluded that changes in ABMR-associated gene expression correlates with the presence of capillary lesions or of DSAs and may predict graft failure independently of C4d staining. Taken together these observations point to the low sensitivity of C4d for the diagnosis of ABMR and support the addition of novel biomarkers of capillary inflammation and endothelial injury, including natural killer cells and macrophages, for the diagnosis algorithm of ABMR[37,38]. This recommendation was officially incorporated into the new Banff 2013 diagnostic criteria for ABMR[39].

The 12th Banff conference on allograft pathology was held in Comandatuba, Brazil in August 2013. The conference led to the following conclusions in the field of ABMR in renal allograft: (1) For acute/active ABMR the following three features must be present for diagnosis, not colon histological evidence of acute tissue injury, evidence of current/recent antibody interaction with vascular endothelium, serologic evidence of DSAs; (2) For chronic/active ABMR the following three features must be present for diagnosis, morphologic evidence of chronic tissue injury, evidence of current/recent antibody interaction with vascular endothelium, serologic evidence of DSAs; and (3) C4d staining without rejection (often accommodation), must include: linear C4d staining in peritubular capillaries, no morphologic lesions by light microscopy and electronic microscopy, no acute cell-mediated rejection.

New techniques involved in rejection diagnosis

Bachelet *et al*[40] with a seminal work demonstrated that DSAs detection in kidney allograft biopsy eluates is a

feasible method to predict the graft outcomes. Indeed, patients with intragraft DSAs displayed more severe ABMR pathology and worse outcome than patients with only DSAs in the serum. According to this work the intragraft DSA detection is a new test to dichotomize HLA antibodies into high and low injurious activity[41].

There are no doubts on the unmet medical need for improvement of diagnostic of renal injury to allow a more personalized therapeutic approach. Therefore, it is believed that the opportunity lies in new technologies such as molecular analysis, as messenger RNA (mRNA) and micro RNA expression from biopsies or even from blood or urine samples[42].

Two reports from the group of Edmonton in 2013 reported the results of molecular analyses of renal allograft biopsies[43,44]. The first report aimed to develop a diagnostic test for the T and B cell-mediated rejection by bootstrapping from the pathology.

The main messages of this paper were: (1) A molecular scoring was developed for diagnosis of rejection; (2) A molecular classification is based on selected genes related to immune cells and their activation products; and (3) The study confirmed certain disagreements among pathologists in applying the golden standard histopathology. In two other studies[45,46] the scoring assessed by the microarray test was validated by the INTERCOM study.

These papers revealed that a previously identified "acute kidney injury signal" early after transplantation was also present in the late kidney biopsies related to late T cell and ABMR, but not to fibrosis.

The multicenter Clinical Trials in Organ Transplantation 04 (CTOT-04) study was designed to investigate whether the urinary-cell mRNA levels encoding immune system proteins implicated in transplant rejection are diagnostic of acute rejection[47]. By logistic regression the authors correlated a three-gene signature of CD3ε mRNA, IP-10 mRNA, and 18S rRNA levels in urinary cells with allograft rejection. This study offers new insight into the possible use of non-invasive diagnostic and prognostic markers for the acute cellular rejection in kidney allograft.

NEW INSIGHTS FOR PANCREAS AND ISLET TRANSPLANTATION

Transplant activity and graft survival

Pancreas and islet cell transplantation (ICTx) confirmed to be the best treatment for diabetes mellitus type I (T1DM). According the OPTN/SRTR data, the number of pancreas transplants has decreased over the past years, most notably the numbers of pancreas after kidney (PAK) and pancreas transplant alone (PTA)[48]. Deceased donor pancreas donation rates have been declining since 2005 and the donation rate remains low. The outcomes of pancreas graft are better for simultaneous pancreas-kidney (SPK) transplantation. The challenges of pancreas transplant are reflected in the high rate of re-hospitalization, most occurring within the first six month post-transplant.

Very recent data[49] confirm the excellent long-term prognosis of SPK transplantation principally in recipients with functioning graft 1-year after transplantation. Patients who receive PTA or PAK grafts have shorter long-term graft survival[50]. Multiple strategies are aimed to be applied to improve immunologic surveillance and to obtain an early diagnosis of the graft rejection in patients receiving PTA.

An interesting study[51] documented an improved patient survival rate for recipients with diabetic end-stage renal disease receiving SPK than that receiving kidney transplant alone (KTA). ICTx remains a hot topic. The collaborative islet transplant registry investigators[52] presented the results of 752 islet allograft recipients with optimal and improving insulin independence rate at 3 years.

Pancreas transplantation for type 2 diabetes mellitus

SPK is widely accepted as an optimal therapeutic option for patients with T1DM and end-stage renal disease, but the indication for patients with type 2 diabetes mellitus (T2DM) is still controversially discussed. Indeed, there is continued uncertainty as to whether to T2DM patients are appropriate pancreas transplant candidates. In an editorial of 2012 Cohen *et al*[53] reviewed the most recent experience with pancreas transplantation in T2DM.

Gruessner *et al*[54] summarized the united network for organ sharing (UNOS) and International Pancreas Transplant Registry and reported no differences in the outcomes of patients with T2DM *vs* T1DM. Orlando *et al*[55] also found equivalent outcomes, regardless of whether the patients were classified as having T1DM or T2DM. Sampaio *et al*[56] reviewing the UNOS database, reported similar results even if T2DM represented only from 4.1% to 7.4% of diabetic patients transplanted.

More recently, Margreiter *et al*[57] reported the outcomes of 21 T2DM recipients receiving SPK and 32 T2DM receiving KTA. Patient and kidney graft survival rates were significantly lower for patients with KTA. The multivariate analysis adjusted for donor and recipient age, body mass index and coronary risk factors, showed that the differences did not remain statistically significant. The authors concluded that, according to the selection criteria proposed by other groups[58], selecting T2DM with an acceptable coronary risk profile and ageing not more than 55 years, is useful to identify those patients that may have a benefit from SPK.

ABMR in pancreas transplantation

ABMR is a recently identified entity. In a recent published paper[59], risk factors for pancreas ABMR were PTA and race mismatch. The diagnosis should be actively sought using C4d staining and DSAs levels in patients with graft dysfunction.

Preliminary studies have been presented at the already mentioned 2013 Banff conference[39]. These studies described the potential association of rejection-related vascular lesions with ABMR. Other studies demonstrated that immunostaining can enhance the understanding of pancreas T cell mediated rejection and ABMR even if the

accurate grade and type of rejection rests principally on the systematic evaluation of morphological features on routinely stained sections[60].

Islet transplantation

ICTx is a modality to treat selected diabetic patients. The "Edmonton Protocol" became a milestone by reporting sustained C-peptide production and high rates of insulin-independence after transplant in T1DM[61].

Long-term analysis of these results indicates that insulin-independence was not durable and most patients returned to moderate amounts of insulin approximately 5-years post-infusion[62]. The causes for this islet graft dysfunction are not completely understood, but are likely associated to several factors as the immune rejection, the autoimmunity or the chronic exposure to diabetogenic immunosuppressant[63].

In the last years relevant progress has occurred testing new immunosuppressant, testing novel devices to provide islets with a safer environments, as well as new transplant sites to overcome the limitations inherent to the current intraportal access[64-68]. The autoimmunity is a limiting factor to the success of ICTx. In a recent study Takita *et al*[69]. documented an early loss of transplanted allergenic islets despite T cell depletion induction. The authors concluded that the T cell depletion with anti-inflammatory regimen can enhance engraftment and survival; however, autoimmune recurrence by islet auto antibodies, principally GAD65 may limit the results.

The revascularization of transplanted pancreatic islets and the role of the transplantation site is another important issue[70]. Indeed, pancreatic islets are highly vascularized, which is important for their ability to secrete insulin in response to changes in blood glucose. The islet isolation process interrupts the connections between the islet vasculature and the systemic circulation. As the revascularization of the ICTx is not immediate, allocating cells in proximity to a good vascular supply is essential. A recent study proved the impaired revascularization of pancreatic islets into the liver[71]. In addition, the portal vein after islets injection undergoes instant blood-mediated inflammatory rejection (IBMIR) which results in an early inflammatory reaction. Therefore, it is essential to avoid this by either identifying a transplant site with minimal interaction with blood or by protecting the vascular grafts from IBMIR[70].

Among other sites, recent studies documented good results with omentum and muscle. The peritoneum offers an unlimited space for transplanted islets and is an attractive site for concurrent use of encapsulated device to protect the islets. A recent study[72] suggests the potential for longevity of islets allocated in the peritoneal cavity. Muscle-skeletal sites offer several advantages. They are easy to access, offer substantial space in which to transplant cells and are highly vascularized making them a very useful area. In a recent study, mice islets were successfully transplanted intramuscularly and the authors concluded that the early hypoxia after transplantation could be overcome by co-implantation of polymerized hemoglobin[73].

Finally, the islet encapsulation has been the issue of a very recent review[74]. Islet encapsulation allows the protection of this tissue without the use of toxic medications and expanding the donor pool to include animal sources. Before the use of this therapy, there are still issues that need to be resolved as the materials to be used, the shapes and sizes of the capsules and the aspects of bioengineering.

NEW INSIGHTS FOR LIVER

Transplant activity and liver graft survival

According the OPTN/SRTR data, in United States the number of adults who registered on the liver transplant waiting list decreased for the first time since 2002. However, the median waiting time for active wait-listed adult candidates increased, as did the number of candidates removed from the list because they were too sick to undergo transplant[75]. Graft survival continues to improve, especially for donation after circulatory death livers.

Since the first liver transplantation, short-term survival has improved rapidly; however, long-term attrition rates have not changed similarly[76]. In 2013 the first publication of European single-center 20-year survival data have been published[77]. The 20-year patient and graft survival rate of 313 patients has been reported. The 20-year patient and graft survival rates were respectively 52.5% and 46.6%. These results were better than two other single center long-term survivals[78,79] and also than the 20-year survival published by the European Liver Transplant Registry[80].

Impaired renal function and re-transplantation had significant impact on patient survival and recurrent diseases. Infections and *de novo* malignancies were the main cause of death. Much work is needed to combat recurrent disease and side effects of immunosuppressants.

The Japanese Liver Transplantation Society analyzed the outcomes of 2224 pediatric patients who underwent living donor liver transplantation[81]. No donor mortality related to transplant has been reported and the 10 and 20-year patient survival rates were 82.8% and 79.6%, respectively.

Primary disease impacts on the outcomes of liver transplantation (LTx). A recent analysis of OPTN/SRTR[82] documented an optimal short and long-term survival of LTx for primary biliary cirrhosis; similar good outcomes were reported for primary sclerosing cholangitis, non-alcoholic steatohepatitis and for hepatitis B virus (HBV). The worst results (HR = 1.5-2.4) were reported for hepatitis C virus (HCV) and hepatocellular carcinoma.

More than one-third of listed potential liver recipients in many western and some Asian countries are infected with the HCV. Recurrence of infection with HCV after LTx is associated with accelerated graft loss and diminished patient survival[83]. Until recently, HCV treatment has been limited to the use of pegylated interferon alpha (Peg IFN) plus ribavirin. In 2012 two direct acting anti-viral drugs, boceprevir and telaprevir were licensed by FDA for the treatment of chronic genotype 1 HCV[84,85]. The use of protease inhibitors (PI) based triple anti HCV

therapy in LTx recipients, is complicated by the known pharmacokinetic effect of the PI on cytochrome P450[86]. Nevertheless, promising small series of HCV recipients treated by PI based triple therapy have been reported[87]. Future approaches rely on the possible use of prophylactic neutralizing monoclonal antibodies to HCV[88].

Ischemia reperfusion injury

IRI is a major cause of morbidity and mortality in LTx. After a transient ischemia, the restoration of blood flow is necessary to restore cellular function, but paradoxically the reperfusion can initiate a cascade of pathways that causes further cellular injury after prolonged ischemia[89].

The lack of oxygen in hepatocytes during ischemia causes adenosine 3 phosphate depletion and alterations in H^+, Na^+ and Ca^{2+} homeostasis that activate hydrolytic enzymes and impair the volume regulation, leading to the swelling of sinusoidal endothelial cells and Kupffer cells (KCs). This fact together with the imbalance between nitric oxide and endothelin production, contributes to the narrowing of the sinusoidal lumen and thus to microcirculatory dysfunction. The activation of KCs releases reactive oxygen species (ROS) and proinflammatory cytokines (TNF alpha and IL 1). Cytokines and chemokines promote neutrophil activation and subsequent release of ROS and proteases. In addition, IL 1 and TNF alpha activate CD4 T-lymphocytes which produce granulocyte-macrophage colony-stimulating factor, IFN gamma and TNF beta. Platelet-activating factor can prime neutrophils for superoxide generation[90].

Several studies in 2013 evaluated different molecules in attempt to attenuate the damage induced by IRI. The most important studies in this field have been extensively reviewed in the work of Akhtar *et al*[91]. Attempt to protect IRI may involve several strategies and several pathways[92-95]. This issue will be described in the therapy chapter.

DSA and acute and chronic liver rejection

The issue of the impact of preformed DSAs on LTx has been a matter of discussion. Early clinical experience showed no differences in patient or graft survival rate[96-98] and DSAs were thought to be an integral part of tolerance development. Later studies documented that patients transplanted with a positive cross-match had an increased risk of early graft loss[99-101]. However, since consistent results are lacking, practice has not changed. In 2013, a study from Kaneku *et al*[102], documented that patients with LTx developing de novo DSAs after transplantation, had significantly lower patient and graft survival rates.

The 2013 Banff conference[39] stated that currently, recognized acute ABMR, occurs in small percentage of sensitized patients and that DSAs can be associated with more progressive fibrosis and an indolent progressive perivascular and subsinusoidal fibrosis. The conference concludes that high titer IgG3 recipients more often show adverse consequences, whereas exclusively not IgG3/IgG1 DSAs appear in some operationally tolerant recipients weaned from immunosuppression.

New tools for rejection diagnosis

Current liver biopsy is the most frequent used technique to evaluate allograft status and is the gold standard for the diagnosis of the acute rejection after orthotopic liver transplantation (OLT). As already described for the kidney, plasma microRNA is now revealing to be a potential biomarker for acute rejection after OLT[103,104].

NEW INSIGHTS FOR HEART

Figures, characteristics and trends for heart transplantation

According to the OPTN/SRTR data, in United States the number of heart transplants performed annually continues to increase gradually, and the number of adult candidates on the waiting list increased by 25% from 2004 to 2012[105]. Heart transplantation (HTx) appears to be more expensive than ventricular assist devices for managing the end-stage heart failure, but is more effective and likely more cost-effective.

By the end of 2013 the data of the Registry of the International Society for Heart and Lung Transplantation (ISHLT) have been published[106]. Cardiomiopathy in recent years has been the leading cause for HTx, followed by coronary artery disease (CAD). This trend has been particularly higher in Europe and the rest of the world than United States, reaching percentage of 57%-60%. Both recipient and donor age statistically increased, as well as the percentage of patients with pre-transplant panel reactive antibodies in the sera > 10%.

In the recent years a highly significant number of patients bridged to transplantation with mechanical circulatory support (MCS) have been registered. Nevertheless, should be outlined that a better survival rate has been reported for patients not on mechanical support prior transplantation.

A progressive and significant increase of Kaplan Meier survival by ERA was reported except for the last two years. Congenital diseases as primary disease attained the best survival rate while re-transplants attained the worst. Importantly long-term freedom from cardiac allograft vasculopathy (CAV) was higher by ERA and by female gender. The causes of death were stable in the last year with prevalence of graft failure, followed by infections.

In 2013, the ISHLT Registry focused a peculiar study on the relevance of age. Interestingly in the recent years, the graft survival rate was not statistically influenced by recipient age, except 18-39 years compared to 60-69 years. On the contrary donor age had significant impact on the graft survival. CAD was the leading cause of HTx for patients aged 60-69 years (53%).

In the recent years an increase of both donor and recipient age has been registered. The most striking variation for elder patients has been observed as the percentage of patients bridged with MCS. By 2012 almost 40% of patients ageing 60-69 years were on MCS prior to transplantation, while only 15% of patients had similar support by 2006. Leading causes of death for patients ageing 60-69 years were graft failure and infections. The elder patients had also more

malignancies and after 10 years only 50% of patients were free from malignancies.

Mechanical circulatory support

As aforementioned in recent years we observed an impressive advance in MCS devices and, overall, newer MCS devices are smaller and more reliable than the first generation of technological devices. Increasing number of reports conclude that in some cases of heart failure, the devices may be used not only as bridges to transplantation, but also as destination therapies[107]. A new device, the Heart Ware Ventricular Assist System is a miniaturized implantable continuous flow blood pump and in 332 patients in a pivotal bridge to transplant demonstrated a high 180-d survival rate[108]. This and other mechanical supports were examined in a recent paper[109] which led to the conclusion that patients with mechanical support, despite being older and less favorable recipients, spent more time in status 1A and had greater waitlist survival.

In a systematic review, Sutcliffe *et al*[110] tried to evaluate the clinical effectiveness and cost effectiveness of last generation MCS as either bridge to transplant (BTT) or alternative to transplant (ATT). The authors concluded that MCS as BTT compared with medical management are effective but with higher cost-effectiveness ratio. MCS as ATT have a reduced cost, but cause reduced quality of life. Considering the wide use of MCS, with the intent to regularize its use, in 2013 ISHLT published the Guidelines for the use of MCS[111].

Prediction of mortality and cardiac allocation score

As a consequence of the aforementioned variables impacting on heart graft survival, several attempts have been made to evaluate the mortality prediction after heart transplantation. In 2013 the Index for Mortality Prediction after Cardiac Transplantation (IMPACT) score was validated using international data[112]. This study validated the use of the IMPACT score as a predictor of short- and long-term mortality after orthotopic heart transplantation.

Other scoring modalities, in addition to the IMPACT score, are the Heart Failure Survival Score, the Seattle Heart Failure Model and the Interagency Registry for Mechanically Assisted Circulatory Support. All these scores were evaluated in a Eurotransplant pilot study for predicting waiting list mortality among heart transplant candidates and among transplanted patients[113]. In non MCS patients all the scores provide accurate risk stratification. The authors conclude that further studies are needed to reveal whether these models should be considered the basis for a new heart allocation policy.

ABMR in heart transplantation

Previous studies have documented that the presence of de novo donor HLA specific antibodies after HTx is an independent predictor of poor survival[114]. Similarly the detection of Luminex positive DSA in pre-transplant serum is a negative predictor of mortality[115] and also IgM non HLA antibodies have been identified as a risk for early allograft failure[116].

Nevertheless in the last Banff Conference[39] it was observed that lacking of search for DSAs or C4d staining are limiting factors to identify ABMR in heart transplantation. While biopsies positive for C4d and C3d are strongly associated with DSAs and allograft dysfunction and represent true ABMR, biopsies only positive for C4d are mostly subclinical. On a morphologic basis, is not possible to designate the latter as accommodation *vs* subclinical ABMR. Moreover there is also uncertainty about the management of subclinical ABMR. To this end the American Heart Association will be publishing a scientific statement evaluating clinical and pathological evidence regarding ABMR.

The ISHLT working formulation for the standardization of nomenclature of ABMR in heart transplantation has published a consensus paper by the end of 2013[117]. As ISHLT itself recognizes is hard to date to make a definitive statement on this issue and there remain numerous challenges and unresolved clinical, immunologic and pathologic questions. Moreover, there is no hard evidence of a direct causality between ABMR and CAV, neither any systematic study of antibody-dependent cellular cytotoxicity as an alternative mechanism linking antibodies to CAV[39].

Chronic cardiac allograft rejection: new insights

Several papers in 2013 have treated new findings on chronic cardiac allograft rejection. A review by Costello *et al*[118] recognized that chronic rejection in the form of CAV is one of the major factors that affect the long-term graft and patient survival. Whereas multiple factors (hyperlipidemia, cytomegalovirus, baseline coronary artery disease) contribute to the development of CAV, immunologic mechanisms play the prevalent role.

Using the intravascular ultrasound (IVUS) to evaluate intimal thickening, some recent studies have validated the use of everolimus (EVR) with reduced-dose cyclosporine (CsA)[119,120]. These studies documented a similar efficacy of EVR with reduced-dose CsA to Mycophenolate Mofetil (MMF) with standard-dose CsA and a reduced intimal proliferation at 12 mo in *de novo* heart transplant recipients. However, these studies have been criticized[121] both because IVUS was made only in a subgroup of patients and because IVUS was performed only at 1 year post-transplant.

Finally, the technique of optical coherence tomography has been proposed to evaluate cardiac allograft vasculopathy[122]. This is a new technique to assess early morphologic changes, but its clinical predictive value remains to be determined.

NEW INSIGHTS FOR LUNG

Figures, characteristics and trends for lung transplantation

In United States lung transplants are increasingly used as treatment for the end-stage lung diseases. Lungs are allocated to adult and adolescent transplant candidates on the basis of age, geography, blood type compatibility and

the Lung Allocation Score (LAS)[123]. The overall median waiting time in 2012 was 4 mo, and 65.3% of candidates underwent transplant within 1-year of listing. Both graft and patient survival rates have continued to improve; survival rates for recipients aged 6-11 years are better than those of younger recipients. Similarly as for the heart by the end of 2013 the data of the ISHLT Registry have been published also for the lung[124].

Obstructive pulmonary diseases (COPD), interstitial pulmonary fibrosis (IPF) and cystic fibrosis (CF) are among the most common causes of LuTx. COPD represents one of the most common indications for LuTx and accounts for one/third of all the procedures[125]. Worldwide a recent analysis of all the recipients reported that 23% had IPF and 3% pulmonary artery hypertension[126]. LuTx has become an excellent treatment option for patients with CF and bronchiectasis. In these patients survival is more favorable than that seen in patients with COPD and IPF[127].

In recent years there has been a significant increase of recipient's age (24% ageing 60-65). As a consequence there was an increase of patients transplanted for COPD, for IPF and for re-transplantation. Though the patients with COPD, IPF and re-transplant have the worst survival, an increase of Kaplan Meier survival by ERA was registered. Recently has been reported an increase of bilateral/double LuTx with respect to single LuTx for all the primary diseases. As double LuTx is associated with an improved graft survival rate for any disease, this could be the cause for the improved survival rate observed in recent years.

Among the side consequences of lung transplantation, both a reduction in renal dysfunction and an increase of hyperlipidemia and diabetes has been registered and probably this fact is related to modification in the dose and type of immunosuppressant[124].

Donor selection and extended criteria donors

The scarcity of suitable donor organs limits lung transplantation[128]. To overcome this problem, recently there was an increased interest towards an expanded donor pool associated with the techniques aimed to evaluate an improve donor lungs as the availability of *ex vivo* lung perfusion (EVLP). The utilization rate of these lungs changed from less than 15% to 50%. It is now quite clear that many of the historical factors used to define a lung as "Extended" do not actually produces significantly worse outcomes.

In a review of the UNOS database[129], the outcomes after LuTx using donors aged 55 to 64 years, were similar to those observed with standard donors. In this review only the donors aged more than 65 years were associated with the decreased intermediate-term survival. In Eurotransplant in 2013 the Hannover center reported its results utilizing lungs turned down for donor-related medical reasons by 3 centers. The authors obtained excellent graft survival similar to the standard lungs and concluded that the rescue allocation donor lungs may be used safely and therefore salvaged for the donor pool[130].

New findings on recipients and LAS

The relevance of size-matching has been evaluated in an extensive study based on evidence-based reviews[131]. Unfortunately the authors conclude that the evidence base that informs the decisions regarding lung size mismatching is limited and composed primarily of small studies with heterogeneous groups of patients.

Currently data are lacking to give the surgeons robust guidelines to conduct decision making for size matching of donors and recipients. Among the pre-transplant variables that affect the survival after LuTx, markers of nutritional status are associated with poorer recipient survival. A recent paper[132] examined several variables associated with the nutrition, including body mass index, body surface area, albumin levels, total proteins and immunoglobulins. Although no nutritional variables were found to be associated with major post-operative complications or infections, a low serum albumin (< 3 mg/dL) was associated with increased risk of death. Even if the results of this study differ slightly from others studies[133]; the body of literature to date suggests that the nutritional status may affect post-transplant outcomes.

The LAS was developed in 2005 to reduce the mortality on the waiting list, to prioritize candidates basing on urgency, to minimize the role of geography and to maximize the transplant benefit. In prioritizing patients with the most urgent status, a new controversy has come into the forefront: whether or not the increased number of critically ill recipients maximizes the transplant benefit. Despite the controversy, the LAS system is an improvement compared with the traditional first-come, first-served system and it has been adopted by UNOS and Eurotransplant[134]. A recent review of the UNOS data[135] concluded that social disparities in lung transplantation have decreased with the implementation of LAS; however, gender disparities (in favor of men) may have actually increased in the LAS ERA.

Primary graft dysfunction, ABMR and chronic allograft dysfunction

Primary graft dysfunction (PGD) is a syndrome encompassing a spectrum of mild to severe lung injury that occurs within the 72 h after LuTx. In addition, PGD has a significant impact on the short and long-term outcomes[136].

The pathogenesis of PGD is complex and influenced by donor, recipient, technical factors and by different combinations of all the above. PGD is driven by an inflammatory response as well as by immunological (both innate and cell mediated) processes[137]. Several strategies have been investigated to prevent and treat PGD[138]. These strategies will be discussed in the therapy chapter.

Allograft rejection is a major cause of a limited survival rate in LuTx. Moreover, the acute rejection represents the principal risk factor for chronic rejection[139]. Acute cellular rejection (ACR) is defined as a perivascular or peribronchiolar lymphocytic infiltrates primarily diagnosed by bronchoscopic transbronchial biopsies[140]. ACR involves several T-cell subtypes and several cytokines.

Data suggest a correlation between acute rejection and effector memory T cells in LuTx and the measurement of peripheral blood CD8+ effector memory T-cells before

LuTx may define the patients at high risk for ACR[141].

The study of Krustrup *et al*[142] documented the association between the distribution of Tregs in the transbronchial biopsies and the level of FoxP3 mRNA in the bronchoalveolar lung fluid (BALF). This indicates that Tregs may play a role in the cellular processes that affect ACR and that looking for FoxP3 mRNA in BALF is a reliable non-invasive method for evaluating the number of Tregs in lung tissue.

Higher values of CXCL10 (IP-10) in BALF are associated with ACR in LuTx suggesting a potential mechanistic role in the pathogenesis of ACR[143]. These results suggest that therapeutic strategies to inhibit CXCL10 (IP-10) and/or its cognate receptor (CXCR3) warrant investigations to prevent and/or treat the ACR in LuTx.

Some retrospective studies conducted and published in 2013 highlighted the relevance of ABMR in LuTx. In one study[144] a clear association between DSAs, ABMR, ACR, bronchiolitis obliterans syndrome (BOS) has been documented. Another study[145] identified ABMR in 21 recipients basing on the presence of HLA-DSAs, the histological evidence of acute lung injury, C4d deposition and clinical allograft dysfunction. In this study the majority of patients who recovered from ABMR, developed chronic lung allograft dysfunction (CLAD) during the follow-up.

Due to the relevance of the syndrome, the Pathology Council of the ISHLT elaborated the Consensus points for pathologic diagnosis of pulmonary ABMR[146]. The conclusions were: (1) The diagnosis of pulmonary ABMR requires a multidisciplinary approach that includes the presence of clinical allograft dysfunction, circulating DSAs and pathologic findings; (2) The histopathology findings in ABMR are non-specific patterns of injury that can be seen also in disorders such as severe ACR, infection, graft preservation injury and drug reaction; and (3) Positive capillary C4d staining should be always reported.

The last Banff conference[39] reviewed the Pathology Council survey and added that the early detection of DSAs following LuTx and the systematic monitoring with sensitive solid-phase platforms are recommended[147]. The overall conclusions revealed that to date survival is poor after ABMR but may improve with the rapid clearance of the antibodies[145].

Important unanswered questions include: (1) How to grade graft dysfunction; (2) What constitutes a significant mean fluorescent intensity of DSAs; (3) How to manage the patient in whom there is discordance between the criteria enumerated; and (4) What's about the non-HLA targets, principally because, according many authors, the BOS is the result of humoral response against non-HLA molecules[148].

CLAD continues to be the major limitation to long-term survival[149]. Its pathogenesis is complex and involves both alloimmune and non-alloimmune pathways. In particular, acute damage to the allograft, including episodes of acute rejection, PGD, cytomegalovirus (CMV), pneumonitis, gastro esophageal reflux and early and late new-onset diffuse alveolar damage have all been shown to increase the risk of CLAD[150].

BOS, characterized by obstructive physiologic changes, is the conventional form of CLAD. Increasing evidence, however suggests that CLAD is a heterogeneous condition and that BOS is not the only form of CLAD. While BOS itself has been recently redefined as neutrophilic reversible allograft dysfunction (NRAD)[151], Sato *et al*[152,153] recently identified a type of CLAD who showed restrictive physiology and peripheral lung fibrosis and named this condition "restrictive allograft syndrome" (RAS). The prognosis of RAS is poor and more severe than that of NRAD.

As already mentioned the pathogenesis is multi-factorial and recently has been documented that acute rejection, lymphocytic bronchiolitis, colonization with Pseudomonas, infection and BALF eosinophilia and neutrophilia are risk factors for both RAS and NRAD[154]. Moreover, immunologic factors as complement activation[155] and the defensins have been implicated in the pathogenesis of CLAD[156].

NEW INSIGHTS ON IMMUNOSUPPRESSIVE THERAPIES IN SOLID ORGAN TRANSPLANTATION

This chapter may be divided into two paragraphs: (1) Old drugs recently revised and used in new strategies; and (2) New drugs recently introduced on the market or still waiting for their approval.

Old drugs recently revised and used in new strategies

The concept that the chronic loose of renal function after kidney transplantation (KTx) should be ascribed to chronic renal calcineurine inhibitors (CNIs) nephrotoxicity, led to a number of trials attempting to avoid or withdraw CNIs from the maintenance immunosuppression therapy.

With the exception of few trials all these attempts documented that to date is not yet the time to give up with CNIs[157]. Moreover, in 2013 a meta-analysis[158] has not documented a favorable effect of CNIs reduction on kidney function in HTx.

Many trials of CNIs reduction have been made thanks to the use of mammalian target of rapamycin inhibitors (mTORIs), a class of drugs devoid of CNIs side-effects. Overall an analysis of 139370 United States kidney transplant recipients documented that the complete substitution of CNIs with mTORIs was associated to a greater risk of allograft failure and death[159].

The use of mTORIs in LTx led to contradictory results. In a phase II prospective randomized trial[160] the use of sirolimus with reduced dose of tacrolimus (TAC) in the de novo liver transplant recipients was associated with higher rates of graft loss, deaths and sepsis when compared to the use of the conventional dose of TAC.

In the recent H2304 trial[161,162] liver transplant patients randomized to EVR with TAC elimination showed strikingly good renal function at 2-year post-transplant, but this treatment group was terminated due to a higher rate of acute rejections. However, there was no significant

difference between the EVR and reduced TAC *vs* TAC control group[163]. The study Preservation of Renal function in liver Transplant recipients with Certican Therapy (PROTECT)[164] documented that an EVR-based CNI-free immunosuppression is feasible following LTx and the patients benefit from sustained preservation of renal function when compared to patients on CNIs, for at least three years.

The discrepancies between the results of H2304 and PROTECT studies could be explained by the use of Il-2 receptor antibody only in the latter study and in the abrupt TAC withdrawal in the former.

The contradictions in the use of mTORIs in LTx have been examined in an editorial of Levitsky *et al*[165]. Probably like any other drug with a narrow therapeutic window, mTORIs must be used in the right amount, right time period and right patient. Right amount is without a loading dose and targeting moderate trough levels. Right time is neither too early nor too late after LTx. The right patient is the one who is at high risk to develop nephrotoxicity.

Several studies document the attenuation of cardiac allograft vasculopathy by mTORIs. A study from Matsuo *et al*[166] documented the usefulness of sirolimus in the case of early initiation. As aforementioned, the recent most important contributions in this field are the Eisen *et al*[119] and Kobashigawa *et al*[120] studies.

They documented the efficacy of EVR with reduced-dose CsA, similar to MMF + standard dose CsA. Patients treated by EVR had reduced intima proliferation. Recently the use of mTORIs in the treatment of lung transplant recipients is an area of active investigation[167,168]. Newer researches involving the use mTORIs or antimetabolites have been made in the treatment and prevention of BOS[169,170]. In a recent review[171], Borro highlights that one of the advantages in LuTx is the administration of the treatments *via* the inhalator route.

A randomized, prospective study of inhalator CsA *vs* placebo documented significant improvements concerning survival and BOS free interval[172]. Inhalator corticosteroids have been suggested in the lymphocytic bronchiolitis, based on the possible reduction of the airway inflammatory markers[173].

Immune modulating and beneficial effect in LuTx have been documented for the statins and Azithromycin. Concerning statins, some groups have considered adding such treatment on a systematic basis in the patients with suspected or confirmed BOS[174]. Principally in patients with an increased bronchoalveolar lavage neutrophilia, azithromycin could prevent BOS, most likely through its interactions with the innate immune system[175].

The finding of the relevance of DSAs in determining ABMR and reduced graft function for any transplanted organ led to search for new strategies in organ immunosupppression. A systematic review[176] on the induction therapy in HTx concluded that acute rejection might be reduced by IL-2R antibodies compared with no induction and by the antithymocyte globulin (ATG) compared with IL-2R antibodies. Similarly, the depleting antibody induction has become the mainstay of immunosuppression in pancreas TX[177].

In KTx the use of ATG is associated with a significant reduction of DSAs and ABMR[178]. The Alemtuzumab induction therapy obtains similarly good results in a systematic review[179]. Further induction trials in the attempt to prevent ABMR with rituximab are ongoing, including the Rituximab Induction in Renal Tx (ReMIND) trial (Clinical-Trials.gov No. NCT01095172)[180,181]. No result has been obtained with Rituximab in the treatment of ABMR as reported from a phase Ⅲ multicenter, randomized, placebo-controlled trial (RITUX ERAH)[182].

New drugs recently introduced in the market or still waiting for approval

Prevention and treatment of ABMR: Eculizumab, the humanized anti C5 antibody is among the new drugs recently used in the prevention of the ABMR in KTx. Its efficacy was recently assessed in one study[183]. There is an ongoing, multicenter, international, randomized trial testing the role of eculizumab that may clarify its utility (NCT00670774)[184].

Limited clinical trial evidence suggests that the proteasome inhibitor Bortezomib may be useful to treat the ABMR following KTx[185]. Agents targeting the B activating factors belonging to the TNF Family (BAFF) pathway which co-stimulates B cell survival and expansion are also in the clinical development as atacicept and belimumab[186].

A further possibility in the field of ABMR is complement inhibition by C1-esterase inhibitors. A trial studying the safety and tolerability of the C1 inhibitor therapy in the prevention of the acute rejection is now ongoing (Clinical Trials gov NCT01134510).

New drugs in KTx: Belatacept, a fusion receptor protein that blocks the co-stimulation pathway CD80/CD86-CD28, was recently approved for the prevention of acute rejection in KTx. In 2013 two papers reported the results at 5 years of immunosuppression with belatacept + MMF and steroids respect to standard CsA maintenance immunosuppression[187,188]. Continued treatment with belatacept was associated with a consistent safety profile and sustained improvement in renal function *vs* CsA overtime.

In a smaller study Kirk *et al*[189] documented the feasibility of an immunosuppressive therapy in KTx with belatacept only, without maintenance steroids or CNIs after alemtuzumab induction. Another co-stimulation pathway is the CD40/CD40L pathway. Humanized anti CD40 antibodies prevented the acute rejection and prolonged the renal graft in non-human primates. In addition, these anti-CD40 antibodies appear safe and effective as maintenance immunosuppressive therapies[190,191]. To date 5 monoclonal antibodies directed against CD40 have been studied for different diseases including KTx (ClinicalTrials.gov NCT01780844).

Alefacept is a recombinant LFA3/IgG1 fusion protein that reduces the number of memory T cells. After its

successful use in psoriasis, a recent study evaluated the efficacy of alefacept when combined with TAC, MMF and steroids in renal transplant patients[192]. Six-month efficacy, safety and tolerability were similar to control group, but the trial was too short to draw conclusions.

Janus kinase (JAKs), are a cytoplasmic tyrosine kinases that participate in the signaling of a broad range of cell surface receptors. JAK3 inhibition by tofacitinib in KTx trials in humans[193,194] have demonstrated tofacitinib to be non inferior to CsA for rejection rates and graft survival, however there was a trend towards more infections.

Sotrastaurin (AEB071) is a small molecular weight immunosuppressant that blocks the early T cell activation through selective inhibition of protein kinase C, crucial for IL-2 and interferon gamma production. In a phase Ⅱ trial[195] sotrastaurin at a dose of at least 200 mg/d + reduced TAC had comparable efficacy to mycophenolic acid (MPA) in prevention of rejection. In another phase Ⅱ study[196] sotrastaurin + everolimus compared to CsA + EVR had higher efficacy rates failure.

New drugs in pancreas Tx and ICTx: In pancreas Tx, after induction therapy the most widely used maintenance protocols are based on TAC and MMF with steroid withdrawal[197]. Considering the recent documented negative impact of DSAs on pancreas Tx, whether promising novel agents such as sotrastaurin, tofacitinib, belatacept, bortezomib or eculizumab will prove to be beneficial for pancreas Tx requires further investigations.

A long-term insulin-independence after ICTx was documented in 10 patients adding efalizumab or belatacept to the standard immunosuppression[64]. In another study[65] efalizumab was compared to belatacept and has been documented that efalizumab increases percentages of the circulating Tregs and profoundly suppresses T-cell reactivity, thus promoting the transplantation tolerance.

Combining anti-inflammatory biologics to maintenance immunosuppression has led to improved success rate. Naziruddin *et al*[66], adding etanercept (TNF alpha antagonist) to immunosuppression obtained protection from inflammatory reaction during the peritransplant period. The same authors obtained an even better protection adding Anakinra (IL-1 beta blocker) to Etanercept[66]. Another group obtained excellent results adding Reparixin (CXCL8 inhibitor) to the immunosuppressive therapy[67,68].

The stabilization of Glucagon-Like-Peptide-1 (GLP-1) by inhibiting Dipeptidyl Peptidase Ⅳ by sitagliptin increases beta cell mass by modulating vascularization[198]. To date two official trials are ongoing on the effect of sitagliptin (NCT00853944 and NCT01186562).

New drugs in LTx: In liver transplantation new drugs have been principally used to protect the IRI. Attempt to protect the IRI may involve several strategies and several pathways[92-95]. Elias-Miro *et al*[92] evaluated antioxidant strategies to reduce the oxidative stress. The positive Pentoxifylline effect seems to be related to the inhibition of

TNF alpha according Genoves *et al*[93].

Tiriveedhi *et al*[94] found a protective effect of Bortezomib on IRI. This proteosoma inhibitor effectively attenuates the IRI by inhibiting the matrix metalloproteinase and the chitinase 3-like 1 (YLK-40) both involved in the extracellular matrix deposition and fibrosis principally in steatotic livers. The complement pathway is also involved in the IRI and a recent and promising study in the mice[95] documented that the C1-esterase inhibitor administration attenuates the liver injury compared to controls.

New drugs in LuTx: New drugs in the field of LuTx are represented by pirfenidone and the C1 esterase inhibitor. Pirfenidone, a small synthetic non peptide molecule demonstrated a potent antifibrotic effect by inhibiting the transforming growth factor beta (TGF beta) and TNF alpha, important mediators of fibrosis and inflammation. Its usefulness has been principally suggested in the lung transplant patients with RAS[199].

Over the last few years, the development of innovative techniques such as EVLP or the refinement in the artificial support methods as Extracorporeal Membrane Oxygenations also contributed to treat and redefine the outcomes of patients with PGD. A very recent study by Sommer *et al*[138] reported a trial with C1-esterase- inhibitor in patients affected by severe PGD. The one year survival was significantly higher than that of not treated patients.

NEW INSIGHTS ON TRANSPLANT TOLERANCE

One of the hallmarks of the adaptive immune system is its ability to recognize a vast number of different antigens. This ability is a consequence of the large lymphocyte repertoire, in which each cell has a different antigen receptor generated by the process of somatic recombination. This process is able to produce an estimate of 10^{15} different lymphocyte clones, each with a different antigen receptor that can hypothetically recognize any naturally occurring structure[200]. Since the somatic recombination is a random process, it generates T cell clones that can recognize self-structures or self-peptides (auto antigens). The mechanism used by the immune system in order to avoid a possible harmful immune response against an individual's own cells and tissues, is known as the immune tolerance and can be classified into central and peripheral tolerance.

Immune tolerance in transplantation is defined as a specific absence of a destructive immune response to a transplanted tissue without immunosuppression. Operative criteria are the complete withdrawal of immunosuppression followed by no evidence for rejection for the transplanted organ for over one year. In humans is characterized by specific *in vitro* non-responsiveness to the donor.

Induction of tolerance differs according the transplanted organ. Indeed, although up to 20% of liver transplant recipients may be successfully withdrawn from immunosuppression[201]; operational tolerance to renal allograft appears to be much

less frequent. In a recent review, Ruiz *et al*[202] reviewed the new strategies to induce the long-term acceptance to organ transplantation. These include: (1) Mixed chimerism as a strategy to induce allograft tolerance; (2) Dendritic cells and Regulatory Macrophages; (3) Exosomes and Phagosomes as tools for alloantigen delivery; (4) Apoptotic cells; (5) Regulatory T cells; and (6) Mesenchimal Stromal/Stem cells.

In the recent American Society of Transplantation (AST) Cutting Edge of Transplantation meeting, held in Arizona (US) February 13th-15th 2014, the best approaches to induce renal allograft tolerance have been reviewed. They are principally two: (1) Tolerance through induction of durable chimerism. In HLA disparate patients the protocols to date principally used are the Massachusetts General Hospital and the Northwestern University protocols; and (2) Immunomodulation through use of donor hematopoietic stem cells, as the Northwestern University protocol.

Mixed chimerism is defined as the coexistence of donor and recipient hematopoietic cells after allogeneic bone marrow transplantation (BMT). To be considered mixed chimerism, donor cells in the blood must represent more than 1% of the total cells. To induce a state of mixed chimerism, it is necessary to perform a conditioning treatment in order to allow the donor bone marrow acceptance. Currently used mixed chimerism protocols induce robust donor-specific tolerance and allow long-term acceptance of fully mismatched skin grafts in murine models[203].

Recently Kawai *et al*[204] reported the results of a study of combined kidney and bone marrow transplantation without maintenance immunosuppression. The conditioning regimen consisted in cyclophosphamide, thymic irradiation, antiCD20 monoclonal antibody and an 8 to 14 mo course of CNIs.

The major problems encountered with these protocols have been "the engraftment syndrome" which causes transient renal dysfunction[205] and the occurrence of low levels of DSAs after discontinuation of immunosuppression. To overcome the engraftment syndrome, the authors have considered the use of low-dose total-body irradiation rather than cyclophosfamide as preconditioning treatment. DSAs occurrence caused an increase of anti CD20 administration.

As myeloablative conditioning is not ethically accepted due to the high risk involved in this type of conditioning, non myeloablative conditioning has emerged as an alternative to induce tolerance through mixed chimerism. Using a simultaneous bone marrow and kidney transplantation and a preconditioning protocol consisting in the co-stimulatory blockade with anti CD154 antibody, Kawai *et al*[206] and Wekerle *et al*[207] achieved the establishment of mixed chimerism in non-human primates. Later on, Kawai *et al*[208] reported tolerance induction using pharmacological immunosuppression and thymic irradiation. The main obstacle remains the presence of the memory T cells that can cross-react with alloantigens[209].

Other immunomodulatory cells with a high potential in future therapies in transplantation are hematopoietic mesenchimal stem cells (MSCs). It is well known that bone-marrow derived MSCs have the capacity to migrate to inflammatory sites and regulate the function of most immune cells through direct contact and/or by cytokine secretion[210].

Leventhal *et al*[211] developed an approach using a bioengineered mobilized cellular product enriched for hematopoietic stem cells (HSC) and tolerogenic CD8 positive/T cell receptor (TCR) graft facilitating cells (FCs), combined with non-myeloablative conditioning. This allows the engraftment, a durable chimerism, and the tolerance induction in highly mismatched related and unrelated donor-recipient pairs.

The same author[212] reported in 2013 an intermediate-term follow up of this phase Ⅱ trial. All 20 patients demonstrated donor specific hypo-responsiveness and were weaned from full-dose immunosuppression. Complete immunosuppression withdrawal at 1 year was successful with durable chimerism in the majority of patients. No graft *vs* host disease or engraftment syndrome has been reported. In all the cited studies a predictive biomarker for success *vs* failure in weaning immunosuppression has not been reliably identified and validated so as to be used as a tool to discontinue immunosuppression.

Leventhal *et al*[213] documented that durable chimerism predicts the outcome. Moreover, the immune/inflammatory gene expression in the peripheral blood and urine were differentially down regulated between tolerant and non tolerant recipients. As aforementioned memory T cells (Tm) represent a major barrier for immunosuppression and tolerance induction after solid organ transplantation. Taking into consideration the critical role of the intrinsic apoptosis pathway in the generation and maintenance of Tm, Cippà *et al*[214] developed a new concept to deplete alloreactive Tm by targeting B Cell Lymphoma-2 (Bcl-2) proteins. The small-molecule Bcl-2/Bcl-XL inhibit ABT-737 efficiently induced apoptosis in alloreactive Tm *in vitro* and *in vivo* and prolonged skin graft survival in sensitized mice. Since Bcl-2 inhibitors yielded encouraging safety results in cancer trials, this novel approach might represent a substantial advance to prevent the allograft rejection and induce tolerance in sensitized recipients.

The mechanisms above mentioned to induce tolerance are almost the same for the liver, even if the liver has particular tolerogenic properties that allow its being spontaneously acceptable in some animal species. The liver structure is considered to favor a tolerogenic environment. Indeed several studies demonstrated that the liver capacity to induce tolerance partly results from the in situ T-cell activation. The hepatocytes, as non-professional antigen presenting cells (APCs), may play key roles in regulating the immune responses and facilitating tolerance induction[215]. Warren *et al*[216] documented that the intrahepatic lymphocytes and the circulating naïve CD8+ cells could interact with the hepatocytes by means of cytoplasmic extensions capable of going through the liver sinusoidal endothelial cells fenestrations. This local activation of T cells by the hepatocytes provides the latter with a significant role as APCs and induces tolerance development in the liver[217]. The peripheral tolerance

mechanisms also play a role in liver graft spontaneous tolerance. As for kidney, also for the liver the most significant mechanism in the tolerance induction is the chimerism[218]. In humans BMT-induced mixed chimerism has been shown to confer the acceptance of donor liver allograft without long-term immunosuppression. However, recipients must be able to withstand the conditioning regimens that allow donor stem cell to engraft.

NEW INSIGHTS ON MAJOR COMPLICATIONS IN TRANSPLANTED PATIENTS: INFECTIONS AND CANCERS

Infections
Infections post solid organ transplantation (SOT) is one of the more important complications. In 2013 many papers have been published on this topic. Among these, the most relevant, in our opinion are: (1) The publication of the third Edition of the American Society of Transplantation on Infectious Disease Guidelines[219]; (2) the publication of the Public Health Service (PHS) Guidelines for Preventing Transmission of Human Immunodeficiency Virus (HIV), HBV and HCV through organ transplantation[220]; (3) the International Consensus Guidelines on the Management of CMV in SOT[221]; and (4) an overview on CMV and the Herpes Viruses in transplantation[222].

Two main factors increase the risk for transplanted patients for infections following transplantation: (1) Risk related to the continuous expanding pool of marginal donors; and (2) Risk related to the requirement to increase immune suppression to treat rejection after SOT. In particular the use of antilymphocyte preparations and many of an increasing diverse list of biologic agents have been associated with an enhanced risk of infection[223].

Overall the risk factors that predisposes to infections in the recipients of SOT may be categorized as being present before transplant within the recipient and those secondary to intraoperative and post-transplant events[224]. Organ transplant recipients are at risk of acquiring pathogens from donors with active or latent infections at the time of the procurement. Examples of pathogens associated with expected donor-derived infections include CMV, Epstein Barr Virus and Toxoplasma. Of greater concern is the development of unexpected donor-derived infections from a growing number of pathogens, including Mycobacterium tubercolosis, Histoplasma, West Nile virus, HBV, HCV and HIV.

Although OPTN policy requires that all potential deceased organ donors are screened for HIV, HBV and HCV by serology, no current policy requires the use of nuclear molecular acid testing (NAT) for donor screening. In 2013 an electronic survey was sent to 58 Organ Procurement Organizations (OPOs) in the United States to assess the current screening practices[225]. All OPOs performed the required serology screening, even if only

52% performed NAT for HIV and HCV. Moreover, respect to a previous survey made in 2008[226], the number of OPOs performing NAT has increased and more OPOs are now testing all donors.

In 2013 the PHS published new Guidelines for reducing HIV, HBV and HCV transmission through organ transplantation[220]. These Guidelines superseded the 1994 PHS Guidelines[227]. Most significant changes are: (1) Expanding the Guideline to include HBV and HCV in addition to HIV; (2) Using factors known to be associated with an increased likelihood of recent HIV, HBV or HCV infection; and (3) Limiting the focus to organs and blood vessel conduit recovered for organ transplantation because the FDA implemented more comprehensive regulations for human cell and tissue products[228].

These guidelines include 34 recommendations on risk assessment of living and deceased donors; informed consent discussion with transplant candidates; testing of recipients' pre and post transplant, collection and/or storage of donor and recipient specimens and tracking and reporting of HIV, HBV and HCV.

Studies on specific pathogens
The human BK polyomavirus is the major cause of polyomavirus-associated nephropathy (PyVAN). Because effective antiviral therapies are lacking, screening kidney transplant patients for BKV replication in urine and blood has become the key recommendation to guide the reduction of immunosuppression in patients with BKV viremia. Retransplantation after PyVAN is largely successful, but requires close monitoring for recurrent BK viremia[229].

Sood *et al*[230] evaluated the relationship of pre-transplantation BK virus-specific donor and recipient serostatus to post-transplant BKV infection. Overall infection was highest in the D+R-group and lowest in the D-R-group. BKV serostatus may be used to risk stratify patients for post-transplantation infection.

CMV remains one of the most common complications affecting SOT, with significant morbidity and occasional mortality. In addition to the direct effects of CMV infection and disease, there are indirect effects, both general and transplant specific, which may significantly impact the outcomes.

An international panel of experts was convened by late 2012 to revise and expand evidence and expert opinion-based consensus guidelines. The reports of such recommendations have been published in 2013[221]. Viral culture of blood or urine has a very limited role for the diagnosis of the disease. Histology/immune-histochemistry is the preferred method for diagnosis of tissue-invasive disease. Quantitative nucleic acid amplification testing (QNAT) is preferred for diagnosis, decision regarding pre-emptive therapy and monitoring response to therapy. If QNAT is not available, antigenemia is an acceptable alternative.

Both universal prophylaxis and pre-emptive strategies are viable approaches for the prevention of CMV disease. For D+R- the use of either prophylaxis or pre-emptive therapy after kidney and liver transplant are recommended. For

D+R- the use of prophylaxis over pre-emptive therapy after heart and lung Tx is recommended. When a pre-emptive therapy strategy is used, it is recommended that the centers develop and validate their local protocol[231]. For non-severe CMV disease, Valganciclovir or intravenous Ganciclovir are recommended as first line treatment, while dose reduction of immunosuppressive therapy should be considered in severe CMV disease.

CMV vaccines are in preclinical, phase I and phase II trials[232,233]. The primary goal of a CMV vaccine should be to prevent or to modulate CMV replication and/or CMV disease. Herpes viruses infect most animal species. Infections due to the eight human herpes viruses (HHV) are exacerbated by immunosuppression in SOT. The special features of the herpes virus life cycle include the ability to establish latent, non-productive infection and the life-long capacity for reactivation to productive lytic infection. Interactions between the latent virus and the immune system determine the frequency and severity of symptomatic infection. In an overview Fishmann[222] reports how the immunologic and cellular effects of herpes virus infections contribute to risk for the opportunistic infections and the graft reactions. Among the most important advances in transplantation are laboratory assays for the diagnosis and monitoring of herpes virus infections and antiviral agents with improved efficacy in the prophylaxis and therapy.

HCV infection is common in SOT recipients and is a significant cause of morbidity and mortality after transplantation. The severity of HCV infection in liver transplantation has been already discussed in the liver chapter. Carbone *et al*[234] reviewed the extent of the problem in donors, kidney, heart and lung transplant candidates.

In HCV-infected kidney allograft recipient, the progression of fibrosis should be evaluated serially. Transplantation of kidneys from HCV positive donors should be restricted to HCV positive recipients. HCV antiviral therapy should be considered for all HCV-RNA positive kidney transplant candidates. The impact of HCV infection on survival in heart and lung transplantation is unclear but even assuming a worse survival in those receiving HCV-infected organs, it has not been evaluated whether they do better or worse than those remaining on the waiting list.

Cancers

Malignancies after SOT are divided into three chapters: donor transmission of cancer, recipients with prior cancer and general epidemiology of cancers after SOT.

Donor transmission of cancers: Xiao *et al*[235] reviewed all case reports, case series and registry studies that described the outcomes of the kidney transplant recipients with donor cancer transmission published up to December 2012. The most common transmitted cancer types were renal cancer, followed by melanoma, lymphoma and lung cancer. Overall the risk of donor transmission of cancer appears low, but there is a high likelihood of reporting bias. The findings of this review support the current recommendation for rejecting organs from donors with a previous history of melanoma and lung cancer, but suggest that the use of donor kidneys with a history of small, incidental renal cell cancer may be reasonable.

At the 2013 American Transplant Congress (ATC), Desai *et al*[236] analyzed data from 30000 recipients of SOT from more than 14000 donors in the National Transplant Registry (NTR) in the United Kingdom to determine whether the risk of cancer transmission from organ donors could be eliminated. They found a very low rate of donor-origin cancer: only 0.6%. The risk of cancer transmission cannot be eliminated because the presence of cancer was not known at donation. This finding is useful to obtain an informed consent for prospective recipients, but in transplants other than kidney and pancreas, the benefits should be planned against the risk of remaining on the waiting list.

In another study the same group looked at donor transmission in a different way, linking donor data to the cancer registries, to determine the risk for donor transmission to the recipients analyzing more than 17000 donors[237,238]. More than 200 (about 1.5%) had a cancer history. Although 61 of these donors were at high risk for transmission, none transmitted their cancer to any of the recipients. These data raise the question about whether we are being too strict and losing potential donors. To put this in context, the death rate on the waiting list is 5% to 15% per year compared with this very low rate of donor transmission of cancer.

At 2013 ATC, Engels *et al*[239,240] analyzed data from the SOT registry in the United States to link donor organs to 15 cancer registries. They concluded that recipients of donors with the cancer did not have significantly increased incidence of cancer compared with the recipients whose donors did not have cancer.

Risk of recurrence of preexisting cancer in organ recipients: Again Desai *et al*[241] analyzed data from NTR in United Kingdom on the issue of the recurrence of a preexisting cancer in an organ transplant recipient. They identified 64 (1.32%) recipients with a history of cancer diagnosed before organ transplantation.

Five recipients developed cancer recurrence and the rate of recurrence within 10 years was 11.9%. This study is interesting because data on this topic are sparse, and it's increasingly become a problem for nephrologists as the ESRD population ages and the burden of co morbidity in KTx candidates is increasing. Although this is a small cohort, the data are useful because this is one of the only contemporary studies of cancer recurrence risk in SOT recipients.

De novo post-transplant malignancies

De novo post-transplant malignancies (PTM) are a serious complication post-transplantation. In an analysis of the US National Transplant Data, Sampaio *et al*[242] analyzed 200000 recipients of kidney, liver, heart and lung. The PTM incidence was 8.03, 11, 14.4 and 19.8 in KTx, LTx, HTx and LuTx respectively. The PTM recipients were older,

mostly white and males in all SOTs.

A cohort study was conducted in Australia using population based, liver and cardiothoracic registries[243]. During a median 5-year follow-up, the risk of any cancer in the liver and cardiothoracic recipients, was significantly elevated compared to the general population (Standardized Incidence Ratio = 2.62). An excess risk was observed for 16 cancer types, predominantly cancers with a viral etiology. The adjusted HR for any cancer in all recipients was higher in heart compared to liver (HR = 1.29). Understanding the factors responsible for the higher cancer incidence in cardiothoracic compared to liver recipients has the potential to lead to targeted cancer prevention strategies in this high risk population.

Two interesting presentations at the ATC 2013 focused the association between the development of a skin cancer and the subsequent development of a solid organ tumor. Cho *et al*[244] analyzed data from OPTN/UNOS database and compared the incidence of solid tumors in organ recipients with and without melanoma skin cancer (NMSC). Developing a skin cancer was a risk factor for developing a solid tumor: 9.4% in those who developed a skin cancer *vs* 3.3% in those who did not.

A very similar study was conducted in Australia. McDonald *et al*[245] analyzed the data from Australia and New Zealand Dialysis and Transplantation (ANZDATA). They found that having a NMSC increased the risk of other cancers by 1.2%. These studies are interesting because skin cancers may be a useful tool to identify people at higher risk for developing other cancers.

The International Transplant Skin Cancer Collaborative (ITSCC) and its European counterpart: Skin Cancer in Organ Transplant Patients Europe (SCOPE) held by the end of 2012 a joint meeting that has been recently published[246].

The cutaneous squamous cell carcinoma (CSCC) incidence has been previously ascribed to immunosuppressive therapies. The decreased immunosurveillance by innate and adapted immune cells has been investigated and the specific role of macrophages. The direct effect of immune suppression on keratinocyte development has been postulated as well. Because of the need of CSCC epidemiology studies, was outlined an international collaboration between ITSCC and SCOPE to prospectively study CSCC in transplant patient.

CONCLUSION

In few fields of human medical knowledge, the science is so rapidly evolving as in organ transplantation. In this review the principal news that occurred by 2013 are described. By, because some news refers to meeting, consensus conference or guidelines held in the late 2012 but published in 2013; others on the contrary were held in 2013, but published in the first months of 2014.

In these conclusions we highlight several points, which in our opinion represent new frontiers in transplantation. While the donor pool is not as large as it would be necessary, the donor shift towards the so called ECD realize new problems

in the organ allocations and in the organ preservation, Relevant news has been found in the field of antibody mediated rejection, both acute and chronic. This kind of rejection involves any solid organ, even if the majority of studies have been done in the kidneys. A new Banff conference has been held in 2013 and new classifications have been made whenever possible.

The ischemia reperfusion injury concerns also any organ. In this field the majority of researches have been made in liver transplantation. The innate immunity is involved and new drugs have been found or are on clinical trials. Pancreas transplantation is now a therapeutic option also for T2DM, even if a limiting factor is the shortage of pancreas available. Islet cell transplantation is improving with new techniques for implantation and for microencapsulation.

Heart transplantation has now optimal graft survival rate and also the MCS is evolving so to represent an alternative to transplantation in addition to bridge to transplantation. New strategies for primary graft dysfunction in lung transplantation have been found as well as a better understanding of the different types of chronic allograft dysfunction. New drugs appear at the horizon, principally for kidney transplantation. In particular, drugs targeting the B cells and the complement pathway are interesting, considering the relevance of ABMR. Other drugs for different organs such as liver, pancreatic islet and lung are being studied in clinical trials. Anti-inflammatory drugs enhance the effect of the immunosuppressant drugs.

The knowledge on tolerance is improving either applying bone marrow cells or mesenchimal stem cells. The infections and the cancers remain among the principal drawbacks in transplantation and several meetings and conferences have been held principally to elaborate guidelines to check and control HCV, HIV, CMV and others HHV.

The need to realize international registries for an improved knowledge of cancer epidemiology has been stressed by several authors. Finally a point of weakness in the field of transplantation is the differences that exist among the countries in the world. The different transplant rate depends also by the fact that in several countries peoples do not reach end stage disease. This probably represents the hardest frontier to be afforded.

REFERENCES

1   OPTN/SRTR 2012 Annual Data Report Introduction. *Am J Transplant* 2014; **14** Suppl 1: 8-10 [DOI: 10.1111/ajt.12578]
2   **Kasiske BL**, Skeans MA, Leighton TR, Ghimire V, Leppke SN, Israni AK. OPTN/SRTR 2011 Annual Data Report: international data. *Am J Transplant* 2013; **13** Suppl 1: 199-225 [PMID: 23237702 DOI: 10.1111/ajt.12026]
3   **Garcia GG**, Harden P, Chapman J. The global role of kidney transplantation. *Lancet* 2012; **379**: e36-e38 [PMID: 22405254 DOI: 10.1016/S0140-6736(12)60202-5]
4   **Israni AK**, Zaun D, Rosendale JD, Snyder JJ, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: deceased organ donation. *Am J Transplant* 2014; **14** Suppl 1: 167-183 [PMID: 24373172 DOI: 10.1111/ajt.12585]
5   **Pondrom S**. Fewer living: a decline in living donors may be due to multiple factors. *Am J Transplant* 2014; **14**: 245-246

[PMID: 24472188 DOI: 10.1111/ajt.12657]

6   **Heaphy EL**, Goldfarb DA, Poggio ED, Buccini LD, Flechner SM, Schold JD. The impact of deceased donor kidney risk significantly varies by recipient characteristics. *Am J Transplant* 2013; **13**: 1001-1011 [PMID: 23406350 DOI: 10.1111/ajt.12154]

7   **Singh SK**, Kim SJ. Does expanded criteria donor status modify the outcomes of kidney transplantation from donors after cardiac death? *Am J Transplant* 2013; **13**: 329-336 [PMID: 23136921 DOI: 10.1111/j.1600-6143.2012.04311.x]

8   **Matsuno N**, Kobayashi E. Challenges in machine perfusion preservation for liver grafts from donation after circulatory death. *Transplant Res* 2013; **2**: 19 [PMID: 24283383 DOI: 10.1186/2047-1440-2-19]

9   **Bruns H**, Schemmer P. Machine perfusion in solid organ transplantation: where is the benefit? *Langenbecks Arch Surg* 2014; **399**: 421-427 [PMID: 24429900 DOI: 10.1007/s00423-014-1161-2]

10   **Rodrigue JR**, Schold JD, Mandelbrot DA. The decline in living kidney donation in the United States: random variation or cause for concern? *Transplantation* 2013; **96**: 767-773 [PMID: 23759882 DOI: 10.1097/TP.0b013e318298fa61]

11   **Tong A**, Chapman JR, Wong G, Josephson MA, Craig JC. Public awareness and attitudes to living organ donation: systematic review and integrative synthesis. *Transplantation* 2013; **96**: 429-437 [PMID: 23677051 DOI: 10.1097/TP.0b013e31829282ac]

12   **Campbell P**. Clinical relevance of human leukocyte antigen antibodies in liver, heart, lung and intestine transplantation. *Curr Opin Organ Transplant* 2013; **18**: 463-469 [PMID: 23838652 DOI: 10.1097/MOT.0b013e3283636c71]

13   **Süsal C**, Roelen DL, Fischer G, Campos EF, Gerbase-DeLima M, Hönger G, Schaub S, Lachmann N, Martorell J, Claas F. Algorithms for the determination of unacceptable HLA antigen mismatches in kidney transplant recipients. *Tissue Antigens* 2013; **82**: 83-92 [PMID: 23718733 DOI: 10.1111/tan.12137]

14   **Böhmig GA**, Fidler S, Christiansen FT, Fischer G, Ferrari P. Transnational validation of the Australian algorithm for virtual crossmatch allocation in kidney paired donation. *Hum Immunol* 2013; **74**: 500-505 [PMID: 23380140 DOI: 10.1016/j.humimm.2013.01.029]

15   **Blumberg JM**, Gritsch HA, Reed EF, Cecka JM, Lipshutz GS, Danovitch GM, McGuire S, Gjertson DW, Veale JL. Kidney paired donation in the presence of donor-specific antibodies. *Kidney Int* 2013; **84**: 1009-1016 [PMID: 23715120 DOI: 10.1038/ki.2013.206]

16   **Garonzik-Wang JM**, Sullivan B, Hiller JM, Cass V, Tchervenkow J, Feldman L, Baran D, Chaudhury P, Cantarovich M, Segev DL, Montgomery RA. International kidney paired donation. *Transplantation* 2013; **96**: e55-e56 [PMID: 24100847 DOI: 10.1097/TP.0b013e3182a68879]

17   **Sharif A**. Unspecified kidney donation--a review of principles, practice and potential. *Transplantation* 2013; **95**: 1425-1430 [PMID: 23778567 DOI: 10.1097/TP.0b013e31829282eb]

18   **Matas AJ**, Smith JM, Skeans MA, Thompson B, Gustafson SK, Schnitzler MA, Stewart DE, Cherikh WS, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: kidney. *Am J Transplant* 2014; **14** Suppl 1: 11-44 [PMID: 24373166 DOI: 10.1111/ajt.12579]

19   **Gondos A**, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation* 2013; **95**: 267-274 [PMID: 23060279 DOI: 10.1097/TP.0b013e3182708ea8]

20   **Willoughby LM**, Fukami S, Bunnapradist S, Gavard JA, Lentine KL, Hardinger KL, Burroughs TE, Takemoto SK, Schnitzler MA. Health insurance considerations for adolescent transplant recipients as they transition to adulthood. *Pediatr Transplant* 2007; **11**: 127-131 [PMID: 17300489 DOI: 10.1111/j.1399-3046.2006.00639.x]

21   **Kennedy J**, Erb C. Prescription noncompliance due to cost among adults with disabilities in the United States. *Am J Public Health* 2002; **92**: 1120-1124 [PMID: 12084695]

22   **Butler JA**, Roderick P, Mullee M, Mason JC, Peveler RC. Frequency and impact of nonadherence to immunosuppressants after renal transplantation: a systematic review. *Transplantation* 2004; **77**: 769-776 [PMID: 15021846]

23   **Woodward RS**, Page TF, Soares R, Schnitzler MA, Lentine KL, Brennan DC. Income-related disparities in kidney transplant graft failures are eliminated by Medicare's immunosuppression coverage. *Am J Transplant* 2008; **8**: 2636-2646 [PMID: 19032227 DOI: 10.1111/j.1600-6143.2008.02422.x]

24   **Schold JD**, Heaphy EL, Buccini LD, Poggio ED, Srinivas TR, Goldfarb DA, Flechner SM, Rodrigue JR, Thornton JD, Sehgal AR. Prominent impact of community risk factors on kidney transplant candidate processes and outcomes. *Am J Transplant* 2013; **13**: 2374-2383 [PMID: 24034708 DOI: 10.1111/ajt.12349]

25   **Cavaillé-Coll M**, Bala S, Velidedeoglu E, Hernandez A, Archdeacon P, Gonzalez G, Neuland C, Meyer J, Albrecht R. Summary of FDA workshop on ischemia reperfusion injury in kidney transplantation. *Am J Transplant* 2013; **13**: 1134-1148 [PMID: 23566221 DOI: 10.1111/ajt.12210]

26   **Pepe MS**, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; **93**: 1054-1061 [PMID: 11459866]

27   **Linkermann A**, Hackl MJ, Kunzendorf U, Walczak H, Krautwald S, Jevnikar AM. Necroptosis in immunity and ischemia-reperfusion injury. *Am J Transplant* 2013; **13**: 2797-2804 [PMID: 24103029 DOI: 10.1111/ajt.12448]

28   **Lau A**, Wang S, Jiang J, Haig A, Pavlosky A, Linkermann A, Zhang ZX, Jevnikar AM. RIPK3-mediated necroptosis promotes donor kidney inflammatory injury and reduces allograft survival. *Am J Transplant* 2013; **13**: 2805-2818 [PMID: 24103001 DOI: 10.1111/ajt.12447]

29   **Mannon RB**. Necroptosis in solid organ transplantation: a missing link to immune activation? *Am J Transplant* 2013; **13**: 2785-2786 [PMID: 24103049 DOI: 10.1111/ajt.12450]

30   **Shuh M**, Bohorquez H, Loss GE, Cohen AJ. Tumor Necrosis Factor-α: Life and Death of Hepatocytes During Liver Ischemia/Reperfusion Injury. *Ochsner J* 2013; **13**: 119-130 [PMID: 23531747]

31   **Djamali A**, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant* 2014; **14**: 255-271 [PMID: 24401076 DOI: 10.1111/ajt.12589]

32   **Taniguchi M**, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE, Catrou PG, Bolin P, Parker K, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. *Am J Transplant* 2013; **13**: 2577-2589 [PMID: 23941128 DOI: 10.1111/ajt.12395]

33   **Giral M**, Foucher Y, Dufay A, Van Huyen JP, Renaudin K, Moreau A, Philippe A, Hegner B, Dechend R, Heidecke H, Brouard S, Cesbron A, Castagnet S, Devys A, Soulillou JP, Dragun D. Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. *Am J Transplant* 2013; **13**: 2567-2576 [PMID: 23919486 DOI: 10.1111/ajt.12397]

34   **Porcheray F**, Fraser JW, Gao B, McColl A, DeVito J, Dargon I, Helou Y, Wong W, Girouard TC, Saidman SL, Colvin RB, Palmisano A, Maggiore U, Vaglio A, Smith RN, Zorn E. Polyreactive antibodies developing amidst humoral rejection of human kidney grafts bind apoptotic cells and activate complement. *Am J Transplant* 2013; **13**: 2590-2600 [PMID: 23919437 DOI: 10.1111/ajt.12394]

35   **Sis B**, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant* 2009; **9**: 2312-2323 [PMID:

19681822 DOI: 10.1111/j.1600-6143.2009.02761.x]

36   **Sellarés J**, Reeve J, Loupy A, Mengel M, Sis B, Skene A, de Freitas DG, Kreepala C, Hidalgo LG, Famulski KS, Halloran PF. Molecular diagnosis of antibody-mediated rejection in human kidney transplants. *Am J Transplant* 2013; **13**: 971-983 [PMID: 23414212 DOI: 10.1111/ajt.12150]

37   **Moreau A**, Varey E, Anegon I, Cuturi MC. Effector mechanisms of rejection. *Cold Spring Harb Perspect Med* 2013; **3**: [PMID: 24186491 DOI: 10.1101/cshperspect.a015461]

38   **Krneta T**, Gillgrass A, Ashkar AA. The influence of macrophages and the tumor microenvironment on natural killer cells. *Curr Mol Med* 2013; **13**: 68-79 [PMID: 22834841 DOI: 10.2174/15665 24011307010068]

39   **Haas M**, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, Castro MC, David DS, David-Neto E, Bagnasco SM, Cendales LC, Cornell LD, Demetris AJ, Drachenberg CB, Farver CF, Farris AB, Gibson IW, Kraus E, Liapis H, Loupy A, Nickeleit V, Randhawa P, Rodriguez ER, Rush D, Smith RN, Tan CD, Wallace WD, Mengel M. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* 2014; **14**: 272-283 [PMID: 24472190 DOI: 10.1111/ajt.12590]

40   **Bachelet T**, Couzi L, Lepreux S, Legeret M, Pariscoat G, Guidicelli G, Merville P, Taupin JL. Kidney intragraft donor-specific antibodies as determinant of antibody-mediated lesions and poor graft outcome. *Am J Transplant* 2013; **13**: 2855-2864 [PMID: 24102857 DOI: 10.1111/ajt.12438]

41   **Sis B**. Intragraft donor-specific antibody detection: a new test to dichotomize HLA alloantibodies into high and low injurious activity. *Am J Transplant* 2013; **13**: 2787-2789 [PMID: 24102879 DOI: 10.1111/ajt.12440]

42   **Volk HD**, Sawitzki B, Reinke P. Molecular analysis of renal allograft biopsies--more than a nice toy for researchers? *Am J Transplant* 2013; **13**: 539-540 [PMID: 23437880 DOI: 10.1111/ajt.12081]

43   **Reeve J**, Sellarés J, Mengel M, Sis B, Skene A, Hidalgo L, de Freitas DG, Famulski KS, Halloran PF. Molecular diagnosis of T cell-mediated rejection in human kidney transplant biopsies. *Am J Transplant* 2013; **13**: 645-655 [PMID: 23356949 DOI: 10.1111/ajt.12079]

44   **Famulski KS**, Reeve J, de Freitas DG, Kreepala C, Chang J, Halloran PF. Kidney transplants with progressing chronic diseases express high levels of acute kidney injury transcripts. *Am J Transplant* 2013; **13**: 634-644 [PMID: 23356967 DOI: 10.1111/ajt.12080]

45   **Halloran PF**, Pereira AB, Chang J, Matas A, Picton M, De Freitas D, Bromberg J, Serón D, Sellarés J, Einecke G, Reeve J. Microarray diagnosis of antibody-mediated rejection in kidney transplant biopsies: an international prospective study (INTERCOM). *Am J Transplant* 2013; **13**: 2865-2874 [PMID: 24119109 DOI: 10.1111/ajt.12465]

46   **Halloran PF**, Pereira AB, Chang J, Matas A, Picton M, De Freitas D, Bromberg J, Serón D, Sellarés J, Einecke G, Reeve J. Potential impact of microarray diagnosis of T cell-mediated rejection in kidney transplants: The INTERCOM study. *Am J Transplant* 2013; **13**: 2352-2363 [PMID: 23915426 DOI: 10.1111/ajt.12387]

47   **Suthanthiran M**, Schwartz JE, Ding R, Abecassis M, Dadhania D, Samstein B, Knechtle SJ, Friedewald J, Becker YT, Sharma VK, Williams NM, Chang CS, Hoang C, Muthukumar T, August P, Keslar KS, Fairchild RL, Hricik DE, Heeger PS, Han L, Liu J, Riggs M, Ikle DN, Bridges ND, Shaked A. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. *N Engl J Med* 2013; **369**: 20-31 [PMID: 23822777 DOI: 10.1056/NEJMoa1215555]

48   **Israni AK**, Skeans MA, Gustafson SK, Schnitzler MA, Wainright JL, Carrico RJ, Tyler KH, Kades LA, Kandaswamy R, Snyder JJ, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: pancreas. *Am J Transplant* 2014; **14** Suppl 1: 45-68 [PMID: 24373167 DOI: 10.1111/ajt.12580]

49   **Schulz T**, Pries A, Caliebe A, Kapischke M. Long-term survival after simultaneous pancreas-kidney transplantation with primary function of at least one year--a single-center experience. *Ann Transplant* 2014; **19**: 106-111 [PMID: 24576894 DOI: 10.12659/AOT.889715]

50   **Montiel-Casado MC**, Pérez-Daga JA, Aranda-Narváez JM, Fernández-Burgos I, Sánchez-Pérez B, León-Díaz FJ, Cabello-Díaz M, Rodríguez-Burgos D, Hernández-Marrero D, Santoyo-Santoyo J. Pancreas graft survival in simultaneous pancreas-kidney versus pancreas-after-kidney and pancreas alone transplantations: a single institution experience. *Transplant Proc* 2013; **45**: 3609-3611 [PMID: 24314973 DOI: 10.1016/j.transproceed.2013.10.019]

51   **Lindahl JP**, Hartmann A, Horneland R, Holdaas H, Reisæter AV, Midtvedt K, Leivestad T, Oyen O, Jenssen T. Improved patient survival with simultaneous pancreas and kidney transplantation in recipients with diabetic end-stage renal disease. *Diabetologia* 2013; **56**: 1364-1371 [PMID: 23549518 DOI: 10.1007/s00125-013-2888-y]

52   CITR Investigators 2011 Update from the Collaborative Islet Transplant Registry. *Am J Transplant* 2012; **12** (Suppl 3): S161 [DOI: 10.1111/j. 1600-6143.2012.04112.x]

53   **Cohen DJ**, Ratner LE. Type 2 diabetes: the best transplant option is still uncertain. *Clin J Am Soc Nephrol* 2012; **7**: 530-532 [PMID: 22442186 DOI: 10.2215/CJN.02120212]

54   **Gruessner AC**, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. *Clin Transplant* 2005; **19**: 433-455 [PMID: 16008587 DOI: 10.1111/j.1399-0012.2005.00378.x]

55   **Orlando G**, Stratta RJ, Light J. Pancreas transplantation for type 2 diabetes mellitus. *Curr Opin Organ Transplant* 2011; **16**: 110-115 [PMID: 21150617 DOI: 10.1097/MOT.0b013e3283424d1f]

56   **Sampaio MS**, Kuo HT, Bunnapradist S. Outcomes of simultaneous pancreas-kidney transplantation in type 2 diabetic recipients. *Clin J Am Soc Nephrol* 2011; **6**: 1198-1206 [PMID: 21441123 DOI: 10.2215/CJN.06860810]

57   **Margreiter C**, Resch T, Oberhuber R, Aigner F, Maier H, Sucher R, Schneeberger S, Ulmer H, Bösmüller C, Margreiter R, Pratschke J, Öllinger R. Combined pancreas-kidney transplantation for patients with end-stage nephropathy caused by type-2 diabetes mellitus. *Transplantation* 2013; **95**: 1030-1036 [PMID: 23407544 DOI: 10.1097/TP.0b013e3182861945]

58   **Sener A**, Cooper M, Bartlett ST. Is there a role for pancreas transplantation in type 2 diabetes mellitus? *Transplantation* 2010; **90**: 121-123 [PMID: 20562677 DOI: 10.1097/TP.0b013e3181e5b7e6]

59   **Niederhaus SV**, Leverson GE, Lorentzen DF, Robillard DJ, Sollinger HW, Pirsch JD, Torrealba JR, Odorico JS. Acute cellular and antibody-mediated rejection of the pancreas allograft: incidence, risk factors and outcomes. *Am J Transplant* 2013; **13**: 2945-2955 [PMID: 24102905 DOI: 10.1111/ajt.12443]

60   **Drachenberg CB**, Torrealba JR, Nankivell BJ, Rangel EB, Bajema IM, Kim DU, Arend L, Bracamonte ER, Bromberg JS, Bruijn JA, Cantarovich D, Chapman JR, Farris AB, Gaber L, Goldberg JC, Haririan A, Honsová E, Iskandar SS, Klassen DK, Kraus E, Lower F, Odorico J, Olson JL, Mittalhenkle A, Munivenkatappa R, Paraskevas S, Papadimitriou JC, Randhawa P, Reinholt FP, Renaudin K, Revelo P, Ruiz P, Samaniego MD, Shapiro R, Stratta RJ, Sutherland DE, Troxell ML, Voska L, Seshan SV, Racusen LC, Bartlett ST. Guidelines for the diagnosis of antibody-mediated rejection in pancreas allografts-updated Banff grading schema. *Am J Transplant* 2011; **11**: 1792-1802 [PMID: 21812920 DOI: 10.1111/j.1600-6143.2011.03670.x]

61   **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl*

J Med 2000; **343**: 230-238 [PMID: 10911004 DOI: 10.1056/NEJM200007273430401]

62   **Jahansouz C**, Jahansouz C, Kumer SC, Brayman KL. Evolution of β-Cell Replacement Therapy in Diabetes Mellitus: Islet Cell Transplantation. *J Transplant* 2011 [DOI: 10.1155/2011/247959]

63   **Pepper AR**, Gala-Lopez B, Ziff O, Shapiro AJ. Current status of clinical islet transplantation. *World J Transplant* 2013; **3**: 48-53 [PMID: 24392308 DOI: 10.5500/wjt.v3.i4.48]

64   **Masharani U**, Moassesfar S, Frassetto L, Szot G, Tavakol M, McElroy J, Ramos M, Johnson K, Stock PG, Posselt A. Long Term Insulin Independence After Islet Transplant Alone (ITA) and Pancreas Transplant Alone (PTA) in Patients with Type 1 Diabetes (T1D) – A Single Institution Experience. *Transplantation* 2013; **96**: 6S, S44

65   **Tang Q**, Posselt A, Szot GL, Melli K, Pauli M, Frasetto L, Masharani U, Fong L, Stock PG. Immunological Impact of Efalizumab Versus Belatacept in Islet Transplantation. *Transplantation* 2013; **96**: 6S, 115

66   **Naziruddin B**, Takita M, Kunnathodi F, Shahbazov R, Kanak M, Lawrence M, Onaca N, Levy M. Early Treatment with Etanercept and Anakinra Prevents Islet Graft Damage in Clinical Islet Auto-transplantation. *Transplantation* 2013; **96**: 6S, 5

67   **Maffi P**, Daffonchio L, Ruffini PA, Allegretti M, Citro A, Magistretti P, Melzi R, Mercalli A, Nano R, Sordi V, Secchi A, Piemonti L. Islet Allotransplantation in Type 1 Diabetes: Phase 2 Pilot Study with CXCL8 Inhibitor (reparixin). *Transplantation* 2013; **96**: 6S, 4

68   **Citro A**, Cantarelli E, Maffi P, Nano R, Melzi R, Mercalli A, Dugnani E, Sordi V, Magistretti P, Daffonchio L, Ruffini PA, Allegretti M, Secchi A, Bonifacio E, Piemonti L. CXCR1/2 inhibition enhances pancreatic islet survival after transplantation. *J Clin Invest* 2012; **122**: 3647-3651 [PMID: 22996693 DOI: 10.1172/JCI63089]

69   **Takita M**, Shahabazov R, Kunnathodi F, Mueller C, Kurup P, Kanak M, Lawrence M, Onaca N, Ueno H, Naziruddin B, Levy M. Early Loss of Transplanted Allogeneic Islets Despite T-Cell Depleting Induction: The Role of Autoimmunity in Clinical Islet Transplantation. *Transplantation* 2013; **96**: 6S, 121

70   **Pepper AR**, Gala-Lopez B, Ziff O, Shapiro AM. Revascularization of transplanted pancreatic islets and role of the transplantation site. *Clin Dev Immunol* 2013 [DOI: 10.1155/2013/352315]

71   **Henriksnäs J**, Lau J, Zang G, Berggren PO, Köhler M, Carlsson PO. Markedly decreased blood perfusion of pancreatic islets transplanted intraportally into the liver: disruption of islet integrity necessary for islet revascularization. *Diabetes* 2012; **61**: 665-673 [PMID: 22315321 DOI: 10.2337/db10-0895]

72   **Bellin M**, Hering B, Balamurugan AN, Parazzoli S, Oseid E, Dunn T, Beilman G, Sutherland D, Robertson RP. Alpha Cells in Intraperitoneal, but not Intra-hepatic, Auto-islet Transplants Function Normally During Hypoglycemia in Humans After Total Pancreatectomy and Islet Auto-transplantation. *Transplantation* 2013; **96**: 6S, 7

73   **Espes D**, Lau J, Quach M, Palmer AF, Carisson PO. Hypoxia in Islets After Intramuscular Transplantation can be Overcome by Co-Implantation of Polymerized Hemoglobin. *Transplantation* 2013; **96**: 6S, 44

74   **Buder B**, Alexander M, Krishnan R, Chapman DW, Lakey JR. Encapsulated islet transplantation: strategies and clinical trials. *Immune Netw* 2013; **13**: 235-239 [PMID: 24385941 DOI: 10.4110/in.2013.13.6.235]

75   **Kim WR**, Smith JM, Skeans MA, Schladt DP, Schnitzler MA, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: liver. *Am J Transplant* 2014; **14** Suppl 1: 69-96 [PMID: 24373168 DOI: 10.1111/ajt.12581]

76   **Lodhi SA**, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant* 2011; **11**: 1226-1235 [PMID: 21564524 DOI: 10.1111/j.1600-6143.2011.03539.x]

77   **Schoening WN**, Buescher N, Rademacher S, Andreou A, Kuehn S, Neuhaus R, Guckelberger O, Puhl G, Seehofer D, Neuhaus P. Twenty-year longitudinal follow-up after orthotopic liver transplantation: a single-center experience of 313 consecutive cases. *Am J Transplant* 2013; **13**: 2384-2394 [PMID: 23915357 DOI: 10.1111/ajt.12384]

78   **Jain A**, Singhal A, Fontes P, Mazariegos G, DeVera ME, Cacciarelli T, Lopez RC, Sindhi R, Humar A, Marsh JW. One thousand consecutive primary liver transplants under tacrolimus immunosuppression: a 17- to 20-year longitudinal follow-up. *Transplantation* 2011; **91**: 1025-1030 [PMID: 21378604 DOI: 10.1097/TP.0b013e3182129215]

79   **Duffy JP**, Kao K, Ko CY, Farmer DG, McDiarmid SV, Hong JC, Venick RS, Feist S, Goldstein L, Saab S, Hiatt JR, Busuttil RW. Long-term patient outcome and quality of life after liver transplantation: analysis of 20-year survivors. *Ann Surg* 2010; **252**: 652-661 [PMID: 20881772 DOI: 10.1097/SLA.0b013e3181f5f23a]

80   **Adam R**, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]

81   **Kasahara M**, Umeshita K, Inomata Y, Uemoto S. Long-term outcomes of pediatric living donor liver transplantation in Japan: an analysis of more than 2200 cases listed in the registry of the Japanese Liver Transplantation Society. *Am J Transplant* 2013; **13**: 1830-1839 [PMID: 23711238 DOI: 10.1111/ajt.12276]

82   **Singal AK**, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* 2013; **95**: 755-760 [PMID: 23370710 DOI: 10.1097/TP.0b013e31827afb3a]

83   **Burton JR**, Everson GT. Management of the transplant recipient with chronic hepatitis C. *Clin Liver Dis* 2013; **17**: 73-91 [PMID: 23177284 DOI: 10.1016/j.cld.2012.09.013]

84   **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

85   **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

86   **Garg V**, van Heeswijk R, Lee JE, Alves K, Nadkarni P, Luo X. Effect of telaprevir on the pharmacokinetics of cyclosporine and tacrolimus. *Hepatology* 2011; **54**: 20-27 [PMID: 21618566 DOI: 10.1002/hep.24443]

87   **Croagh CM**, Lubel J. Advances in the management of hepatitis C. *Intern Med J* 2013; **43**: 1265-1271 [PMID: 24330361 DOI: 10.1111/imj.12304]

88   **Sautto GA**, Diotti RA, Clementi M. New therapeutic options for HCV infection in the monoclonal antibody era. *New Microbiol* 2012; **35**: 387-397 [PMID: 23109006]

89   **Datta G**, Fuller BJ, Davidson BR. Molecular mechanisms of liver ischemia reperfusion injury: insights from transgenic knockout models. *World J Gastroenterol* 2013; **19**: 1683-1698 [PMID: 23555157 DOI: 10.3748/wjg.v19.i11.1683]

90   **Mendes-Braz M**, Elias-Miró M, Jiménez-Castro, MB, Casillas-Ramírez A, Ramalho FS, Peralta C. The current state of knowledge of hepatic ischemia-reperfusion injury based on

its study in experimental models. *J Biomed Biotechnol* 2012; [DOI: 10.1155/2012/298657]

91 **Akhtar MZ**, Henderson T, Sutherland A, Vogel T, Friend PJ. Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. *Transplant Proc* 2013; **45**: 2083-2092 [PMID: 23953517 DOI: 10.1016/j.transproceed.2013.04.004]

92 **Elias-Miró M**, Jiménez-Castro MB, Rodés J, Peralta C. Current knowledge on oxidative stress in hepatic ischemia/reperfusion. *Free Radic Res* 2013; **47**: 555-568 [PMID: 23738581 DOI: 10.3109/10715762.2013.811721]

93 **Genovés P**, García D, Cejalvo D, Martin A, Zaragoza C, Toledo AH, Toledo-Pereyra LH, Lloris-Carsi JM. Pentoxifylline in liver ischemia and reperfusion. *J Invest Surg* 2014; **27**: 114-124 [PMID: 24143911 DOI: 10.3109/08941939.2013.835454]

94 **Tiriveedhi V**, Upadhya GA, Busch RA, Gunter KL, Dines JN, Knolhoff BL, Jia J, Sarma NJ, Ramachandran S, Anderson CD, Mohanakumar T, Chapman WC. Protective role of bortezomib in steatotic liver ischemia/reperfusion injury through abrogation of MMP activation and YKL-40 expression. *Transpl Immunol* 2014; **30**: 93-98 [PMID: 24380732 DOI: 10.1016/j.trim.2013.12.003]

95 **Saidi RF**, Rajeshkumar B, Shariftabrizi A, Dresser K, Walter O. Human C1 inhibitor attenuates liver ischemia-reperfusion injury and promotes liver regeneration. *J Surg Res* 2014; **187**: 660-666 [PMID: 24433870 DOI: 10.1016/j.jss.2013.09.009]

96 **Iwatsuki S**, Rabin BS, Shaw BW, Starzl TE. Liver transplantation against T cell-positive warm crossmatches. *Transplant Proc* 1984; **16**: 1427-1429 [PMID: 6390842]

97 **Gordon RD**, Fung JJ, Markus B, Fox I, Iwatsuki S, Esquivel CO, Tzakis A, Todo S, Starzl TE. The antibody crossmatch in liver transplantation. *Surgery* 1986; **100**: 705-715 [PMID: 3532391]

98 **Ruiz R**, Tomiyama K, Campsen J, Goldstein RM, Levy MF, McKenna GJ, Onaca N, Susskind B, Tillery GW, Klintmalm GB. Implications of a positive crossmatch in liver transplantation: a 20-year review. *Liver Transpl* 2012; **18**: 455-460 [PMID: 22139972 DOI: 10.1002/lt.22474]

99 **Takaya S**, Bronsther O, Iwaki Y, Nakamura K, Abu-Elmagd K, Yagihashi A, Demetris AJ, Kobayashi M, Todo S, Tzakis AG. The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors. *Transplantation* 1992; **53**: 400-406 [PMID: 1371195]

100 **Ogura K**, Koyama H, Takemoto S, Terasaki PI, Busuttil RW. Significance of a positive crossmatch on outcome in human liver transplantation. *Transplant Proc* 1992; **24**: 1465 [PMID: 1496620]

101 **O'Leary JG**, Kaneku H, Demetris AJ, Marr JD, Shiller SM, Susskind BM, Tillery GW, Terasaki PI, Klintmalm GB. Antibody-mediated rejection as a contributor to previously unexplained early liver allograft loss. *Liver Transpl* 2014; **20**: 218-227 [PMID: 24382837 DOI: 10.1002/lt.23788]

102 **Kaneku H**, O'Leary JG, Banuelos N, Jennings LW, Susskind BM, Klintmalm GB, Terasaki PI. De novo donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. *Am J Transplant* 2013; **13**: 1541-1548 [PMID: 23721554 DOI: 10.1111/ajt.12212]

103 **Hu J**, Wang Z, Tan CJ, Liao BY, Zhang X, Xu M, Dai Z, Qiu SJ, Huang XW, Sun J, Sun QM, He YF, Song K, Pan Q, Wu Y, Fan J, Zhou J. Plasma microRNA, a potential biomarker for acute rejection after liver transplantation. *Transplantation* 2013; **95**: 991-999 [PMID: 23466638 DOI: 10.1097/TP.0b013e31828618d8]

104 **Wei L**, Gong X, Martinez OM, Krams SM. Differential expression and functions of microRNAs in liver transplantation and potential use as non-invasive biomarkers. *Transpl Immunol* 2013; **29**: 123-129 [PMID: 24001411 DOI: 10.1016/j.trim.2013.08.005]

105 **Colvin-Adams M**, Smith JM, Heubner BM, Skeans MA, Edwards LB, Waller C, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2011 Annual Data Report: heart. *Am J Transplant* 2013; **13** Suppl 1: 119-148 [PMID: 23237699 DOI: 10.1111/ajt.12023]

106 **Lund LH**, Edwards LB, Kucheryavaya AY, Dipchand AI, Benden C, Christie JD, Dobbels F, Kirk R, Rahmel AO, Yusen RD, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report--2013; focus theme: age. *J Heart Lung Transplant* 2013; **32**: 951-964 [PMID: 24054804 DOI: 10.1016/j.healun.2013.08.006]

107 **Pondrom S**. Heart failure options broaden. *Am J Transplant* 2013; **13**: 827-828 [PMID: 23551626 DOI: 10.1111/ajt.12231]

108 **Slaughter MS**, Pagani FD, McGee EC, Birks EJ, Cotts WG, Gregoric I, Howard Frazier O, Icenogle T, Najjar SS, Boyce SW, Acker MA, John R, Hathaway DR, Najarian KB, Aaronson KD. HeartWare ventricular assist system for bridge to transplant: combined results of the bridge to transplant and continued access protocol trial. *J Heart Lung Transplant* 2013; **32**: 675-683 [PMID: 23796152 DOI: 10.1016/j.healun.2013.04.004]

109 **Taghavi S**, Jayarajan SN, Komaroff E, Mangi AA. Continuous flow left ventricular assist device technology has influenced wait times and affected donor allocation in cardiac transplantation. *J Thorac Cardiovasc Surg* 2014; **147**: 1966-1971, 1971.e1 [PMID: 24613158 DOI: 10.1016/j.jtcvs.2014.02.001]

110 **Sutcliffe P**, Connock M, Pulikottil-Jacob R, Kandala NB, Suri G, Gurung T, Grove A, Shyangdan D, Briscoe S, Maheswaran H, Clarke A. Clinical effectiveness and cost-effectiveness of second- and third-generation left ventricular assist devices as either bridge to transplant or alternative to transplant for adults eligible for heart transplantation: systematic review and cost-effectiveness model. *Health Technol Assess* 2013; **17**: 1-499, v-vi [PMID: 24280231 DOI: 10.3310/hta17530]

111 **Feldman D**, Pamboukian SV, Teuteberg JJ, Birks E, Lietz K, Moore SA, Morgan JA, Arabia F, Bauman ME, Buchholz HW, Deng M, Dickstein ML, El-Banayosy A, Elliot T, Goldstein DJ, Grady KL, Jones K, Hryniewicz K, John R, Kaan A, Kusne S, Loebe M, Massicotte MP, Moazami N, Mohacsi P, Mooney M, Nelson T, Pagani F, Perry W, Potapov EV, Eduardo Rame J, Russell SD, Sorensen EN, Sun B, Strueber M, Mangi AA, Petty MG, Rogers J. The 2013 International Society for Heart and Lung Transplantation Guidelines for mechanical circulatory support: executive summary. *J Heart Lung Transplant* 2013; **32**: 157-187 [PMID: 23352391 DOI: 10.1016/j.healun.2012.09.013]

112 **Kilic A**, Allen JG, Weiss ES. Validation of the United States-derived Index for Mortality Prediction After Cardiac Transplantation (IMPACT) using international registry data. *J Heart Lung Transplant* 2013; **32**: 492-498 [PMID: 23474362 DOI: 10.1016/j.healun.2013.02.001]

113 **Smits JM**, de Vries E, De Pauw M, Zuckermann A, Rahmel A, Meiser B, Laufer G, Reichenspurner H, Strueber M. Is it time for a cardiac allocation score? First results from the Eurotransplant pilot study on a survival benefit-based heart allocation. *J Heart Lung Transplant* 2013; **32**: 873-880 [PMID: 23628111 DOI: 10.1016/j.healun.2013.03.015]

114 **Smith JD**, Banner NR, Hamour IM, Ozawa M, Goh A, Robinson D, Terasaki PI, Rose ML. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. *Am J Transplant* 2011; **11**: 312-319 [PMID: 21219570 DOI: 10.1111/j.1600-6143.2010.03383.x]

115 **Smith JD**, Hamour IM, Banner NR, Rose ML. C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. *Am J Transplant* 2007; **7**: 2809-2815 [PMID: 17908268 DOI: 10.1111/j.1600-6143.2007.01991.x]

116 **Smith JD**, Hamour IM, Burke MM, Mahesh B, Stanford RE, Haj-Yahia S, Robinson DR, Kaul P, Yacoub MH, Banner NR, Rose ML. A reevaluation of the role of IgM Non-HLA antibodies in cardiac transplantation. *Transplantation* 2009; **87**: 864-871 [PMID: 19300190 DOI: 10.1097/TP.0b013e31819a65fa]

117 **Berry GJ**, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, Goddard M, Hammond EH, Leone O, Marboe C, Miller D, Neil D, Rassl D, Revelo MP, Rice A, Rene Rodriguez E, Stewart S, Tan CD, Winters GL, West L, Mehra

MR, Angelini A. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant* 2013; **32**: 1147-1162 [PMID: 24263017 DOI: 10.1016/j.healun.2013.08.011]

118 **Costello JP**, Mohanakumar T, Nath DS. Mechanisms of chronic cardiac allograft rejection. *Tex Heart Inst J* 2013; **40**: 395-399 [PMID: 24082367]

119 **Eisen HJ**, Kobashigawa J, Starling RC, Pauly DF, Kfoury A, Ross H, Wang SS, Cantin B, Van Bakel A, Ewald G, Hirt S, Lehmkuhl H, Keogh A, Rinaldi M, Potena L, Zuckermann A, Dong G, Cornu-Artis C, Lopez P. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. *Am J Transplant* 2013; **13**: 1203-1216 [PMID: 23433101 DOI: 10.1111/ajt.12181]

120 **Kobashigawa JA**, Pauly DF, Starling RC, Eisen H, Ross H, Wang SS, Cantin B, Hill JA, Lopez P, Dong G, Nicholls SJ. Cardiac allograft vasculopathy by intravascular ultrasound in heart transplant patients: substudy from the Everolimus versus mycophenolate mofetil randomized, multicenter trial. *JACC Heart Fail* 2013; **1**: 389-399 [PMID: 24621971 DOI: 10.1016/j.jchf.2013.07.002]

121 **Mehra MR**. Advancing immunosuppression in heart transplantation: "one step forward, two steps back. *Am J Transplant* 2013; **13**: 1119-1120 [PMID: 23621159 DOI: 10.1111/ajt.12177]

122 **Khandhar SJ**, Yamamoto H, Teuteberg JJ, Shullo MA, Bezerra HG, Costa MA, Selzer F, Lee JS, Marroquin OC, McNamara DM, Mulukutla SR, Toma C. Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTCAV study). *J Heart Lung Transplant* 2013; **32**: 596-602 [PMID: 23499356 DOI: 10.1016/j.healun.2013.02.005]

123 **Valapour M**, Skeans MA, Heubner BM, Smith JM, Schnitzler MA, Hertz MI, Edwards LB, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: lung. *Am J Transplant* 2014; **14** Suppl 1: 139-165 [PMID: 24373171 DOI: 10.1111/ajt.12584]

124 **Yusen RD**, Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Lund LH, Rahmel AO, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Adult Lung and Heart-Lung Transplant Report--2013; focus theme: age. *J Heart Lung Transplant* 2013; **32**: 965-978 [PMID: 24054805 DOI: 10.1016/j.healun.2013.08.007]

125 **Shah RJ**, Kotloff RM. Lung transplantation for obstructive lung diseases. *Semin Respir Crit Care Med* 2013; **34**: 288-296 [PMID: 23821504 DOI: 10.1055/s-0033-1348468]

126 **Gottlieb J**. Lung transplantation for interstitial lung diseases and pulmonary hypertension. *Semin Respir Crit Care Med* 2013; **34**: 281-287 [PMID: 23821503 DOI: 10.1055/s-0033-1348462]

127 **Corris PA**. Lung transplantation for cystic fibrosis and bronchiectasis. *Semin Respir Crit Care Med* 2013; **34**: 297-304 [PMID: 23821505 DOI: 10.1055/s-0033-1348469]

128 **Snell GI**, Paraskeva M, Westall GP. Donor selection and management. *Semin Respir Crit Care Med* 2013; **34**: 361-370 [PMID: 23821510 DOI: 10.1055/s-0033-1348464]

129 **Bittle GJ**, Sanchez PG, Kon ZN, Claire Watkins A, Rajagopal K, Pierson RN, Gammie JS, Griffith BP. The use of lung donors older than 55 years: a review of the United Network of Organ Sharing database. *J Heart Lung Transplant* 2013; **32**: 760-768 [PMID: 23664760 DOI: 10.1016/j.healun.2013.04.012]

130 **Sommer W**, Kühn C, Tudorache I, Avsar M, Gottlieb J, Boethig D, Haverich A, Warnecke G. Extended criteria donor lungs and clinical outcome: results of an alternative allocation algorithm. *J Heart Lung Transplant* 2013; **32**: 1065-1072 [PMID: 23953918 DOI: 10.1016/j.healun.2013.06.021]

131 **Barnard JB**, Davies O, Curry P, Catarino P, Dunning J,

Jenkins D, Sudarshan C, Nair S, Tsui S, Parmar J. Size matching in lung transplantation: an evidence-based review. *J Heart Lung Transplant* 2013; **32**: 849-860 [PMID: 23953814 DOI: 10.1016/j.healun.2013.07.002]

132 **Chamogeorgakis T**, Mason DP, Murthy SC, Thuita L, Raymond DP, Pettersson GB, Blackstone EH. Impact of nutritional state on lung transplant outcomes. *J Heart Lung Transplant* 2013; **32**: 693-700 [PMID: 23664761 DOI: 10.1016/j.healun.2013.04.005]

133 **Shah P**, Orens JB. Impact of nutritional state on lung transplant outcomes: the weight of the evidence. *J Heart Lung Transplant* 2013; **32**: 755-756 [PMID: 23856213 DOI: 10.1016/j.healun.2013.06.001]

134 **McShane PJ**, Garrity ER. Impact of the lung allocation score. *Semin Respir Crit Care Med* 2013; **34**: 275-280 [PMID: 23821502 DOI: 10.1055/s-0033-1348461]

135 **Wille KM**, Harrington KF, deAndrade JA, Vishin S, Oster RA, Kaslow RA. Disparities in lung transplantation before and after introduction of the lung allocation score. *J Heart Lung Transplant* 2013; **32**: 684-692 [PMID: 23582477 DOI: 10.1016/j.healun.2013.03.005]

136 **Suzuki Y**, Cantu E, Christie JD. Primary graft dysfunction. *Semin Respir Crit Care Med* 2013; **34**: 305-319 [PMID: 23821506 DOI: 10.1055/s-0033-1348474]

137 **Bertani A**. Overcoming primary graft dysfunction after lung transplantation: are we finding our way there? *Transplantation* 2014; **97**: 1100-1101 [PMID: 24573115]

138 **Sommer W**, Tudorache I, Kühn C, Avsar M, Salman J, Ius F, Gras C, Weber P, Welte T, Gottlieb J, Haverich A, Warnecke G. C1-esterase-inhibitor for primary graft dysfunction in lung transplantation. *Transplantation* 2014; **97**: 1185-1191 [PMID: 24573112 DOI: 10.1097/TP.0000000000000034]

139 **McManigle W**, Pavlisko EN, Martinu T. Acute cellular and antibody-mediated allograft rejection. *Semin Respir Crit Care Med* 2013; **34**: 320-335 [PMID: 23821507 DOI: 10.1055/s-0033-1348471]

140 **Glanville AR**. The role of surveillance bronchoscopy post-lung transplantation. *Semin Respir Crit Care Med* 2013; **34**: 414-420 [PMID: 23821514 DOI: 10.1055/s-0033-1348466]

141 **San Segundo D**, Ballesteros MÁ, Naranjo S, Zurbano F, Miñambres E, López-Hoyos M. Increased numbers of circulating CD8 effector memory T cells before transplantation enhance the risk of acute rejection in lung transplant recipients. *PLoS One* 2013; **8**: e80601 [PMID: 24236187 DOI: 10.1371/journal.pone.0080601]

142 **Krustrup D**, Madsen CB, Iversen M, Engelholm L, Ryder LP, Andersen CB. The number of regulatory T cells in transbronchial lung allograft biopsies is related to FoxP3 mRNA levels in bronchoalveolar lavage fluid and to the degree of acute cellular rejection. *Transpl Immunol* 2013; **29**: 71-75 [PMID: 23969200 DOI: 10.1016/j.trim.2013.08.002]

143 **Husain S**, Resende MR, Rajwans N, Zamel R, Pilewski JM, Crespo MM, Singer LG, McCurry KR, Kolls JK, Keshavjee S, Liles WC. Elevated CXCL10 (IP-10) in bronchoalveolar lavage fluid is associated with acute cellular rejection after human lung transplantation. *Transplantation* 2014; **97**: 90-97 [PMID: 24025324 DOI: 10.1097/TP.0b013e3182a6ee0a]

144 **Lobo LJ**, Aris RM, Schmitz J, Neuringer IP. Donor-specific antibodies are associated with antibody-mediated rejection, acute cellular rejection, bronchiolitis obliterans syndrome, and cystic fibrosis after lung transplantation. *J Heart Lung Transplant* 2013; **32**: 70-77 [PMID: 23260706 DOI: 10.1016/j.healun.2012.10.007]

145 **Witt CA**, Gaut JP, Yusen RD, Byers DE, Iuppa JA, Bennett Bain K, Alexander Patterson G, Mohanakumar T, Trulock EP, Hachem RR. Acute antibody-mediated rejection after lung transplantation. *J Heart Lung Transplant* 2013; **32**: 1034-1040 [PMID: 23953920 DOI: 10.1016/j.healun.2013.07.004]

146 **Berry G**, Burke M, Andersen C, Angelini A, Bruneval P, Calabrese F, Fishbein MC, Goddard M, Leone O, Maleszewski J, Marboe C, Miller D, Neil D, Padera R, Rassl D, Revello

M, Rice A, Stewart S, Yousem SA. Pathology of pulmonary antibody-mediated rejection: 2012 update from the Pathology Council of the ISHLT. *J Heart Lung Transplant* 2013; **32**: 14-21 [PMID: 23260701 DOI: 10.1016/j.healun.2012.11.005]

147　**Snyder LD**, Wang Z, Chen DF, Reinsmoen NL, Finlen-Copeland CA, Davis WA, Zaas DW, Palmer SM. Implications for human leukocyte antigen antibodies after lung transplantation: a 10-year experience in 441 patients. *Chest* 2013; **144**: 226-233 [PMID: 23328795 DOI: 10.1378/chest.12-0587]

148　**Glanville AR**. Antibody-mediated rejection in lung transplantation: turning myth into reality. *J Heart Lung Transplant* 2013; **32**: 12-13 [PMID: 23260700 DOI: 10.1016/j.healun.2012.11.015]

149　**Weigt SS**, DerHovanessian A, Wallace WD, Lynch JP, Belperio JA. Bronchiolitis obliterans syndrome: the Achilles' heel of lung transplantation. *Semin Respir Crit Care Med* 2013; **34**: 336-351 [PMID: 23821508 DOI: 10.1055/s-0033-1348467]

150　**Saito T**, Takahashi H, Kaneda H, Binnie M, Azad S, Sato M, Waddell TK, Cypel M, Liu M, Keshavjee S. Impact of cytokine expression in the pre-implanted donor lung on the development of chronic lung allograft dysfunction subtypes. *Am J Transplant* 2013; **13**: 3192-3201 [PMID: 24164971 DOI: 10.1111/ajt.12492]

151　**Verleden SE**, Vandermeulen E, Ruttens D, Vos R, Vaneylen A, Dupont LJ, Van Raemdonck DE, Vanaudenaerde BM, Verleden GM. Neutrophilic reversible allograft dysfunction (NRAD) and restrictive allograft syndrome (RAS). *Semin Respir Crit Care Med* 2013; **34**: 352-360 [PMID: 23821509 DOI: 10.1055/s-0033-1348463]

152　**Sato M**, Ohmori-Matsuda K, Saito T, Matsuda Y, Hwang DM, Waddell TK, Singer LG, Keshavjee S. Time-dependent changes in the risk of death in pure bronchiolitis obliterans syndrome (BOS). *J Heart Lung Transplant* 2013; **32**: 484-491 [PMID: 23433813 DOI: 10.1016/j.healun.2013.01.1054]

153　**Sato M**, Hwang DM, Waddell TK, Singer LG, Keshavjee S. Progression pattern of restrictive allograft syndrome after lung transplantation. *J Heart Lung Transplant* 2013; **32**: 23-30 [PMID: 23260703 DOI: 10.1016/j.healun.2012.09.026]

154　**Verleden SE**, Ruttens D, Vandermeulen E, Vaneylen A, Dupont LJ, Van Raemdonck DE, Verleden GM, Vanaudenaerde BM, Vos R. Bronchiolitis obliterans syndrome and restrictive allograft syndrome: do risk factors differ? *Transplantation* 2013; **95**: 1167-1172 [PMID: 23425818 DOI: 10.1097/TP.0b013e318286e076]

155　**Suzuki H**, Lasbury ME, Fan L, Vittal R, Mickler EA, Benson HL, Shilling R, Wu Q, Weber DJ, Wagner SR, Lasaro M, Devore D, Wang Y, Sandusky GE, Lipking K, Pandya P, Reynolds J, Love R, Wozniak T, Gu H, Brown KM, Wilkes DS. Role of complement activation in obliterative bronchiolitis post-lung transplantation. *J Immunol* 2013; **191**: 4431-4439 [PMID: 24043901 DOI: 10.4049/jimmunol.1202242]

156　**Tiriveedhi V**, Banan B, Deepti S, Nataraju A, Hachem R, Trulock E, Alexander PG, Thalachallour M. Role of defensins in the pathogenesis of chronic lung allograft rejection. *Hum Immunol* 2014; **75**: 370-377 [PMID: 24380698 DOI: 10.1016/j.humimm.2013.12.014]

157　**Salvadori M**, Bertoni E. Is it time to give up with calcineurin inhibitors in kidney transplantation? *World J Transplant* 2013; **3**: 7-25 [PMID: 24175203 DOI: 10.5500/wjt.v3.i2.7]

158　**Cornu C**, Dufays C, Gaillard S, Gueyffier F, Redonnet M, Sebbag L, Roussoulières A, Gleissner CA, Groetzner J, Lehmkuhl HB, Potena L, Gullestad L, Cantarovich M, Boissonnat P. Impact of the reduction of calcineurin inhibitors on renal function in heart transplant patients: a systematic review and meta-analysis. *Br J Clin Pharmacol* 2014; **78**: 24-32 [PMID: 24251918 DOI: 10.1111/bcp.12289]

159　**Isakova T**, Xie H, Messinger S, Cortazar F, Scialla JJ, Guerra G, Contreras G, Roth D, Burke GW, Molnar MZ, Mucsi I, Wolf M. Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. *Am J Transplant* 2013; **13**: 100-110 [PMID: 23025566 DOI: 10.1111/j.1600-6143.2012.04281.x]

160　**Asrani SK**, Wiesner RH, Trotter JF, Klintmalm G, Katz E, Maller E, Roberts J, Kneteman N, Teperman L, Fung JJ, Millis JM. De novo sirolimus and reduced-dose tacrolimus versus standard-dose tacrolimus after liver transplantation: the 2000-2003 phase II prospective randomized trial. *Am J Transplant* 2014; **14**: 356-366 [PMID: 24456026 DOI: 10.1111/ajt.12543]

161　**Saliba F**, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Jonas S, Sudan D, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Dong G, Lopez PM, Fung J, Junge G. Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study. *Am J Transplant* 2013; **13**: 1734-1745 [PMID: 23714399 DOI: 10.1111/ajt.12280]

162　**Junge G**, Dumortier T, Schwende H, Fung J. mTOR inhibition in liver transplantation: how to dose for effective/ safe CNI reduction? *Transplant Proc* 2013; **45**: 1979-1980 [PMID: 23769088 DOI: 10.1016/j.transproceed.2013.02.102]

163　**Bhat M**, Charlton M. Preserving flow in liver transplant recipients: mTOR inhibitors everolimus and sirolimus are not peas from a pod. *Am J Transplant* 2013; **13**: 1633-1635 [PMID: 23802724 DOI: 10.1111/ajt.12290]

164　**Sterneck M**, Kaiser GM, Heyne N, Richter N, Rauchfuss F, Pascher A, Schemmer P, Fischer L, Klein CG, Nadalin S, Lehner F, Settmacher U, Neuhaus P, Gotthardt D, Loss M, Ladenburger S, Paulus EM, Mertens M, Schlitt HJ. Everolimus and early calcineurin inhibitor withdrawal: 3-year results from a randomized trial in liver transplantation. *Am J Transplant* 2014; **14**: 701-710 [PMID: 24502384 DOI: 10.1111/ajt.12615]

165　**Levitsky J**, Feng S. Sirolimus and mTOR inhibitors in liver transplantation: the wheel has come full circle. *Am J Transplant* 2014; **14**: 249-250 [PMID: 24620373 DOI: 10.1111/ajt.12545]

166　**Matsuo Y**, Cassar A, Yoshino S, Flammer AJ, Li J, Gulati R, Topilsky Y, Raichlin E, Lennon RJ, Lerman LO, Rihal CS, Kushwaha SS, Lerman A. Attenuation of cardiac allograft vasculopathy by sirolimus: Relationship to time interval after heart transplantation. *J Heart Lung Transplant* 2013; **32**: 784-791 [PMID: 23856215 DOI: 10.1016/j.healun.2013.05.015]

167　**Witt CA**, Hachem RR. Immunosuppression: what's standard and what's new? *Semin Respir Crit Care Med* 2013; **34**: 405-413 [PMID: 23821513 DOI: 10.1055/s-0033-1348465]

168　**Gurk-Turner C**, Manitpisitkul W, Cooper M. A comprehensive review of everolimus clinical reports: a new mammalian target of rapamycin inhibitor. *Transplantation* 2012; **94**: 659-668 [PMID: 22986894 DOI: 10.1097/TP.0b013e31825b411c]

169　**Da Dalt L**, Bressan S, Martinolli F, Perilongo G, Baraldi E. Treatment of bronchiolitis: state of the art. *Early Hum Dev* 2013; **89** Suppl 1: S31-S36 [PMID: 23809346 DOI: 10.1016/S0378-3782(13)70011-2]

170　**Sacher VY**, Fertel D, Srivastava K, Panos A, Nguyen D, Baxter T, Shafazand S, Pham SM. Effects of prophylactic use of sirolimus on bronchiolitis obliterans syndrome development in lung transplant recipients. *Ann Thorac Surg* 2014; **97**: 268-274 [PMID: 24119986 DOI: 10.1016/j.athoracsur.2013.07.072]

171　**Borro JM**. Advances in immunosuppression after lung transplantation. *Med Intensiva* 2013; **37**: 44-49 [PMID: 22854620 DOI: 10.1016/j.medin.2012.05.011]

172　**Iacono AT**, Johnson BA, Grgurich WF, Youssef JG, Corcoran TE, Seiler DA, Dauber JH, Smaldone GC, Zeevi A, Yousem SA, Fung JJ, Burckart GJ, McCurry KR, Griffith BP. A randomized trial of inhaled cyclosporine in lung-transplant recipients. *N Engl J Med* 2006; **354**: 141-150 [PMID: 16407509 DOI: 10.1056/NEJMoa043204]

173　**Whitford H**, Walters EH, Levvey B, Kotsimbos T, Orsida B, Ward C, Pais M, Reid S, Williams T, Snell G. Addition of inhaled corticosteroids to systemic immunosuppression after lung transplantation: a double-blind, placebo-controlled trial. *Transplantation* 2002; **73**: 1793-1799 [PMID: 12085003]

174 **Johnson BA**, Iacono AT, Zeevi A, McCurry KR, Duncan SR. Statin use is associated with improved function and survival of lung allografts. *Am J Respir Crit Care Med* 2003; **167**: 1271-1278 [PMID: 12615629 DOI: 10.1164/rccm.200205-410OC]

175 **Vos R**, Vanaudenaerde BM, Verleden SE, Ruttens D, Vaneylen A, Van Raemdonck DE, Dupont LJ, Verleden GM. Anti-inflammatory and immunomodulatory properties of azithromycin involved in treatment and prevention of chronic lung allograft rejection. *Transplantation* 2012; **94**: 101-109 [PMID: 22461039 DOI: 10.1097/TP.0b013e31824db9da]

176 **Penninga L**, Møller CH, Gustafsson F, Gluud C, Steinbrüchel DA. Immunosuppressive T-cell antibody induction for heart transplant recipients. *Cochrane Database Syst Rev* 2013; **12**: CD008842 [PMID: 24297433 DOI: 10.1002/14651858.CD008842]

177 **Stratta RJ**, Farney AC, Rogers J, Orlando G. Immunosuppression for pancreas transplantation with an emphasis on antibody induction strategies: review and perspective. *Expert Rev Clin Immunol* 2014; **10**: 117-132 [PMID: 24236648 DOI: 10.1586/1744666X.2014.853616]

178 **Mako M,** Hager D, Muth B, Kaufman D, Ellis T, Djamali A. Thymoglobulin is associated with a significant reduction in de novo donor-specific antibodies and antibody mediated rejection in moderately sensitized renal transplant recipients. *Am J Transplant* 2013; **13**: S113

179 **Morgan RD**, O'Callaghan JM, Knight SR, Morris PJ. Alemtuzumab induction therapy in kidney transplantation: a systematic review and meta-analysis. *Transplantation* 2012; **93**: 1179-1188 [PMID: 22660659 DOI: 10.1097/TP.0b013e318257ad41]

180 **Barnett AN**, Hadjianastassiou VG, Mamode N. Rituximab in renal transplantation. *Transpl Int* 2013; **26**: 563-575 [PMID: 23414100 DOI: 10.1111/tri.12072]

181 **Kohei N**, Hirai T, Omoto K, Ishida H, Tanabe K. Chronic antibody-mediated rejection is reduced by targeting B-cell immunity during an introductory period. *Am J Transplant* 2012; **12**: 469-476 [PMID: 22054413 DOI: 10.1111/j.1600-6143.2011.03830.x]

182 **Sautenet B,** Blancho G, Buchler M. One year results of the effects of rituximab on acute humoral rejection in renal transplantation: RITUX ERAH, a multicenter randomized placebo controlled trial. *Am J Transplant* 2013; **13**: S112

183 **Stegall MD**, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405-2413 [PMID: 21942930 DOI: 10.1111/j.1600-6143.2011.03757.x]

184 A service of the U.S. National Institutes of Health. Cited: 2013-06-03. Available from: URL: http: //www.clinicaltrials.gov/

185 **Fehr T**, Gaspert A. Antibody-mediated kidney allograft rejection: therapeutic options and their experimental rationale. *Transpl Int* 2012; **25**: 623-632 [PMID: 22394269 DOI: 10.1111/j.1432-2277.2012.01453.x]

186 **Page EK**, Dar WA, Knechtle SJ. Biologics in organ transplantation. *Transpl Int* 2012; **25**: 707-719 [PMID: 22420711 DOI: 10.1111/j.1432-2277.2012.01456.x]

187 **Charpentier B**, Medina Pestana JO, Del C Rial M, Rostaing L, Grinyó J, Vanrenterghem Y, Matas A, Zhang R, Mühlbacher F, Pupim L, Florman S. Long-term exposure to belatacept in recipients of extended criteria donor kidneys. *Am J Transplant* 2013; **13**: 2884-2891 [PMID: 24103072 DOI: 10.1111/ajt.12459]

188 **Rostaing L**, Vincenti F, Grinyó J, Rice KM, Bresnahan B, Steinberg S, Gang S, Gaite LE, Moal MC, Mondragón-Ramirez GA, Kothari J, Pupim L, Larsen CP. Long-term belatacept exposure maintains efficacy and safety at 5 years: results from the long-term extension of the BENEFIT study. *Am J Transplant* 2013; **13**: 2875-2883 [PMID: 24047110 DOI: 10.1111/ajt.12460]

189 **Kirk AD**, Guasch A, Xu H, Cheeseman J, Mead SI, Ghali A, Mehta AK, Wu D, Gebel H, Bray R, Horan J, Kean LS, Larsen CP, Pearson TC. Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *Am J Transplant* 2014; **14**: 1142-1151 [PMID: 24684552 DOI: 10.1111/ajt.12712]

190 **Goldwater R**, Keirns J, Blahunka P, First R, Sawamoto T, Zhang W, Kowalski D, Kaibara A, Holman J. A phase 1, randomized ascending single-dose study of antagonist anti-human CD40 ASKP1240 in healthy subjects. *Am J Transplant* 2013; **13**: 1040-1046 [PMID: 23356210 DOI: 10.1111/ajt.12082]

191 **Watanabe M**, Yamashita K, Suzuki T, Kamachi H, Kuraya D, Koshizuka Y, Ogura M, Yoshida T, Aoyagi T, Fukumori D, Shimamura T, Okimura K, Maeta K, Miura T, Sakai F, Todo S. ASKP1240, a fully human anti-CD40 monoclonal antibody, prolongs pancreatic islet allograft survival in nonhuman primates. *Am J Transplant* 2013; **13**: 1976-1988 [PMID: 23841873 DOI: 10.1111/ajt.12330]

192 **Rostaing L**, Charpentier B, Glyda M, Rigotti P, Hettich F, Franks B, Houbiers JG, First R, Holman JM. Alefacept combined with tacrolimus, mycophenolate mofetil and steroids in de novo kidney transplantation: a randomized controlled trial. *Am J Transplant* 2013; **13**: 1724-1733 [PMID: 23730730 DOI: 10.1111/ajt.12303]

193 **Vincenti F**, Tedesco Silva H, Busque S, O'Connell P, Friedewald J, Cibrik D, Budde K, Yoshida A, Cohney S, Weimar W, Kim YS, Lawendy N, Lan SP, Kudlacz E, Krishnaswami S, Chan G. Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. *Am J Transplant* 2012; **12**: 2446-2456 [PMID: 22682022 DOI: 10.1111/j.1600-6143.2012.04127.x]

194 **Wojciechowski D**, Vincenti F. Tofacitinib in kidney transplantation. *Expert Opin Investig Drugs* 2013; **22**: 1193-1199 [PMID: 23841583 DOI: 10.1517/13543784.2013.811231]

195 **Russ GR**, Tedesco-Silva H, Kuypers DR, Cohney S, Langer RM, Witzke O, Eris J, Sommerer C, von Zur-Mühlen B, Woodle ES, Gill J, Ng J, Klupp J, Chodoff L, Budde K. Efficacy of sotrastaurin plus tacrolimus after de novo kidney transplantation: randomized, phase II trial results. *Am J Transplant* 2013; **13**: 1746-1756 [PMID: 23668931 DOI: 10.1111/ajt.12251]

196 **Tedesco-Silva H**, Kho MM, Hartmann A, Vitko S, Russ G, Rostaing L, Budde K, Campistol JM, Eris J, Krishnan I, Gopalakrishnan U, Klupp J. Sotrastaurin in calcineurin inhibitor-free regimen using everolimus in de novo kidney transplant recipients. *Am J Transplant* 2013; **13**: 1757-1768 [PMID: 23659755 DOI: 10.1111/ajt.12255]

197 **Kimelman M**, Brandacher G. Trends in immunosuppression after pancreas transplantation: what is in the pipeline? *Curr Opin Organ Transplant* 2013; **18**: 76-82 [PMID: 23254700 DOI: 10.1097/MOT.0b013e32835c6eda]

198 **Samikannu B**, Chen C, Lingwal N, Padmasekar M, Engel FB, Linn T. Dipeptidyl peptidase IV inhibition activates CREB and improves islet vascularization through VEGF-A/VEGFR-2 signaling pathway. *PLoS One* 2013; **8**: e82639 [PMID: 24349326 DOI: 10.1371/journal.pone.0082639]

199 **Vos R**, Verleden SE, Ruttens D, Vandermeulen E, Yserbyt J, Dupont LJ, Van Raemdonck DE, De Raedt N, Gheysens O, De Jong PA, Verleden GM, Vanaudenaerde BM. Pirfenidone: a potential new therapy for restrictive allograft syndrome? *Am J Transplant* 2013; **13**: 3035-3040 [PMID: 24102752 DOI: 10.1111/ajt.12474]

200 **Bonarius HP**, Baas F, Remmerswaal EB, van Lier RA, ten Berge IJ, Tak PP, de Vries N. Monitoring the T-cell receptor repertoire at single-clone resolution. *PLoS One* 2006; **1**: e55 [PMID: 17183685 DOI: 10.1371/journal.pone.0000055]

201 **Levitsky J**. Operational tolerance: past lessons and future prospects. *Liver Transpl* 2011; **17**: 222-232 [PMID: 21384504 DOI: 10.1002/lt.22265]

202 **Ruiz P**, Maldonado P, Hidalgo Y, Gleisner A, Sauma D, Silva C, Saez JJ, Nuñez S, Rosemblatt M, Bono MR. Transplant tolerance: new insights and strategies for long-term allograft acceptance. *Clin Dev Immunol* 2013; **2013**: 210506 [PMID: 23762087 DOI: 10.1155/2013/210506]

203 **Sachs DH**, Sykes M, Kawai T, Cosimi AB. Immuno-intervention for the induction of transplantation tolerance through mixed chimerism. *Semin Immunol* 2011; **23**: 165-173 [PMID: 21839648 DOI: 10.1016/j.smim.2011.07.001]

204 **Kawai T**, Sachs DH, Sykes M, Cosimi AB. HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 2013; **368**: 1850-1852 [PMID: 23656665 DOI: 10.1056/NEJMc1213779]

205 **Farris AB**, Taheri D, Kawai T, Fazlollahi L, Wong W, Tolkoff-Rubin N, Spitzer TR, Iafrate AJ, Preffer FI, Locascio SA, Sprangers B, Saidman S, Smith RN, Colvin AB, Sykes M, Sachs DH, Colvin RB. Acute renal endothelial injury during marrow recovery in a cohort of combined kidney and bone marrow allografts. *Am J Transplant* 2011; **11**: 1464-1477 [PMID: 21668634 DOI: 10.1111/j.1600-6143.2011.03572.x]

206 **Kawai T**, Sogawa H, Boskovic S, Abrahamian G, Smith RN, Wee SL, Andrews D, Nadazdin O, Koyama I, Sykes M, Winn HJ, Colvin RB, Sachs DH, Cosimi AB. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. *Am J Transplant* 2004; **4**: 1391-1398 [PMID: 15307826 DOI: 10.1111/j.1600-6143.2004.00523.x]

207 **Wekerle T**, Kurtz J, Ito H, Ronquillo JV, Dong V, Zhao G, Shaffer J, Sayegh MH, Sykes M. Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nat Med* 2000; **6**: 464-469 [PMID: 10742157 DOI: 10.1038/74731]

208 **Kawai T**, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL, Shaffer J, Preffer FI, Ding R, Sharma V, Fishman JA, Dey B, Ko DS, Hertl M, Goes NB, Wong W, Williams WW, Colvin RB, Sykes M, Sachs DH. HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 2008; **358**: 353-361 [PMID: 18216355 DOI: 10.1056/NEJMoa071074]

209 **Adams AB**, Williams MA, Jones TR, Shirasugi N, Durham MM, Kaech SM, Wherry EJ, Onami T, Lanier JG, Kokko KE, Pearson TC, Ahmed R, Larsen CP. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest* 2003; **111**: 1887-1895 [PMID: 12813024 DOI: 10.1172/JCI200317477]

210 **Casiraghi F**, Perico N, Remuzzi G. Mesenchymal stromal cells to promote solid organ transplantation tolerance. *Curr Opin Organ Transplant* 2013; **18**: 51-58 [PMID: 23254705 DOI: 10.1097/MOT.0b013e32835c5016]

211 **Leventhal J**, Abecassis M, Miller J, Gallon L, Ravindra K, Tollerud DJ, King B, Elliott MJ, Herzig G, Herzig R, Ildstad ST. Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation. *Sci Transl Med* 2012; **4**: 124ra28 [PMID: 22399264 DOI: 10.1126/scitranslmed.3003509]

212 **Leventhal J**, Abecassis M, Miller J, Gallon L, Tollerud D, Elliott MJ, Bozulic LD, Houston C, Sustento-Reodica N, Ildstad ST. Tolerance induction in HLA disparate living donor kidney transplantation by donor stem cell infusion: durable chimerism predicts outcome. *Transplantation* 2013; **95**: 169-176 [PMID: 23222893 DOI: 10.1097/TP.0b013e3182782fc1]

213 **Leventhal JR**, Mathew JM, Salomon DR, Kurian SM, Suthanthiran M, Tambur A, Friedewald J, Gallon L, Charette J, Levitsky J, Kanwar Y, Abecassis M, Miller J. Genomic biomarkers correlate with HLA-identical renal transplant tolerance. *J Am Soc Nephrol* 2013; **24**: 1376-1385 [PMID: 23787913 DOI: 10.1681/ASN.2013010068]

214 **Cippà PE**, Gabriel SS, Kraus AK, Chen J, Wekerle T, Guimezanes A, Wüthrich RP, Fehr T. Bcl-2 inhibition to overcome memory

cell barriers in transplantation. *Am J Transplant* 2014; **14**: 333-342 [PMID: 24472193 DOI: 10.1111/ajt.12554]

215 **Lau AH**, de Creus A, Lu L, Thomson AW. Liver tolerance mediated by antigen presenting cells: fact or fiction? *Gut* 2003; **52**: 1075-1078 [PMID: 12865260]

216 **Warren A**, Le Couteur DG, Fraser R, Bowen DG, McCaughan GW, Bertolino P. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. *Hepatology* 2006; **44**: 1182-1190 [PMID: 17058232 DOI: 10.1002/hep.21378]

217 **McAvoy EF**, Kubes P. Holey endothelium: gateways for naïve T cell activation. *Hepatology* 2006; **44**: 1083-1085 [PMID: 17058224 DOI: 10.1002/hep.21421]

218 **Wu SL**, Pan CE. Tolerance and chimerism and allogeneic bone marrow/stem cell transplantation in liver transplantation. *World J Gastroenterol* 2013; **19**: 5981-5987 [PMID: 24106398 DOI: 10.3748/wjg.v19.i36.5981]

219 **Blumberg EA**, Danziger-Isakov L, Kumar D, Michaels MG, Razonable RR. The American Society of Transplantation Infectious Diseases Guidelines. 3rd ed. *Am J Transplant* 2013; **13**, S 4: 1-371 [DOI: 10.1111/ajt.12129]

220 **Seem DL**, Lee I, Umscheid CA, Kuehnert MJ. Excerpt from PHS guideline for reducing HIV, HBV and HCV transmission through organ transplantation. *Am J Transplant* 2013; **13**: 1953-1962 [PMID: 23890284 DOI: 10.1111/ajt.12386]

221 **Kotton CN**, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, Humar A. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation* 2013; **96**: 333-360 [PMID: 23896556 DOI: 10.1097/TP.0b013e31829df29d]

222 **Fishman JA**. Overview: cytomegalovirus and the herpesviruses in transplantation. *Am J Transplant* 2013; **13** Suppl 3: 1-8; quiz 8 [PMID: 23347210 DOI: 10.1111/ajt.12002]

223 **Humar A**, Michaels M. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am J Transplant* 2006; **6**: 262-274 [PMID: 16426310 DOI: 10.1111/j.1600-6143.2005.01207.x]

224 **Green M**. Introduction: Infections in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 3-8 [PMID: 23464993 DOI: 10.1111/ajt.12093]

225 **Theodoropoulos N**, Jaramillo A, Ladner DP, Ison MG. Deceased organ donor screening for HIV, hepatitis B, and hepatitis C viruses: a survey of organ procurement organization practices. *Am J Transplant* 2013; **13**: 2186-2190 [PMID: 23711196 DOI: 10.1111/ajt.12260]

226 **Orlowski J**, Alexander C, Ison M, Rosendale J, Chabalewski F. NAT Survey Workgroup, Richmond, VA. Nucleic Acid Testing (NAT) for HIV, HBV, and HCV: Current Practices of 58 US Organ Procurement Organizations (OPOs). *Am J Transplant* 2009; **9**: S2 197 [DOI: 10.1111/j.1600-6143.2009.02658.x]

227 **Rogers MF**, Simonds RJ, Lawton KE, Moseley RR, Jones WK. Guidelines for preventing transmission of human immunodeficiency virus through transplantation of human tissue and organs. *MMWR Recomm Rep* 1994; **43**: 1-17

228 Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products. Available from: URL: http://www.fda.gov/downloads/Biologics Blood Vaccines/Guidance ComplianceRegulatoryInformation/Guidances/Tissue/ucm091345.pdf

229 **Hirsch HH**, Randhawa P. BK polyomavirus in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 179-188 [PMID: 23465010 DOI: 10.1111/ajt.12110]

230 **Sood P**, Senanayake S, Sujeet K, Medipalli R, Van-Why SK, Cronin DC, Johnson CP, Hariharan S. Donor and recipient BKV-specific IgG antibody and posttransplantation BKV infection: a prospective single-center study. *Transplantation* 2013; **95**: 896-902

[PMID: 23511214 DOI: 10.1097/TP.0b013e318282ba83]

231 **Greiner M**, Cusini A, Ruesch M, Schiesser M, Ledergerber B, Fehr T, Mueller NJ. A stringent preemptive protocol reduces cytomegalovirus disease in the first 6 months after kidney transplantation. *Infection* 2012; **40**: 669-675 [PMID: 23015480 DOI: 10.1007/s15010-012-0343-5]

232 **Griffiths PD**, Stanton A, McCarrell E, Smith C, Osman M, Harber M, Davenport A, Jones G, Wheeler DC, O'Beirne J, Thorburn D, Patch D, Atkinson CE, Pichon S, Sweny P, Lanzman M, Woodford E, Rothwell E, Old N, Kinyanjui R, Haque T, Atabani S, Luck S, Prideaux S, Milne RS, Emery VC, Burroughs AK. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. *Lancet* 2011; **377**: 1256-1263 [PMID: 21481708 DOI: 10.1016/S0140-6736(11)60136-0]

233 **Kharfan-Dabaja MA**, Boeckh M, Wilck MB, Langston AA, Chu AH, Wloch MK, Guterwill DF, Smith LR, Rolland AP, Kenney RT. A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2012; **12**: 290-299 [PMID: 22237175 DOI: 10.1016/S1473-3099(11)70344-9]

234 **Carbone M**, Mutimer D, Neuberger J. Hepatitis C virus and nonliver solid organ transplantation. *Transplantation* 2013; **95**: 779-786 [PMID: 23172130 DOI: 10.1097/TP.0b013e318273fec4]

235 **Xiao D**, Craig JC, Chapman JR, Dominguez-Gil B, Tong A, Wong G. Donor cancer transmission in kidney transplantation: a systematic review. *Am J Transplant* 2013; **13**: 2645-2652 [PMID: 24034231 DOI: 10.1111/ajt.12430]

236 **Desai R**, Collett D, Watson CJ, Johnson P, Evans T, Neuberger J. Cancer transmission from organ donors-unavoidable but low risk. *Transplantation* 2012; **94**: 1200-1207 [PMID: 23269448 DOI: 10.1097/TP.0b013e318272df41]

237 **Desai R,** Collett D, Watson CJ, Johnson P, Evans T, Neuberger J. Death on waiting-list and risk of transmission of cancer: where is the right balance? *Am J Transplant* 2013; **13** (suppl 5): S140

238 **Desai R**, Collett D, Watson CJ, Johnson P, Evans T, Neuberger J. Estimated risk of cancer transmission from organ donor to graft recipient in a national transplantation registry. *Br J Surg* 2014; **101**: 768-774 [PMID: 24771410 DOI: 10.1002/bjs.9460]

239 **Engels E.** Risk for transmission of cancer from solid organ donors in the United States. *Am J Transplant* 2013; **13** (suppl 5): S536

240 **Engels EA**, Castenson D, Pfeiffer RM, Kahn A, Pawlish K, Goodman MT, Nalesnik MA, Israni AK, Snyder J, Kasiske B. Cancers among US organ donors: a comparison of transplant and cancer registry diagnoses. *Am J Transplant* 2014; **14**: 1376-1382 [PMID: 24712385 DOI: 10.1111/ajt.12683]

241 **Desai R**, Collett D, Watson CJ, Johnson P, Evans T, Neuberger J. Risk of recurrence of preexisting cancer in organ recipients. *Am J Transplant* 2013; **13** (suppl 5): S98

242 **Sampaio MS**, Cho YW, Qazi Y, Bunnapradist S, Hutchinson IV, Shah T. Posttransplant malignancies in solid organ adult recipients: an analysis of the U.S. National Transplant Database. *Transplantation* 2012; **94**: 990-998 [PMID: 23085553 DOI: 10.1097/TP.0b013e318270bc7b]

243 **Na R**, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM. Comparison of de novo cancer incidence in Australian liver, heart and lung transplant recipients. *Am J Transplant* 2013; **13**: 174-183 [PMID: 23094788 DOI: 10.1111/j.1600-6143.2012.04302.x]

244 **Cho Y**, Sampaio M, Shah T, Qazi Y, Fong T, Hutchinson IV. Association between de novo post-transplant nonmelanoma skin cancer and solid tumor after organ transplantation – an analysis of OPTN/UNOS data. *Am J Transplant* 2013; **13** (suppl 5): S536

245 **McDonald S,** Carroll R. The occurrence of non-melanoma skin cancer predicts risk of subsequent solid organ cancer. *Am J Transplant* 2013; **13** (suppl 5): S97

246 **Hanlon A**, Colegio OR. The cutting edge of skin cancer in transplant recipients: scientific retreat of international transplant Skin Cancer Collaborative and Skin Cancer in Organ Transplant Patients Europe. *Am J Transplant* 2014; **14**: 1012-1015 [PMID: 24612476 DOI: 10.1111/ajt12681]

TOPIC HIGHLIGHT

Maurizio Salvadori, Professor, *Series Editor*

Psychopathological aspects of kidney transplantation: Efficacy of a multidisciplinary team

Concetta De Pasquale, Massimiliano Veroux, Luisa Indelicato, Nunzia Sinagra, Alessia Giaquinta, Michele Fornaro, Pierfrancesco Veroux, Maria L Pistorio

Concetta De Pasquale, Massimiliano Veroux, Luisa Indelicato, Nunzia Sinagra, Alessia Giaquinta, Pierfrancesco Veroux, Maria L Pistorio, Vascular Surgery and Organ Transplant Unit, Department of Surgery, Transplantation and Advanced Technologies, University Hospital of Catania, 95124 Catania, Italy
Michele Fornaro, Department of Formative Sciences, University of Catania, 95124 Catania, Italy
Author contributions: De Pasquale C, Veroux M, Indelicato L, Sinagra N, Giaquinta A, Fornaro M, Veroux P and Pistorio ML all contributed to this paper.
Correspondence to: Massimiliano Veroux, MD, PhD, Vascular Surgery and Organ Transplant Unit, Department of Surgery, Transplantation and Advanced Technologies, University Hospital of Catania, Via S. Sofia, 86-95123 Catania, Italy. veroux@unict.it
Telephone: +39-95-3782384 Fax: +39-95-3782948
Received: March 14, 2014   Revised: May 2, 2014
Accepted: May 29, 2014
Published online: December 24, 2014

Abstract

Renal transplantation is a well established treatment for end-stage renal disease, allowing most patients to return to a satisfactory quality of life. Studies have identified many problems that may affect adaptation to the transplanted condition and post-operative compliance. The psychological implications of transplantation have important consequences even on strictly physical aspects. Organ transplantation is very challenging for the patient and acts as an intense stressor stimulus to which the patient reacts with neurotransmitter and endocrine-metabolic changes. Transplantation can result in a psychosomatic crisis that requires the patient to mobilize all bio-psycho-social resources during the process of adaptation to the new foreign organ which may result in an alteration in self-representation and identity, with possible psychopathologic repercussions. These reactions are feasible in mental disorders, *e.g.*, post-traumatic stress disorder, adjustment disorder, and psychosomatic disorders. In organ transplantation, the fruitful collaboration between professionals with diverse scientific expertise, calls for both a guarantee for mental health and greater effectiveness in challenging treatments for a viable association between patients, family members and doctors. Integrated and multidisciplinary care should include uniform criteria and procedures for standard assessments, for patient autonomy, adherence to therapy, new coping strategies and the adoption of more appropriate lifestyles.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Psychiatric consultation; Psychological care; Kidney transplantation; Therapeutic compliance; Social and family support

Core tip: Kidney transplantation is now an established clinical technique, although the emotional experiences and the psychological and psychopathological complications related to organ donation and transplantation should not be underestimated. Following transplantation, problems related to the physical integration of a foreign body can arise. On the one hand, the "Life-Extending" process creates a kind of symbolic rebirth with euphoric aspects, and on the other hand, the patient can develop a kind of emotional vulnerability with body image and self-representation disorders, or paranoid reactions to a panic crisis due to the presence of a foreign object (transplanted organ). In fact, the transplanted patient may experience a reactive psychopathologic process (depression, anxiety, dissociative disorder) both due to transplanted organ acceptance difficulties and immunosuppressive therapy complications. The study of psychological aspects and their evaluation using a multidisciplinary approach are important to avoid issues not adequately recognized, which can undermine the transplant success, and/or lead to psychological

distress and psychological suffering in the patient. Transplanted patient re-employment and social and family reintegration requires psychotherapeutic support to implement new coping strategies.

INTRODUCTION

Renal transplantation is a well-established treatment for end-stage renal disease, allowing most patients to return to a satisfactory quality of life. Advances in medical science and technology in this field are impressive. However, there are still some difficulties that limit the number of transplants performed and the positive outcomes of the interventions. In addition to the insufficient number of donated organs from deceased and living donors, a major difficulty is the result of transplant course management often exclusively medical-surgical, ignoring the close interaction between mind and body.

In recent years there has been a gradual increase in integration between medical and psychological disciplines and psychological support to patients at all stages of the transplantation and to the donor's family, which is now a fairly well-established method of intervention[1-6]. In the case of deceased organ donation, the medical-surgical process is conditioned by the death of another human being, and this raises biological, moral, religious, psychological and social questions.

On the one hand, the donation and removal of organs bring out strong feelings in the relatives of donors, such as demoralization, loneliness, pain and anguish. On the other hand, the person receiving the transplant has feelings of hope, joy, desire for life and rebirth. The inability to mourn and to accept the loss in donor relatives (usually mothers) may result in the so-called "syndrome of the hound". This is a state of mental suffering that involves some people who remain in a state of denial and in mourning, and who show an irresistible desire to know the identity of the transplanted person[7].

In the case of a living donor, the family takes on the responsibility of donation. Feelings of guilt, any need of repair and symbiotic relationships between family members are sometimes reasons that prevent the specialist from granting suitability for transplantation. Psychotherapy has a very important function as it helps the patient to deal with reality, giving a different meaning to the motivations that lead to transplantation.

With regard to the psychological aspects of the recipient with chronic kidney disease, kidney transplantation, although it represents for many patients the "liberation" from the restrictions imposed by the "dialysis addiction", it can also arouse doubts, anxiety and distress which can become, in the post-operative period, fear of infections, worries of rejection and of the unpredictable outcome. In fact, transplant patients can develop emotional distress and affective disorders, such as anxiety and depression, associated with a compromised quality of life[8-12].

Transplantation can also result in a psychosomatic crisis that requires the patient to mobilize all their bio-psycho-social resources during the process of adaptation to the new foreign organ which may result in an alteration in self-representation and identity, with possible psychopathologic repercussions[13-15].

This article will review relevant research on the psychopathological aspects of kidney transplantation. The topics analyzed include body image, personality, post-transplant psychopathological risk, and therapeutic compliance.

BODY IMAGE IN KIDNEY TRANSPLANTATION

The human being has a mental representation of one's body. This, as only a small part is innate, is something that is formed in early childhood, which can change during a person's lifetime and varies in health and disease. The body, therefore, is also a mentally complex construct.

In Schilder's theory (1935), organic disease is a factor of fundamental importance in the evolution and organization of our body schema. Disease in an organ can facilitate a "psychosomatic crisis", a crisis in which the somatic and the psychic aspects are of equal importance, and influence each other[16,17].

In transplantation, if surgery rapidly restores the anatomical and physiological function, cognitive and emotional integration is required: "psychic transplantation[10,18-20].

In this context, the contributions from psychosomatic aspects refer to the complex task of mind reconstruction which the transplanted subject must perform in their own image. This is a difficult process of reconstruction, which allows the acceptance and psychic integration of the new organ[21-23].

During the course of transplantation, the wholeness and unity of the body image is broken. This "Life-Extending" process can develop a kind of emotional vulnerability with body image and self-representation disorders, or paranoid reactions to a panic crisis due to the presence of a foreign object (transplanted organ). This reconstruction process is long and difficult and requires psychic integration of the transplanted organ. According to Castelnuovo-Tedesco (1981), during the organ integration process there are three stages: (1) phase of the foreign body, in which the transplanted organ as foreign can cause persecutory anxieties, or on the contrary idealization; (2) phase of partial incorporation, in which the patient begins to integrate the organ; and (3) phase of total incorporation, in which the organ is acquired automatically, therefore, spontaneous consciousness of the same is absent[18]. Therefore, following

transplantation the "foreign" organ is integrated leading to good harmonization of body image in the recipient[24-26].

PERSONALITY AND RECIPIENT EMOTIONAL PATTERNS

The affective profile in transplanted patients should be more extensively examined to review all aspects of their mental and emotional assessment, as the emotional pattern constitutes a critical clinical feature of these patients[27]. Receiving an organ requires the death of the donor, or at best, living donor surgery, and even if voluntary, this may be the cause of guilt fantasies expressed by transplant subjects[28,29].

Another important aspect to be taken into consideration concerns the psychological attitudes in the stages preceding the transplant, as the patient may have "unrealistic expectations" that will be an obstacle in dealing with transplant procedures and consequences[30-32].

Equally disappointing may be the "traumatic" discovery that the transplant did not provide a good "restitutio ad integrum", with the onset of depressive dynamics and difficulties in accepting the therapeutic post-transplant program[33-37].

This lack of motivation must be identified and possibly corrected before transplantation, as it can lead to rejection resulting in a waste of resources and equipment. If the patient is motivated and understands all the implications of kidney disease in the terminal phase of uremia, the patient feels a responsibility to himself, his family and hopes to improve, following transplantation, his quality of life and his own mental and physical balance[38-42].

De Pasquale *et al*[23] explored personality characteristics in patients undergoing renal transplantation and confirmed the hypothesis that transplantation can pose a potential risk to the patient's psychological balance. The analyzed psychological variables showed a "hysterical personality" characterized by immaturity and self-centeredness, impulsive behavior, dependency, inferiority feelings, hypercontrol and superficial interpersonal relationships. This mental condition is well established in transplanted subjects who tend to be egocentric, dependent on caregivers and focus only on their own needs and the new physical condition, thus changing relationship quality, emotions and self-esteem.

In determining hysterical phenomenology, congenital factors as well as acquired factors related to the environment, suffering, stress and electrolyte changes (K/Ca) are important[43]. Organ transplantation is very challenging in patients and acts as an intense stressor stimulus to which the patient reacts with neurotransmitter and endocrine-metabolic changes. These reactions can result in mental disorders, *e.g.*, post-traumatic stress disorder, adjustment disorder, and psychosomatic disorders.

Pistorio *et al*[30] investigated other personality traits which may emerge in transplant patients and found borderline personality and obsessive-compulsive personality, which are traits negatively correlated with good quality of life. They concluded that it is important to identify patients who have shown pathologic personality traits in order to provide adequate psychologic-psychiatric support and follow-up.

LIVING KIDNEY TRANSPLANTATION

Kidney donation from a living donor is the best solution for end-stage renal failure, both in terms of cost-effectiveness and quality of life, and has many advantages compared with cadaveric transplantation. However, medical practice has long been questioned on ethical, legal and psychological aspects related to living donation.

In this regard, it is important to remember altruistic or "Samaritan" organ donation, only allowed for kidney donation, which follows the National Bioethics Committee of April 23, 2010 and Board of Health of May 4, 2010 guidelines, in compliance with the law n. 458/67 and its implementing regulation n. 116 of April 16, 2010. The Samaritan donor's clinical suitability evaluation follows the same procedures as recommended for standard living donation. Personality dimensions are an essential prerequisite for suitability assessment in transplantation[44,45].

Both recipient and donor affective disorders diagnosed by diagnostic and statistical manual of mental disorders IV TR Axis I personality disorders, substance or benzodiazepine addiction and cognitive deficits should be excluded to avoid psychological and psychiatric post-donation complications[46,47].

Studies have identified many issues which may affect adaptation to the transplanted condition and post-operative compliance[21,48].

The decision to choose living donor transplantation is determined by a particular condition characterized by strong mental and emotional distress in the patient and his family, compounded by the fact that the donor is almost always a family member. Living kidney transplantation creates a particular donor-recipient relationship, characterized by mutual emotional support, which is useful in dealing with this delicate situation[49].

Several authors point out that the reasons for living donation seem to be linked to the suffering of their relative due to progressive renal failure, dialysis and its side effects and long waiting times for deceased donor transplant. Attention should also be paid to the indirect benefits that donation brings to the donor in terms of improvements in self-esteem and self-image.

It is necessary to explore the development of motivation for living donation in order to achieve and maintain a harmonious relationship with the recipient, while respecting their individuality.

In the intra-family selection process for donor identification, the donor is most often the mother enforcing the "maternal privilege" of being the only one eligible for donation[50-52].

In identifying the donor it is necessary to assess the risks of an "impulsive" or poorly cognitively and affectively processed decision, caused by excessive "moral obligation" feelings, "hypomania" and "megalomania" aspects[31,53,54].

Several studies have shown the presence of reluctance on the part of the sick person to accept the donation from a relative. The reasons for this reluctance are different and vary

from one individual to another, and transplant failure can result in intense guilt feelings in the recipient[28,55-57].

With regard to the couple (donor-recipient), some studies have reported an improvement in this relationship, while others have defined it as stable[58-62].

According to a study conducted in 2006 in The Netherlands, the main factor leading to the increase in the number of consents in favor of living donation was being properly informed about the surgical procedures and any risks to themselves and to the donor through specific interviews and questionnaires[63,64].

The risk of problems in recipient sexual identity may occur in people who show sexual identity problems or in adolescents. In these cases, kidney adaptation and integration processes may be more difficult if the donor is of the opposite sex[65].

Therefore, the psychological coping process involved in living kidney donation demands a reconstitution of the body self[66].

De Pasquale *et al*[31] (2013) analyzed living kidney donor personality by examining a sample of 18 living kidney donors using the Millon Clinical Multiaxial Inventory-Ⅲ; they found the presence of narcissistic, histrionic and obsessive-compulsive personality traits in living kidney donors.

POST-TRANSPLANT PSYCHOPATHOLOGICAL RISK

The emotional impact of transplantation can be a traumatic event that interrupts the sense of continuity and personal integrity, eliciting strong emotions.

The experience of negative and disorganized contents makes the person unable to cope with the stressors, including hospitalization, surgery, and invasive treatments, which can be encoded in a distorted way and experienced as terrifying perceptions[67,68].

The threat to the "physical integrity" can then turn into a threat to the "mind integrity", giving rise to psychopathological reactions of different nature and gravity[69-72].

Several international studies showed physical functions and overall post-transplant quality of life improvement: uremic symptoms, sleep disturbances and appetite disorders disappeared, and hematocrit and hemoglobin levels increased significantly, as well as improvements in cognitive function[73-80]. However, despite these improvements and a reduction in total symptom distress, many studies also found a risk of psychopathological and psychosocial malaise[75,81-83].

In the period immediately following surgery, the patient may present a confusional psychosis with anxiety, restlessness, confusion, agitation, hallucinations, confabulation and emotional lability. The frequency of this confusional psychosis varies (20%-40%) and the use of steroids may prolong the psychotic state resulting in "steroid psychosis" with the prevalence of paranoid and hallucination reactions[65].

In the subsequent post-transplant period, liberation feelings, intense emotionalism, euphoria and a sense of rebirth may be prevalent. This phase, which is defined as the "honeymoon", also presents negative symptoms including rejection fear, post-transplant complications, existential uncertainty and gratitude feelings, but also guilt feelings towards the donor[84,85].

In the case where "healing" expectations are amplified, both for a lack of information and for a state of post-operative euphoria, anxious-depressive states may be present in the post-transplant phase[86,87].

The hospital discharge, return to the family and social context require an adaptation process lasting 6 mo to a year, the "life by sick" and dependence on others waiver. The perception of loss of support from physicians can make readjustment to the outside world difficult for transplant patients. This experience is more noticeable in people with a weak perception of their personal abilities and autonomy, for example, after a long period of dialysis[88].

The acceptance of transplant status change is often difficult for family members who have had to redefine roles within the family and recognize the effective autonomy skills of their relative. The process is complex and can present moments of opposition to change, with a need to recover the pre-transplant relations system[44].

The state of post-transplant well-being may be hindered by the following factors: (1) late shock effects/surgery stress (6 mo-1 year), which can lead to cognitive disorders, insomnia, anxiety and depression; (2) anti-rejection therapy side effects: tremors and ataxia due to cyclosporine, changes in body image; (3) anxiety for regular medical checks; (4) emotional crises for complications or rejection episodes with fear, anguish, dejection and anger; and (5) organic or psychological sexual dysfunction[23,65,87-89]. In summary, for better post-transplant rehabilitation and given the obvious risks of psychopathology, the development of interdisciplinary interventions such as socio-medical and psychotherapeutic programs, without which adaptation after transplantation may be difficult and with inevitable repercussions on quality of life[90].

THE ROLE OF A MULTIDISCIPLINARY TEAM ON ADHERENCE IN KIDNEY TRANSPLANTATION

Transplantation results in a significant improvement in expectations and quality of life, even if possible adaptation difficulties may be present such as psychopathological disorders, problems with compliance and adherence to treatment protocols. Such non-adherence seems to predict morbidity and mortality[91-93].

After transplantation, regular immunosuppressive drug administration is crucial, and even small deviations from the prescribed regimen are associated with an increased risk of rejection. The eventual resumption of dialysis replacement therapy after transplantation affects not only patient physical function, but especially his personal, daily and social life. Strong feelings of discomfort, especially

in females, with a "resignation to a life of eternal sick", a reduction in self-esteem due to the change in their role in the family have been reported in the literature[94-101].

A strong concern for the future of himself and of his family prevails, in addition to a strong psychological stress condition that leads to anger and depression. The sense of self-efficacy, coping with the disease and self-monitoring, fosters respect for prescriptions. Patients with a higher self-efficacy show a greater ability to self-manage their own health, with better physical health, a satisfactory quality of life and a decreased risk of complications[95,102-109]. Other studies have shown a positive correlation between self-efficacy and several indicators of health: better control of diabetes, fewer depressive symptoms, lower use of health care institutions and long-term adherence to prescribed drug therapy[110-113]. The beneficial effect of exercise on allograft function and its positive correlation with better health and quality of life were also demonstrated.

Another problem observed concerning psychiatric disorders prior to transplantation is related to non-optimal post-transplant therapeutic compliance[114-120]. Depression pre-or post-transplantation is associated with an increased risk of non-adherence to medical prescriptions, as well as high levels of anxiety and hostility and the presence of unstable personality traits. An excessive perception of "restored health" can lead to promiscuity, abuse of various substances and non-adherence to prescribed treatment in transplant patients, which has a significant impact on post-transplant recovery[65,121,122].

The perceived consequences of living with a chronic medical condition (such as a renal transplant) likely affect adherence and psychological outcomes. Among investigations in adults with a chronic illness, more severe perceived consequences have been found to be associated with greater use of avoidance coping strategies, denial, and behavioral disengagement[123-125]. Medication non-adherence is a common problem in organ transplantation patients with severe consequences for the patients' health[126].

A better understanding of the perceived adversity associated with different aspects of living with a chronic illness may clarify possible interventions to improve illness outcomes. According to recent literature, patients who receive a protocol of psychological support before transplantation and during post-transplant follow-up, this leads to improved treatment compliance and quality of life with modifications related to the physical, emotional and psychological aspects[127]. In this context, consultation and liaison psychiatry has played, and continues to play, a role in stimulating research and fostering the integration between psychiatry and other medical and surgical disciplines.

In a hospital environment, there is a growing need for liaison between operators, and doctors and nurses from different specialties. More use should be made of the Consultation-Liaison Psychiatry facilities, particularly where there is a strong emotional impact on the relationship between operator and patient, such as the intensive care

unit, *etc.*, where psychiatrists and psychologists should encourage the involvement of the various stakeholders in patient management, and encourage the exchange of knowledge and experience in appropriate and useful liaison activities to prevent burn-out[128].

It is also necessary to include discussions on clinical cases as part of the multidisciplinary team and to promote training sessions and supervision, which are useful in planning cognitive and psychosocial rehabilitation, and psychotherapy both for the patient and his family.

Assessment of quality of life is one of the key indicators for monitoring coping strategies acquired by the transplanted patient and/or the donor-recipient pair. In fact, although it constitutes a subjective variable, quality of life constantly changes in relation to the short- and long-term therapeutic results, and with recipient and donor expectations[119,129,130].

Integrated and multidisciplinary care should also include uniform criteria and procedures for standard assessments, patient autonomy studies, adherence to therapy, new coping strategies and the adoption of more appropriate lifestyles. Only through a "working network" is it possible to monitor the re-employment, family and social reintegration of transplant patients, as health is the result of a number of social, environmental, psychological, economic and genetic determinants[1,48].

REFERENCES

1　**House R**, Trzepacz PT, Thompson TL. Psychiatric consultation to organ transplant services. In: Tasman A; Goldfinger SM, Kaufman CA, editors. Review of psychiatry volume 9. Washington, DC: American Psychiatric Press, 1990: 515-535

2　**Klapheke MM**. The role of the psychiatrist in organ transplantation. *Bull Menninger Clin* 1999; **63**: 13-39

3　**Levenson JL**, Olbrisch ME. Psychosocial screening and selection of candidates for organ transplantation. In: Trzepacz P, DiMartini A, editors. The Transplant Patient: biological, psychiatric and ethical issues in organ transplantation. UK: Cambridge University Press, 2000: 21-41 [DOI: 10.1017/CBO9780511544385.003]

4　**Olbrisch ME**, Benedict SM, Ashe K, Levenson JL. Psychological assessment and care of organ transplant patients. *J Consult Clin Psychol* 2002; **70**: 771-783 [PMID: 12090382 DOI: 10.1037/0022-006X.70.3.771]

5　**Schweitzer J**, Seidel-Wiesel M, Verres R, Wiesel M. Psychological consultation before living kidney donation: finding out and handling problem cases. *Transplantation* 2003; **76**: 1464-1470 [PMID: 14657687 DOI: 10.1097/01.TP.0000084320.57817.32]

6　**López-Navas A**, Ríos A, Riquelme A, Martínez-Alarcón L, Pons JA, Miras M, Sanmartín A, Febrero B, Ramírez P, Parrilla P. Psychological care: social and family support for patients awaiting a liver transplant. *Transplant Proc* 2011; **43**: 701-704 [PMID: 21486578 DOI: 10.1016/j.transproceed.2011.01.095]

7　**Lovera G**, Basile A, Bertolotti M, Comazzi AM, Clerici CA, Gandione M, Mazzoldi M, Rupolo G, Feltrin A, Ponton P. Psychological assistance in organ transplantation. *Ann Ist Super Sanità* 2000; **36**: 225-246 [PMID: 11213654]

8　**De Vito Dabbs A**, Dew MA, Stilley CS, Manzetti J, Zullo T, McCurry KR, Kormos RL, Iacono A. Psychosocial vulnerability, physical symptoms and physical impairment after lung and heart-lung transplantation. *J Heart Lung*

Transplant 2003; **22**: 1268-1275 [PMID: 14585388 DOI: 10.1016/S1053-2498(02)01227-5]

9   **Surman OS**. Psychiatric aspects of liver transplantation. *Psychosomatics* 1994; **35**: 297-307 [PMID: 8036258 DOI: 10.1016/S0033-3182(94)71777-1]

10   **Dew MA**, Roth LH, Schulberg HC, Simmons RG, Kormos RL, Trzepacz PT, Griffith BP. Prevalence and predictors of depression and anxiety-related disorders during the year after heart transplantation. *Gen Hosp Psychiatry* 1996; **18**: 48S-61S [PMID: 8937923 DOI: 10.1016/S0163-8343(96)00077-1]

11   **Dew MA**, Kormos RL, DiMartini AF, Switzer GE, Schulberg HC, Roth LH, Griffith BP. Prevalence and risk of depression and anxiety-related disorders during the first three years after heart transplantation. *Psychosomatics* 2001; **42**: 300-313 [PMID: 11496019 DOI: 10.1176/appi.psy.42.4.300]

12   **Erim Y**, Beckmann M, Valentin-Gamazo C, Malago M, Frilling A, Schlaak JF, Gerken G, Broelsch CE, Senf W. Quality of life and psychiatric complications after adult living donor liver transplantation. *Liver Transpl* 2006; **12**: 1782-1790 [PMID: 17133566 DOI: 10.1002/lt.20907]

13   **Dew MA**. Behavioral factors in heart transplantation: Quality of life and medical compliance. *J APPL BIOBEHAV RES* 1999; **2**: 28-54 [DOI: 10.1111/j.1751-9861.1994.tb00037.x]

14   **Dew MA**, Switzer GE, DiMartini AF, Matukaitis J, Fitzgerald MG, Kormos RL. Psychosocial assessments and outcomes in organ transplantation. *Prog Transplant* 2000; **10**: 239-259; quiz 260-261 [PMID: 11232552]

15   **Griva K**, Ziegelmann JP, Thompson D, Jayasena D, Davenport A, Harrison M, Newman SP. Quality of life and emotional responses in cadaver and living related renal transplant recipients. *Nephrol Dial Transplant* 2002; **17**: 2204-2211 [PMID: 12454234 DOI: 10.1093/ndt/17.12.2204]

16   **Schilder P**. The Image and Appearance of the Human Body: Studies in the Constructive Energies of the Psyche. Madison: International Universities Press, 1950

17   **Chiesa S**. Il trapianto d'organo: crisi e adattamento psicologico. *Psichiatria e Medicina* 1989; **10**: 15-21

18   **Castelnuovo-Tedesco P**. Transplantation: psychological implications of changes in body image. In Levy Psychonephrology, Psychological factors in hemodialysis and transplantation. New York: Plenum Press, 1981

19   **Castelnuovo-Tedesco P**. Organ transplant, body image, psychosis. *Psychoanal Q* 1973; **42**: 349-363 [PMID: 4579589]

20   **Fukunishi I**, Sugawara Y, Takayama T, Makuuchi M, Kawarasaki H, Kita Y, Aikawa A, Hasegawa A. Psychiatric problems in living-related transplantation (II): the association between paradoxical psychiatric syndrome and guilt feelings in adult recipients after living donor liver transplantation. *Transplant Proc* 2002; **34**: 2632-2633 [PMID: 12431555 DOI: 10.1016/S0041-1345(02)03455-3]

21   **Rupolo G**, Poznanski C. Psicologia e psichiatria del trapianto d'organi. Masson: Elsevier, 1999: 1-188

22   **Lefebvre P**, Crombez JC, LeBeuf J. Psychological dimension and psychopathological potential of acquiring a kidney. *Can Psychiatr Assoc J* 1973; **18**: 495-500 [PMID: 4128311]

23   **De Pasquale C**, Pistorio ML, Sorbello M, Parrinello L, Corona D, Gagliano M, Giuffrida G, Giaquinta A, Sinagra N, Zerbo D, Veroux P, Veroux M. Body image in kidney transplantation. *Transplant Proc* 2010; **42**: 1123-1126 [PMID: 20534240 DOI: 10.1016/j.transproceed.2010.03.071]

24   **Lastrico A**, Politi PL, Barale F. Sul vissuto del trapianto cardiaco. *Minerva Psichiatr* 1994; **35**: 139-145 [PMID: 7968410]

25   **Bertini M**. Problemi psicologici nei cosiddetti procedimenti 'life extending'. In: AAVV Rigetto psicologico di protesi, innesti, trapianti e plastiche. Roma: Istituto Italiano di Medice Sociale, 1972

26   **Plassman R**. Organ worlds: outline of an analytical psychology of the body. *Psychoanalitic Inquiry* 1998; **18**: 344-367 [DOI: 10.1080/07351699809534197]

27   **Chisholm MA**. Identification of medication-adherence barriers and strategies to increase adherence in recipients of renal transplants. *Manag Care Interface* 2004; **17**: 44-48 [PMID: 15521285]

28   **Kranenburg L**, Zuidema W, Weimar W, Ijzermans J, Passchier J, Hilhorst M, Busschbach J. Postmortal or living related donor: preferences of kidney patients. *Transpl Int* 2005; **18**: 519-523 [PMID: 15819799 DOI: 10.1111/j.1432-2277.2005.00081.x]

29   **Martínez-Alarcón L**, Ríos A, Conesa C, Ramírez P. Postmortal or living related donor: preferences of kidney patients. *Transpl Int* 2006; **19**: 598-599; author reply 600 [PMID: 16764640 DOI: 10.1111/j.1432-2277.2006.00290.x]

30   **Pistorio ML**, Veroux M, Corona D, Sinagra N, Giaquinta A, Zerbo D, Giacchi F, Gagliano M, Tallarita T, Veroux P, De Pasquale C. The study of personality in renal transplant patients: possible predictor of an adequate social adaptation? *Transplant Proc* 2013; **45**: 2657-2659 [PMID: 24034016 DOI: 10.1016/j.transproceed.2013.07.003]

31   **De Pasquale C**, Veroux M, Corona D, Sinagra N, Giaquinta A, Zerbo D, Cimino S, Gagliano M, Giuffrida G, Veroux P, Pistorio ML. The concept of self and emotional involvement in living kidney donation: a psychometric investigation. *Transplant Proc* 2013; **45**: 2604-2606 [PMID: 24034001 DOI: 10.1016/j.transproceed.2013.08.00]

32   **Morris PL**, Jones B. Transplantation versus dialysis: a study of quality of life. *Transplant Proc* 1988; **20**: 23-26 [PMID: 3278459]

33   **Kerr S**, Johnson E, Pandian K, Gillingham K, Matas A. Psychological impact of a failed kidney transplant. *Transplant Proc* 1997; **29**: 1573 [PMID: 9123430 DOI: 10.1016/S0041-1345(96)00680]

34   **Fauci AS**, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. *Ann Intern Med* 1976; **84**: 304-315 [PMID: 769625 DOI: 10.7326/0003-4819-84-3-304]

35   **Strauss B**, Thormann T, Strenge H, Biernath E, Foerst U, Stauch C, Torp U, Bernhard A, Speidel H. Psychosocial, neuropsychological and neurological status in a sample of heart transplant recipients. *Qual Life Res* 1992; **1**: 119-128 [PMID: 1301119 DOI: 10.1007/BF00439719]

36   **Deshields TL**, McDonough EM, Mannen RK, Miller LW. Psychological and cognitive status before and after heart transplantation. *Gen Hosp Psychiatry* 1996; **18**: 62S-69S [PMID: 8937924 DOI: 10.1016/S0163-8343(96)00078-3]

37   **Chisholm MA**, Mulloy LL, Jagadeesan M, DiPiro JT. Impact of clinical pharmacy services on renal transplant patients' compliance with immunosuppressive medications. *Clin Transplant* 2001; **15**: 330-336 [PMID: 11678959 DOI: 10.1034/j.1399-0012.2001.150505.x]

38   **Kizilisik AT**, Shokouh-Amiri MH, Tombazzi CR, Desmukh S, Grewal HP, Vera SR, Gaber AO. Psychiatric complications after liver transplantation. *Transplant Proc* 2001; **33**: 3697 [PMID: 11750575 DOI: 10.1016/S0041-1345(01)02508-8]

39   **Köllner V**, Schade I, Maulhardt T, Maercker A, Joraschky P, Gulielmos V. Posttraumatic stress disorder and quality of life after heart or lung transplantation. *Transplant Proc* 2002; **34**: 2192-2193 [PMID: 12270360 DOI: 10.1016/S0041-1345(02)03198-6]

40   **Favaro A**, Gerosa G, Caforio AL, Volpe B, Rupolo G, Zarneri D, Boscolo S, Pavan C, Tenconi E, d'Agostino C, Moz M, Torregrossa G, Feltrin G, Gambino A, Santonastaso P. Posttraumatic stress disorder and depression in heart transplantation recipients: the relationship with outcome and adherence to medical treatment. *Gen Hosp Psychiatry* 2011; **33**: 1-7 [PMID: 21353121 DOI: 10.1016/j.genhosppsych.2010.10.001]

41   **Depasquale C**, Pistorio ML, Corona D, Mistretta A, Zerbo D, Sinagra N, Giaquinta A, Tallarita T, Esker B, Mociskyte D, Leonardi A, Gula R, Veroux P, Veroux M. Correlational study between psychic symptoms and quality of life among hemodialysis patients older than 55 years of age. *Transplant Proc* 2012; **44**: 1876-1878 [PMID: 22974860 DOI: 10.1016/j.transproceed.2012.06.015]

42   **Waterman AD**, Robbins ML, Paiva AL, Peipert JD, Davis LA,

Hyland SS, Schenk EA, Baldwin KA, Amoyal NR. Measuring kidney patients' motivation to pursue living donor kidney transplant: Development of Stage of Change, Decisional Balance and Self-Efficacy measures. *J Health Psychol* 2013 Oct 22; Epub ahead of print [PMID: 24155194]

43   **Halligan PW**, David AS, eds. Conversion Hysteria: Towards a Cognitive Neuropsychological Account. *Psychology* (Press Hove) 1999 [DOI: 10.1093/brain/124.1.232]

44   **Fidel Kinori SG**, Alcántara Tadeo A, Castan Campanera E, Costa Requena G, Diez Quevedo C, Lligoña Garreta A, Lopez Lazcano A, Martínez Del Pozo M, Rangil Muñoz T, Peri Nogués JM; en nombre del Grupo de Trabajo de evaluación psiquiátrica y psicológica de la Organización Catalana de Trasplantes. Unified Protocol for psychiatric and psychological assessment of candidates for transplantation of organs and tissues, PSI-CAT. *Rev Psiquiatr Salud Ment* 2014 Apr 6; Epub ahead of print [PMID: 24717548]

45   **Anand-Kumar V**, Kung M, Painter L, Broadbent E. Impact of organ transplantation in heart, lung and liver recipients: assessment of positive life changes. *Psychol Health* 2014; **29**: 687-697 [PMID: 24460459 DOI: 10.1080/08870446.2014.882922]

46   **Cramond WA**, Knight PR, Lawrence JR, Higgins BA, Court JH, MacNamara FM, Clarkson AR, Miller CD. Psychological aspects of the management of chronic renal failure. *Br Med J* 1968; **1**: 539-543 [PMID: 4868553 DOI: 10.1136/bmj.1.5591.539]

47   **American psychiatric Association**. Diagnostic and Statistical manual of Mental Disorders. 4th ed. Washington, DC: America Psychiatric Association, 1994: Text Revision, 2000

48   **Levenson JL**, Olbrisch ME. Psychosocial evaluation of organ transplant candidates. A comparative survey of process, criteria, and outcomes in heart, liver, and kidney transplantation. *Psychosomatics* 1993; **34**: 314-323 [PMID: 8351306 DOI: 10.1016/S0033-3182(93)71865-4]

49   **Franklin PM**, Crombie AK. Live related renal transplantation: psychological, social, and cultural issues. *Transplantation* 2003; **76**: 1247-1252 [PMID: 14578766 DOI: 10.1097/01.TP.0000087833.48999.3D]

50   **Binet I**, Bock AH, Vogelbach P, Gasser T, Kiss A, Brunner F, Thiel G. Outcome in emotionally related living kidney donor transplantation. *Nephrol Dial Transplant* 1997; **12**: 1940-1948 [PMID: 9306347 DOI: 10.1093/ndt/12.9.1940]

51   **Pradel FG**, Mullins CD, Bartlett ST. Exploring donors' and recipients' attitudes about living donor kidney transplantation. *Prog Transplant* 2003; **13**: 203-210 [PMID: 14558635]

52   **Sajjad I**, Baines LS, Salifu M, Jindal RM. The dynamics of recipient-donor relationships in living kidney transplantation. *Am J Kidney Dis* 2007; **50**: 834-854 [PMID: 17954298 DOI: 10.1053/j.ajkd.2007.07.029]

53   **Heinrich TW**, Marcangelo M. Psychiatric issues in solid organ transplantation. *Harv Rev Psychiatry* 2009; **17**: 398-406 [PMID: 19968454 DOI: 10.3109/10673220903463259]

54   **Trzcinska M**, Włodarczyk Z. Psychological Aspects of Kidney Transplantation. *UROL NEPHRO* 2011 [DOI: 10.5772/16725]

55   **Waterman AD**, Stanley SL, Covelli T, Hazel E, Hong BA, Brennan DC. Living donation decision making: recipients' concerns and educational needs. *Prog Transplant* 2006; **16**: 17-23 [PMID: 16676669]

56   **Murray LR**, Conrad NE, Bayley EW. Perceptions of kidney transplant by persons with end stage renal disease. *ANNA J* 1999; **26**: 479-483; discussion 484 [PMID: 10776075]

57   **de Groot IB**, Schipper K, van Dijk S, van der Boog PJ, Stiggelbout AM, Baranski AG, Marang-van de Mheen PJ. Decision making around living and deceased donor kidney transplantation: a qualitative study exploring the importance of expected relationship changes. *BMC Nephrol* 2012; **13**: 103 [PMID: 22958636 DOI: 10.1186/1471-2369-13-103]

58   **Clemens KK**, Thiessen-Philbrook H, Parikh CR, Yang RC, Karley ML, Boudville N, Ramesh Prasad GV, Garg AX. Psychosocial health of living kidney donors: a systematic review. *Am J Transplant* 2006; **6**: 2965-2977 [PMID: 17294524

DOI: 10.1111/j.1600-6143.2006.01567.x]

59   **Andersen MH**, Bruserud F, Mathisen L, Wahl AK, Hanestad BR, Fosse E. Follow-up interviews of 12 living kidney donors one yr after open donor nephrectomy. *Clin Transplant* 2007; **21**: 702-709 [PMID: 17988262 DOI: 10.1111/j.1399-0012.2007.00726.x]

60   **Neuhaus TJ**, Wartmann M, Weber M, Landolt MA, Laube GF, Kemper MJ. Psychosocial impact of living-related kidney transplantation on donors and partners. *Pediatr Nephrol* 2005; **20**: 205-209 [PMID: 15627165]

61   **Duque JL**, Loughlin KR, Kumar S. Morbidity of flank incision for renal donors. *Urology* 1999; **54**: 796-801 [PMID: 10565736 DOI: 10.1016/S0090-4295(99)00274-5]

62   **Giessing M**, Reuter S, Schönberger B, Deger S, Tuerk I, Hirte I, Budde K, Fritsche L, Morgera S, Neumayer HH, Loening SA. Quality of life of living kidney donors in Germany: a survey with the Validated Short Form-36 and Giessen Subjective Complaints List-24 questionnaires. *Transplantation* 2004; **78**: 864-872 [PMID: 15385806 DOI: 10.1097/01.TP.0000133307.00604.86]

63   **Kranenburg L**, Zuidema W, Weimar W, IJzermans J, Passchier J, Hilhorst M, Busschbach J. Postmortal or living related donor: preferences of kidney patients. Authors' reply. *Transplant Int* 2006; **19**: 600 [DOI: 10.1111/j.1432-2277.2006.00305.x]

64   **Klapheke MM**. The role of the psychiatrist in organ transplantation. *Bull Menninger Clin* 1999; **63**: 13-39 [PMID: 10028744 DOI: 10.1002/1098]

65   **Ilić S**, Avramović M. Psychological aspects of living donor kidney transplantation. *Med Biol* 2002; **9**: 195-200

66   **Kumnig M**, Beck T, Höfer S, König P, Schneeberger S, Weißenbacher A, Bunzel B, Schüßler G, Rumpold G. Preoperative evaluation of the body concept of donors and recipients of living kidney donations. *Z Psychosom Med Psychother* 2013; **59**: 3-12 [PMID: 23467994]

67   **Cotugno A**. Trauma, attaccamento e sviluppo psicologico della personalità. *Psicobiettivo* 1999; **19:** 15-26

68   **Costantini E**, Monticelli F. Catastrofe e emozione veemente. *Psicobiettivo* 1999; **19**: 3

69   **Stukas AA**, Dew MA, Switzer GE, DiMartini A, Kormos RL, Griffith BP. PTSD in heart transplant recipients and their primary family caregivers. *Psychosomatics* 1999; **40**: 212-221 [PMID: 10341533 DOI: 10.1016/S0033-3182(99)71237-5]

70   **Manne S**, DuHamel K, Nereo N, Ostroff J, Parsons S, Martini R, Williams S, Mee L, Sexson S, Wu L, Difede J, Redd WH. Predictors of PTSD in mothers of children undergoing bone marrow transplantation: the role of cognitive and social processes. *J Pediatr Psychol* 2002; **27**: 607-617 [PMID: 12228332 DOI: 10.1093/jpepsy/27.7.607]

71   **Stuber ML**, Shemesh E. Post-traumatic stress response to life-threatening illnesses in children and their parents. *Child Adolesc Psychiatr Clin N Am* 2006; **15**: 597-609 [PMID: 16797441 DOI: 10.1016/j.chc.2006.02.006]

72   **Gattinara P**, Andovini C, Costantini E, Morganti G, Onofri A. I trapianti d'organo nella prospettiva cognitivo-evoluzionistica. *Psicobiettivo* 2005; **XXV**: 18-20 [DOI: 10.1400/64499]

73   **Johnson JP**, McCauley CR, Copley JB. The quality of life of hemodialysis and transplant patients. *Kidney Int* 1982; **22**: 286-291 [PMID: 6757524 DOI: 10.1038/ki.1982.167]

74   **Christensen AJ**, Holman JM, Turner CW. A prospective study of quality of life in end stage renal disease: effects of cadaveric renal transplantation. *Clin Transplant* 1991; **5**: 40-47

75   **Witzke O**, Becker G, Franke G, Binek M, Philipp T, Heemann U. Kidney transplantation improves quality of life. *Transplant Proc* 1997; **29**: 1569-1570 [PMID: 9123428 DOI: 10.1016/S0041-1345(96)00678-1]

76   **Tsuji-Hayashi Y**, Fukuhara S, Green J, Takai I, Shinzato T, Uchida K, Oshima S, Yamazaki C, Maeda K. Health-related quality of life among renal-transplant recipients in Japan. *Transplantation* 1999; **68**: 1331-1335 [PMID: 10573072 DOI: 10.0041-1337/99/6809-1331/0]

77   **Mingardi G**. Quality of life and end stage renal disease

therapeutic programs. DIA-QOL Group. Dialysis quality of life. *Int J Artif Organs* 1998; **21**: 741-747 [PMID: 9894753]

78   **Bakewell AB**, Higgins RM, Edmunds ME. Does ethnicity influence perceived quality of life of patients on dialysis and following renal transplant? *Nephrol Dial Transplant* 2001; **16**: 1395-1401 [PMID: 11427631 DOI: 10.1093/ndt/16.7.1395]

79   **van der Mei SF**, Groothoff JW, van Sonderen EL, van den Heuvel WJ, de Jong PE, van Son WJ. Clinical factors influencing participation in society after successful kidney transplantation. *Transplantation* 2006; **82**: 80-85 [PMID: 16861945 DOI: 10.1097/01.tp.0000225768.56981.aa]

80   **Kramer L**, Madl C, Stockenhuber F, Yeganehfar W, Eisenhuber E, Derfler K, Lenz K, Schneider B, Grimm G. Beneficial effect of renal transplantation on cognitive brain function. *Kidney Int* 1996; **49**: 833-838 [PMID: 8648927 DOI: 10.1038/ki.1996.115]

81   **Min SK**, Kim KH, Shin JH, Han JO, Lee KY, Kang WR. Psychiatric aspects of hemodialysis and kidney transplantation. *Yonsei Med J* 1984; **25**: 122-132 [PMID: 6400060]

82   **Grady KL**, Jalowiec A, White-Williams C. Improvement in quality of life in patients with heart failure who undergo transplantation. *J Heart Lung Transplant* 1996; **15**: 749-757 [PMID: 8878757]

83   **Chilcot J**, Spencer BW, Maple H, Mamode N. Depression and kidney transplantation. *Transplantation* 2014; **97**: 717-721 [PMID: 24342977 DOI: 10.1097/01.TP.0000438212.72960.ae]

84   **Surman OS**. Psychiatric aspects of organ transplantation. *Am J Psychiatry* 1989; **146**: 972-982 [PMID: 2665527]

85   **Christopherson LK**. Cardiac transplantation: a psychological perspective. *Circulation* 1987; **75**: 57-62 [DOI: 10.1161/01. CIR.75.1.57]

86   **Fukunishi I**, Hasegawa A, Ohara T, Aikawa A, Hatanaka A, Suzuki J, Kikuchi M, Amagasaki K. Kidney transplantation and liaison psychiatry, part I: anxiety before, and the prevalence rate of psychiatric disorders before and after, transplantation. *Psychiatry Clin Neurosci* 1997; **51**: 301-304 [PMID: 9413877]

87   **Kalman TP**, Wilson PG, Kalman CM. Psychiatric morbidity in long-term renal transplant recipients and patients undergoing hemodialysis. A comparative study. *JAMA* 1983; **250**: 55-58 [PMID: 6343654 DOI: 10.1001/jama.1983.03340010037025]

88   **Cavallero P**, Ferrari MG, Verbena V, Mosca F. Vicinanza e risorse nelle persone sottoposte a trapianto d'organo e loro familiari. *Psychofenia* 2007; **X**: 17 [DOI: 10.1285/ i17201632vXn17p71]

89   **Rupolo G**, Bertolini C. Modalità di coping nei pazienti sottoposti a trapianto d'organo. Riv. *Psichiatria* 1996; **3**: 94-102

90   **Lovera G**, Basile A, Bertolotti M, Comazzi AM, Clerici CA, Gandione M, Mazzoldi M, Rupolo G, Feltrin A, Ponton P. [Psychological assistance in organ transplantation]. *Ann Ist Super Sanita* 2000; **36**: 225-246 [PMID: 11213654]

91   **Chisholm MA**, Lance CE, Mulloy LL. Patient factors associated with adherence to immunosuppressant therapy in renal transplant recipients. *Am J Health Syst Pharm* 2005; **62**: 1775-1781 [PMID: 16120736 DOI: 10.2146/ajhp040541]

92   **Chisholm-Burns MA**, Spivey CA, Wilks SE. Social support and immunosuppressant therapy adherence among adult renal transplant recipients. *Clin Transplant* 2010; **24**: 312-320 [PMID: 19694770 DOI: 10.1111/j.1399-0012.2009.01060.x]

93   **Burra P**, Germani G, Gnoato F, Lazzaro S, Russo FP, Cillo U, Senzolo M. Adherence in liver transplant recipients. *Liver Transpl* 2011; **17**: 760-770 [PMID: 21384527 DOI: 10.1002/ lt.22294]

94   **Hawkins DW**, Fiedler FP, Douglas HL, Eschbach RC. Evaluation of a clinical pharmacist in caring for hypertensive and diabetic patients. *Am J Hosp Pharm* 1979; **36**: 1321-1325 [PMID: 507073]

95   **De Geest S**, Borgermans L, Gemoets H, Abraham I, Vlaminck H, Evers G, Vanrenterghem Y. Incidence, determinants, and consequences of subclinical noncompliance with immunosuppressive therapy in renal transplant recipients. *Transplantation* 1995; **59**: 340-347 [PMID: 7871562 DOI: 10.1097/

00007890-199502000-00006]

96   **De Geest S**, Abraham I, Moons P, Vandeputte M, Van Cleemput J, Evers G, Daenen W, Vanhaecke J. Late acute rejection and subclinical noncompliance with cyclosporine therapy in heart transplant recipients. *J Heart Lung Transplant* 1998; **17**: 854-863 [PMID: 9773856]

97   **De Geest S**, Abraham I, Dunbar-Jacob J, Vanhaecke J. Behavioral strategies for long-term survival of transplant recipients. In: Metry, J.; Meyer, U., editors. Drug Regimen Compliance: Issues in Clinical Trials and Patient Management. Chichester NY: John Wiley & Sons, 1999: 163-180 [DOI: 10.1002/047084633X.ch12]

98   **Desmyttere A**, Dobbels F, Cleemput I, De Geest S. Noncompliance with immunosuppressive regimen in organ transplantation: is it worth worrying about? *Acta Gastroenterol Belg* 2005; **68**: 347-352 [PMID: 16268422]

99   **Rao PS**, Schaubel DE, Jia X, Li S, Port FK, Saran R. Survival on dialysis post-kidney transplant failure: results from the Scientific Registry of Transplant Recipients. *Am J Kidney Dis* 2007; **49**: 294-300 [PMID: 17261432 DOI: 10.1053/j.ajkd.2006.11.022]

100   **Takemoto SK**, Pinsky BW, Schnitzler MA, Lentine KL, Willoughby LM, Burroughs TE, Bunnapradist S. A retrospective analysis of immunosuppression compliance, dose reduction and discontinuation in kidney transplant recipients. *Am J Transplant* 2007; **7**: 2704-2711 [PMID: 17868065 DOI: 10.1111/ j.1600-6143.2007.01966.x]

101   **Mennilli S**, Vosolo MN, Di Liberato L, Bonomini M. Quando il trapianto renale fallisce: necessità di pianificazione di un percorso assistenziale al paziente che riprende il trattamento sostitutivo dialitico. *Giornale di Tecniche Nefrologiche & Dialitiche Wichting Editore* 2010; **22**: 17-21 [DOI: 10.5301/GTND.2012.10129]

102   **Tsay SL**, Healstead M. Self-care self-efficacy, depression, and quality of life among patients receiving hemodialysis in Taiwan. *Int J Nurs Stud* 2002; **39**: 245-251 [PMID: 11864647 DOI: 10.1016/ S0020-7489(01)00030-X]

103   **Weng LC**, Dai YT, Huang HL, Chiang YJ. Self-efficacy, self-care behaviours and quality of life of kidney transplant recipients. *J Adv Nurs* 2010; **66**: 828-838 [PMID: 20423370 DOI: 10.1111/j.1365-2648.2009.05243.x]

104   **Maeda U**, Shen BJ, Schwarz ER, Farrell KA, Mallon S. Self-efficacy mediates the associations of social support and depression with treatment adherence in heart failure patients. *Int J Behav Med* 2013; **20**: 88-96 [PMID: 22212607 DOI: 10.1007/ s12529-011-9215-0]

105   **Gaines JM**, Talbot LA, Metter EJ. The relationship of arthritis self-efficacy to functional performance in older men and women with osteoarthritis of the knee. *Geriatr Nurs* 2002; **23**: 167-170 [PMID: 12075283 DOI: 10.1067/mgn.2002.125420]

106   **Bodenheimer T**, Lorig K, Holman H, Grumbach K. Patient self-management of chronic disease in primary care. *JAMA* 2002; **288**: 2469-2475 [PMID: 12435261 DOI: 10.1001/ jama.288.19.2469]

107   **Linnell K**. Chronic disease self-management: one successful program. *Nurs Econ* 2005; **23**: 189-191; 196-198 [PMID: 16189984]

108   **Christensen AJ**, Ehlers SL, Raichle KA, Bertolatus JA, Lawton WJ. Predicting change in depression following renal transplantation: effect of patient coping preferences. *Health Psychol* 2000; **19**: 348-353 [PMID: 10907653 DOI: 10.1037/0278-6 133.19.4.348]

109   **Baines LS**, Joseph JT, Jindal RM. Compliance and late acute rejection after kidney transplantation: a psycho-medical perspective. *Clin Transplant* 2002; **16**: 69-73 [PMID: 11982618 DOI: 10.1034/j.1399-0012.2002.00111.x]

110   **Ikeda K**, Aoki H, Saito K, Muramatsu Y, Suzuki T. Associations of blood glucose control with self-efficacy and rated anxiety/depression in type II diabetes mellitus patients. *Psychol Rep* 2003; **92**: 540-544 [PMID: 12785638]

111   **Weng LC**, Dai YT, Wang YW, Huang HL, Chiang YJ. Effects of self-efficacy, self-care behaviours on depressive symptom of Taiwanese kidney transplant recipients. *J Clin*

Nurs 2008; **17**: 1786-1794 [PMID: 18266845 DOI: 10.1111/j.1365-2702.2007.02035.x]

112 **Mancusi F**. Le risorse di fronteggiamento dell'insufficienza renale cronica: una ricerca sperimentale in un centro di dialisi. *Psychomed* 2011; **6**: 61-65

113 **Lorig KR**, Sobel DS, Ritter PL, Laurent D, Hobbs M. Effect of a self-management program on patients with chronic disease. *Eff Clin Pract* 2001; **4**: 256-262 [PMID: 11769298]

114 **Painter PL**, Hector L, Ray K, Lynes L, Paul SM, Dodd M, Tomlanovich SL, Ascher NL. Effects of exercise training on coronary heart disease risk factors in renal transplant recipients. *Am J Kidney Dis* 2003; **42**: 362-369 [PMID: 12900820 DOI: 10.1016/S0272-6386(03)00673-5]

115 **van den Berg-Emons RJ**, van Ginneken BT, Nooijen CF, Metselaar HJ, Tilanus HW, Kazemier G, Stam HJ. Fatigue after liver transplantation: effects of a rehabilitation program including exercise training and physical activity counseling. *Phys Ther* 2014; **94**: 857-865 [PMID: 24557657]

116 **Shirali AC**, Bia MJ. Management of cardiovascular disease after kidney transplantation. *Clin J Am Soc Nephrol* 2008 [DOI: 10.2215CJN.05081107]

117 **Mosconi G**, Panicali L, Corsini S. Physical activity in solid organ transplant recipients. *Transplant Int* 2009; **22**: 175-176

118 **McAuley E**. The role of efficacy cognitions in the prediction of exercise behavior in middle-aged adults. *J Behav Med* 1992; **15**: 65-88 [PMID: 1583674]

119 **Denhaerynck K**, Dobbels F, Steiger J, De Geest S. Quality of life after kidney transplantation. In Veroux M, Veroux P: Kidney Transplantation: challenging the future, United Arab Emirates. Bentham: Bentham Publisher, 2012: 71-84

120 **Shapiro PA**, Williams DL, Foray AT, Gelman IS, Wukich N, Sciacca R. Psychosocial evaluation and prediction of compliance problems and morbidity after heart transplantation. *Transplantation* 1995; **60**: 1462-1466 [PMID: 8545875]

121 **Cukor D**, Newville H, Jindal R. Depression and immunosuppressive medication adherence in kidney transplant patients. *Gen Hosp Psychiatry* 2008; **30**: 386-387 [PMID: 18585547 DOI: 10.1016/j.genhosppsych.2007.12.003]

122 **Dew MA**, Roth LH, Thompson ME, Kormos RL, Griffith BP. Medical compliance and its predictors in the first year after heart transplantation. *J Heart Lung Transplant* 1996; **15**: 631-645 [PMID: 8794030]

123 **Kemp S**, Morley S, Anderson E. Coping with epilepsy: do illness representations play a role? *Br J Clin Psychol* 1999; **38** (Pt 1): 43-58 [PMID: 10212736 DOI: 10.1348/014466599162656]

124 **Morris R**, Petrie K, Weinman J. Functioning in chronic fatigue syndrome: do illness perceptions play a regulatory role? *BJHP* 1996; **1**: 15-25 [DOI: 10.1111/j.2044-8287.1996.tb00488.x]

125 **Hagger MS**, Orbell S. A meta-analytic review of the common-sense model of illness representation. *Psychol Health* 2003; **18**: 141-184 [DOI: 10.1080/088704403100081321]

126 **Zelikovsky N**, Schast AP. Eliciting accurate reports of adherence in a clinical interview: development of the Medical Adherence Measure. *Pediatr Nurs* 2008; **34**: 141-146 [PMID: 18543839]

127 **De Pasquale C**, Pistorio ML, Veroux P, Giuffrida G, Sinagra N, Ekser B, Zerbo D, Corona D, Giaquinta A, Veroux M. Quality of life in kidney transplantation from marginal donors. *Transplant Proc* 2011; **43**: 1045-1047 [PMID: 21620049 DOI: 10.1016/j.transproceed.2011.01.156]

128 **Roussel MG**, Gorham N, Wilson L, Mangi AA. Improving recovery time following heart transplantation: the role of the multidisciplinary health care team. *J Multidiscip Healthc* 2013; **6**: 293-302 [PMID: 24009423]

129 **Comazzi AM**. Indagine psicologica sulla disponibilità alla donazione d organi per trapianti. Min. *Psich Psicol* 1974; 15: 27-31

130 **Comazzi AM**, Clerici CA, Facchetti E, Pizzi C, Scalamogna M, Sirchia G. Psychological support for donor families in the North Italy Transplant Program (NITp). VA: American Psychiatric Press, 1990: 515-535

REVIEW

Diagnosis and management of coronary allograft vasculopathy in children and adolescents

Nathalie Dedieu, Gerald Greil, James Wong, Matthew Fenton, Michael Burch, Tarique Hussain

Nathalie Dedieu, Matthew Fenton, Michael Burch, Great Ormond Street Hospital for Children NHS Foundation Trust, WC1 3JH London, United Kingdom
Nathalie Dedieu, Gerald Greil, James Wong, Tarique Hussain, King's College London, Division of Imaging Sciences and Biomedical Engineering, St Thomas Hospital, SE1 7EH London, United Kingdom
Author contributions: All authors contributed to the paper.
Correspondence to: Nathalie Dedieu, MD, King's College London, Division of Imaging Sciences and Biomedical Engineering, St Thomas Hospital, Westminster Bridge Road, 4th Floor Lambeth wing, SE1 7EH London,
United Kingdom. nathaliededieu@hotmail.com
Telephone: +44-20-71889617 Fax: +44-20-71885442
Received: June 9, 2014　　Revised: August 12, 2014
Accepted: September 16, 2014
Published online: December 24, 2014

Abstract

Coronary allograft vasculopathy remains one of the leading causes of death beyond the first year post transplant. As a result of denervation following transplantation, patients lack ischaemic symptoms and presentation is often late when the graft is already compromised. Current diagnostic tools are rather invasive, or in case of angiography, significantly lack sensitivity. Therefore a non-invasive tool that could allow early diagnosis would be invaluable.This paper review the disease form its different diagnosis techniques,including new and less invasive diagnostic tools to its pharmacological management and possible treatments.

Key words: Cardiac transplantation; Allograft vasculopathy; Paediatrics; Diagnosis; Management which reflect the content of the study

Core tip: Coronary allograft vasculopathy remains the leading cause of great loss in children after the first year post transplant. This paper offers a state of the art review of the disease from diagnosis including most recent and less invasive tools to management.

DEFINITION AND PHYSIOPATHOLOGY

In children, coronary allograft vasculopathy (CAV) remains the main limiting survival factor after heart transplantation and the major cause of mortality after the first year post transplant leading ultimately to graft loss[1,2].

One elegant etiological description of CAV is that of "immunologic mechanisms operating in a milieu of non-immunologic risk factors"[3]. The process is believed to start off as a response to endothelial injury in the graft, originated by a complex interaction of multiple donor and recipient factors. The resulting endothelial dysfunction, leads to altered endothelial permeability and subsequent intimal hyperplasia as a consequence of the vascular remodeling originated by the inflammatory response. The immunologic events constitute the original trigger and non-inflammatory events such as cytomegalovirus infection, ischemic time (reperfusion injury), increased donor age and classical cardiovascular risk factors (*i.e.*, diabetes, dyslipidemias, smoking and hypertension), perpetuate the inflammatory response and increase the endothelial injury[4].

Typical lesions (Figures 1 and 2) consist of diffuse intimal proliferation leading to the development of luminal stenosis and small vessels occlusion which then limits blood supply to the graft causing chronic vascular injury and ultimately myocardial ischemia[5]. The lesions develop earlier and quicker than atherosclerotic lesions.

Figure 1 Stenotic coronary artery macroscopic aspect in post mortem study of explanted heart.
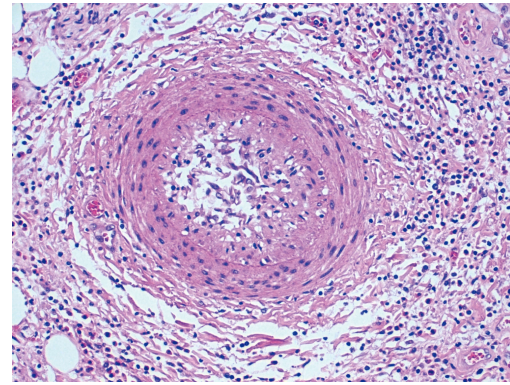

Figure 2 Histopathologic example of stenotic microvasculopathy: Medial thickening and endothelial swelling with evidence of luminal stenosis (hematoxylin-eosin stain).

In addition, progression is often silent due to the lack of ischemic symptoms from the denervated heart and often, the first clinical manifestation is an adverse cardiac event[6].

The real incidence of CAV among the pediatric population remains unknown, with a reported incidence varying between studies from 3% to 43%[7]. According to an angiographic multicenter study, the incidence of CAV would be 2%, 9% and 17% at 1, 3 and 5 years[1]. Looking at the incidence reported in studies that use intravascular ultrasound (IVUS), this is even higher, with 75% incidence of detectable intimal thickening at 5 years, with half of these representing at least mild disease[8]. The most current angiographic data estimates the incidence of CAV in the pediatric cohort of 13% at 5 years, 25% at 10 years and 54% at 15 years[9].

According to the ISHLT registry, using angiographic definitions, 65% of recipients are free of CAV at 10 years, but after a diagnosis of CAV, the 2-year graft survival rate is less than 50%[2].

Age at transplantation has a strong influence on survival with a 74% 8-year freedom of CAV in younger recipients compared to 56% in recipients older than 10 years[10].

As CAV lesions are preceded by endothelial dysfunction, it is essential to identify and characterize this as early as possible for targeted therapy and ultimately to improve patient survival.

DIAGNOSIS

The diagnosis of CAV is challenging. As a result of the denervation inherent to heart transplantation, patients fail to display classical clinical warning signs of angina[11]. The ability of early diagnosis is essential but unfortunately, the majority of the diagnostic techniques lack sensitivity or are rather invasive. A reliable and repeatable non-invasive method that detects CAV and its functional significance would have a huge impact on the follow up of heart transplant recipients. However, sensitivity and specificity of the currently available non-invasive tests remain limited.

Screening protocols vary among centers and the majority of units use a combination of diagnostic modalities, depending mainly on local preferences and expertise.

Angiography

For many years, until the introduction of IVUS, this has been the cornerstone of CAV diagnosis[12,13]. Despite its relatively low sensitivity[14,15] and resulting delay in diagnosis, coronary angiography remains the most widely used diagnostic technique for CAV in the majority of transplant centers.

Angiography is known to underestimate the disease[16]. Adults series display a low sensitivity and negative predictive value. St Goar *et al*[14] found that 50% of patients with normal angiographies had moderate to severe intima thickening on IVUS. In a series by Tuzcu *et al*[17] the sensitivity of angiography for CAV detection (defined by maximal intimal thickness > 0.5 mm) was 43%, specificity was however high with 95%.

Similarly in a most recent paper, Gregory *et al*[18], using the same definition, showed a sensitivity even lower of 11% with a negative predictive value of 57%. Defining CAV as mean intimal thickness > 0.3 mm, Störk *et al*[19] found a sensitivity of 44% and a negative predictive value of 28% when compared to the IVUS data.

Its main limitation arises from the fact that it assesses the vessel lumen. The contrast fills the patent lumen without direct visualisation of the vessel wall. By the time a filling defect appears and there is significant stenosis, the graft is already compromised. CAV tends to be diffuse and concentric affecting large and medium size vessels as well as the microvasculature[14,20]. Typically there is initial vessel expansion: as the intima thickens, the external elastic membrane expands preserving initially the lumen area (Glagov-type positive remodeling)[21-24]. This explains why the coronary angiography result can be normal in the presence of significant disease demonstrated by IVUS. Nevertheless, angiography is inexpensive, readily available across centers and findings have proven prognostic implications regarding graft survival and adverse cardiac events[25,26].

One of the largest experiences in pediatric patients has been published by Pahl *et al*[1] in 2005 and included

Table 1 ISHLT consensus grading for coronary allograft vasculopathy (Mehra *et al*[13] 2010)

Grade	
0 (Not significant)	No detectable angiographic lesion
I (Mild)	Angiographic LM < 50% stenosis, or primary vessel with maximal lesion of < 70%, or any branch stenosis of < 70% (including diffuse narrowing)
II (Moderate)	Angiographic LM 50%-69% stenosis, a single primary vessel ≥ 70% stenosis, or isolated branch stenosis of ≥ 70%in branches of 2 systems
III (Severe)	Angiographic LM ≥ 70%, or 2 or more primary vessels ≥ 70% stenosis, or isolated branch stenosis of ≥ 70% in all 3 systems, or mild/moderate angiographic disease with LVEF < 45% or evidence of significant restrictive physiology (*i.e.*, symptomatic heart failure with echocardiographic E to A velocity ratio > 2 (> 1.5 in children), shortened isovolumetric relaxation time (< 60 ms), shortened deceleration time (< 150 ms), or restrictive hemodynamic values (Right Atrial Pressure > 12 mmHg, Pulmonary Capillary Wedge Pressure > 25 mmHg, Cardiac Index < 2l min/m²)

LM: Left main.

Table 2 Stanford score (severity based on the localization of the most severe disease

Grade	Severity	Intimal thickness
I	Minimal	< 0.3 mm and < 180 degrees
II	Mild	< 0.3 mm and > 180 degrees
II	Moderate	0.3-0.5 mm OR 0.5-1 mm and < 180 degrees
IV	Severe	> 1 mm OR 0.5-1 mm and > 180 degrees

Figure 3 Left coronary angiography showing severe epicardial disease with multiple stenosis in left anterior descending artery and left circumflex artery.
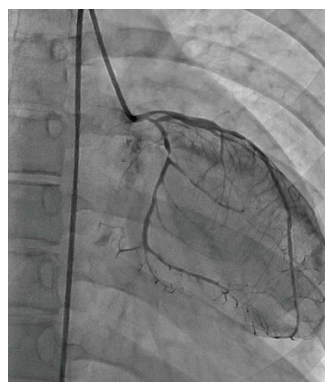
multicenter data proceeding from the Pediatric Heart Transplant Study database. Two thousand and forty-nine angiograms from 751 patients were analysed. The incidence of angiographic abnormalities at 5 years was 17%. However, moderate-to-severe disease occurred in only 6% at 5 years[1]. The use of IVUS in children is limited and they showed a sensitivity of angiography to detect CAV when compared to IVUS data between 18% and 30%[8,27].

In 2010, the ISHLT published new guidelines for CAV including a new classification (Table 1) in view to provide a more refined definition and prognostic value[13]. Figures 3 and 4 showed angiography of two grats with severe disease.

IVUS
IVUS is more sensitive than angiography for early CAV detection and allows delineation of the vessel wall as well as measurement of intimal thickness[14]. Even if it might provide an oversimplified picture of the disease process, the intimal thickening measured *via* IVUS remains the most sensitive diagnostic modality available[13].

As mentioned above, Glagov-type positive remodeling occurs in response to the vessel wall disease. This serves to maintain initial lumen patency and the angiographic appearance of the vessel can therefore be normal despite significant CAV. This is particularly significant in the first year post transplantation. Later on in the disease process, constrictive negative remodeling of the vessel will occur and lead to the stenosis of the vessel[23].

IVUS parameters reported in the literature include: intimal thickness, mean intimal index (ratio of the mean intimal area to the sum of the mean intimal and luminal areas), total atheroma volume and percentage of atheroma volume. In 1995, the Rickenbacher *et al*[28] demonstrated that, in an adult cohort, moderate to severe intimal thickening diagnosed by IVUS was predictive of the future development of angiographically detectable disease (Table 2). This article describes CAV as being present when maximal intimal thickness is ≥ 0.3 mm. A further finding was that maximal intimal thickness (MIT) ≥ 0.3 at 1 year was associated with a 4 year survival of 73% compared to 96% within the group of MIT < 0.3 mm[28]. Two more recent studies published in 2005[17,29] reported that a change of MIT ≥ 0.5 mm over the first year post-transplant was an independent predictor for subsequent angiographic development of CAV; for myocardial infarction and for all-cause death at 5-years post-transplant. Patients with a change in MIT > 0.5 mm had a 5-year incidence of 21% for death or graft loss, 46% for all major adverse events and 65% for the development of subsequent angiographic disease compared to 6%, 17% and 35% respectively for patients without a 0.5 mm change[29].

Interestingly, however, intimal proliferation evaluated in IVUS does not always correlate with microvascular or small artery disease in biopsies specimens[13,30]. Looking specifically at Pediatric data, IVUS has not shown yet
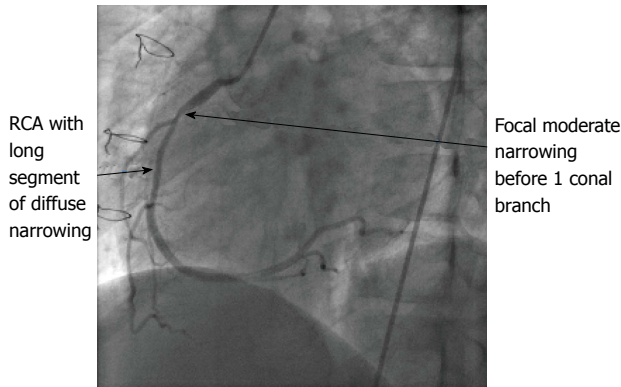
Figure 4 RCA angiography.

RCA with long segment of diffuse narrowing

Focal moderate narrowing before 1 conal branch

Figure 5 Intravascular ultrasound still frame showing severe intimal thickening (Stanford grade IV).

Intima thickened

Catheter

Vessel lumen

impact on prognosis[27] and this probably relates to the limited number of studies, each with differing analysis methodology.

According to published data, sensitivity increases with the number of vessels imaged[31]. However, our experience in children suggests that this is not the case and multi-vessel imaging increases risk without substantially altering sensitivity. Therefore, in our usual practice, we only image the left main and proximal left anterior descending. We use automatic pullback to enhance consistent sampling and identification of branch vessels that are used as landmarks in order to be able to compare serial investigations. We analyze 30 cross-section images taken at 1.5 mm intervals and identified (as mentioned above) by branch points. Additionally, image analysis is performed during mid-diastolic rest period for consistency. In addition to maximal intimal thickness, mean intimal thickness, and mean intimal index, Stanford grading score (Table 2) and percentage of atheroma are recorded. We also use a semi-automatic interactive edge detection software (QIVUS) to improve reproducibility of measurements[32] (Figure 5).

Unfortunately, IVUS remains rather unused in clinical routine: the higher cost and potential morbidity added to the requirement of a trained operator, limits its use currently. This is particularly true in the pediatric population, where the size of the patient is an additional limitation. Nicolas *et al*[27], have reported feasibility in patients ≥ 10 kg but in our institution, we normally do not proceed in patients under 10 years of age[8,27].

Echocardiography

The usefulness and accuracy of several echocardiographic techniques, as diagnostic methods for CAV have been explored. Published data have shown disparate results but more recent reports involving dobutamine stress echocardiography have demonstrated greater prognostic value[33-40].

Dobutamine stress echocardiography (DSE) allows assessment of wall motion, inducible ischemia and viability. Nevertheless, the sensitivity, specificity positive predictive value and negative predictive value vary significantly among these st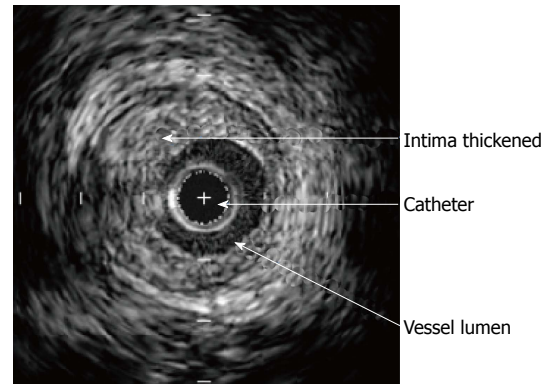udies. Despite these limitations, Spes *et al*[35] noted, in an adult cohort, that in patients with abnormal DSE, 90% had significant CAV by IVUS, but only 49% by angiography again demonstrating the relative insensitivity of angiography. Furthermore, they showed that a normal pharmacological stress echocardiography after heart transplantation has a high negative predictive value for any major adverse cardiovascular event. This suggests that if a strict DSE protocol is followed, a selective invasive angiography/IVUS policy may be adopted[34,35,37,38,41]. This was corroborated in a Pediatric cohort by Pahl *et al*[39]. Some authors have pointed out that endothelial dysfunction might be the cause of abnormal wall motion detected by DSE and normal angiography[42].

In children, the variability when compared to angiography, is even higher than that showed in adult series. Sensitivity rates vary between 35% and 71%, specificity between 80% and 94%, positive predictive value between 45% and 91% and negative predictive value between 81% and 92%[37,43,44]. If reliability within a given department is established, then it certainly appears to be an attractive option for children due to its non-invasive nature. However, it does require a good set up, effective sedation, expertise in images acquisition, expertise in interpretation and a standardised, reproducible protocol.

Sensitivity and specificity of stress echocardiography techniques can be improved by quantitative analysis using strain imaging. This modality can quantify regions of wall motion abnormality, (*i.e.*, a reduction in peak systolic strain % will be seen in LV segments associated with inducible ischemia and accurate measurements of time to peak strain may also give information on regional wall motion abnormalities). Eroglu *et al*[40], showed that, in adults, the accuracy of DSE can be improved using strain analysis (Figure 6).

Combined use of contrast-enhanced echocardiography with adenosine mediated hyperemia in order to assess coronary flow reserve has shown encouraging results in adults. Tona *et al*[45] demonstrated feasibility and prognostic value of coronary flow reserve measured by contrast enhanced echocardiography with good correlation with major acute cardiac events. Severe Coronary Flow Reserve (CFR) alteration was shown to precede acute cardiac event
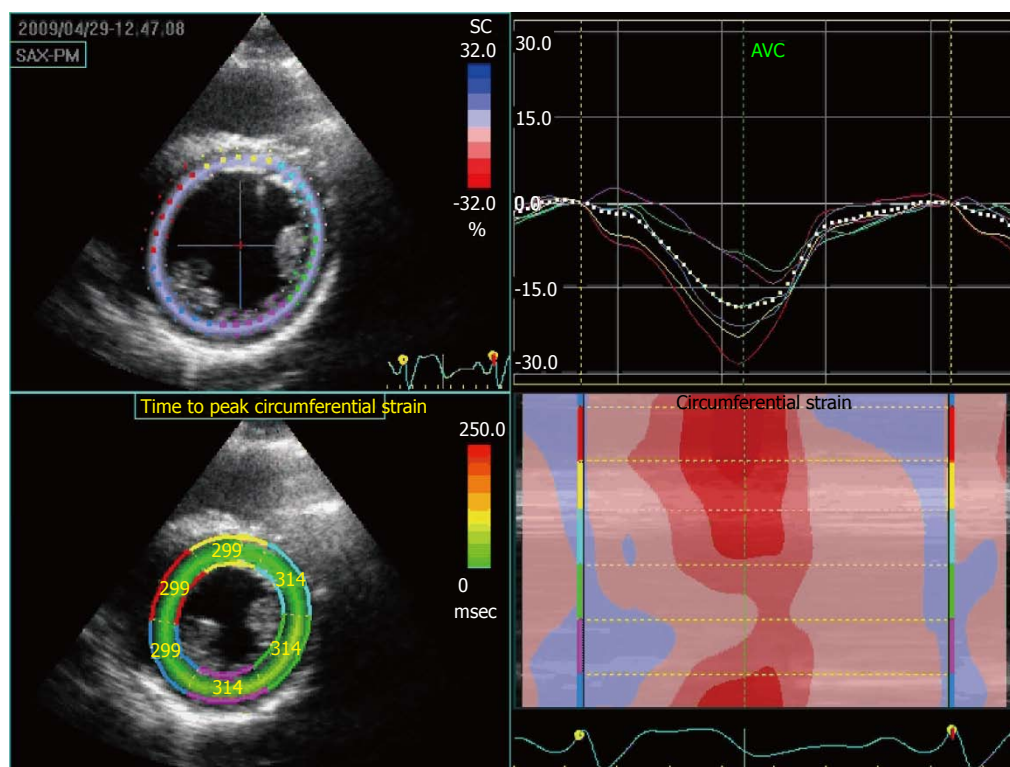
Figure 6 Speckle-tracking echocardiographic analysis of myocardial deformation showing circumferential strain in a patient with coronary allograft vasculopathy. On the top right image we can appreciate the dyssinchrony (later contraction compared to the rest of the segments) and lower contractility of the green and purple segment corresponding to LCX territory. On the bottom right figure, we can appreciate how the same green and purple segments have significant lower contractility than the others (red corresponds to the maximum contractility and blue to the absence of it). The left superior panel shows the color coding for each of the segments. The left inferior panel shows the time interval between beginning of QRS and maximal strain value.

onset. On a more recent study, the same group, showed high sensitivity and specificity for this technique in the detection of significant CAV (defined by Media Intimal Thickness > 0.5 in IVUS)[46]. Although these results are really encouraging, more studies are needed to establish the reproducibility. Interestingly, a separate small study in adults showed that transesophageal echocardiographic measurement of CFR impairment could identify CAV but it did not allow grading of severity[47]. However, this approach will be more difficult to implement in children, owing to difficulty in imaging due to the small size of the coronary arteries and the need for sedation in many patients.

The application of tissue Doppler techniques to the transplant population is also worth mentioning. Dandel *et al*[48,49] showed the utility of power Doppler TDI for the diagnosis of CAV in adults. Systolic Tissue Doppler Imaging (TDI) parameters at basal lateral LV wall level showed the highest diagnostic accuracy. Peak systolic motion velocity (Sm) and time to peak systole (TSm) differed significantly between patients with and without CAV as identified by IVUS. Furthermore, with Sm > 11 cm/s and TSm > 110 cm/s^2, angiographic disease can be excluded and, in the absence of any rejection, an Sm < 10 cm/s has a positive predictive value of over 97% for CAV (as detected by IVUS or angiography)[48,49]. The main limitations for the widespread use of this technique arise from the inter-observer and inter-departmental variability. These techniques have been applied to adult cohorts mainly and the available literature in the pediatric population is still very limited. One small retrospective study has shown that tricuspid annulus velocity was the best predictor of graft failure in pre-terminal patients. However, conventional echocardiographic parameters such as increase in tricuspid regurgitation severity and a reduction in left ventricular ejection fraction were also associated with increased mortality[50]. However, another recent study in a pediatric cohort showed poor correlation between TDI and hemodynamics parameters[51], highlighting the need for further confirmatory studies in children.

Exercise stress echocardiography (ESE) in adult patients was initially found to have unacceptably low sensitivity for the detection of CAV[33,52]. However, Chen *et al*[53] showed recently a sensitivity higher than 88% with almost 92% specificity in detecting significant epicardial angiographic CAD among pediatric heart transplant recipients. The positive predictive value of ESE was 72.7%, and the negative predictive value was 97.1%[53]. These results need wider confirmation prior to consideration as a screening tool.

Invasive coronary hemodynamics

CAV is a complex and diffuse process that leads to concentric luminal stenosis and occlusion of epicardial large and medium sized vessels. It also affects the intramyocardial microvasculature. Microvascular disease is present in heart transplant recipients early after transplant, even in
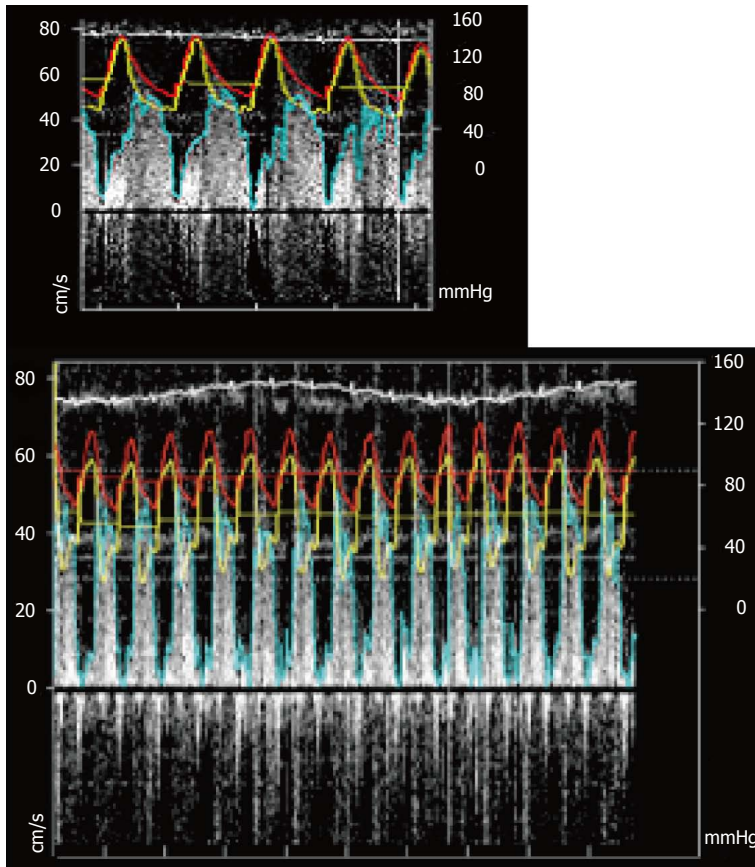
asymptomatic patients[20,54] and it is known to be associated with CAV, ischemia and death.

Fractional flow reserve (FFR) is defined as the ratio of maximum flow in the presence of a stenosis to normal maximum flow. It is a lesion-specific index of stenosis severity that can be calculated by simultaneous measurement of mean arterial, distal coronary, and central venous pressure, during pharmacological vasodilation. FFR is a well established tool to assess hemodynamic significance of coronary focal stenosis and has been recommended since 2010 by European Society of Cardiology for the physiological assessment of moderate coronary stenosis when functional information is lacking[55] in atherosclerotic disease.

In such cases, pressure gradients and FFR are recorded throughout the length of the artery through a pull back of the wire during maximum pharmacologically-induced hyperemia.

The Combowire® XT also allows simultaneous measurement of flow and pressure and FFR simultaneously to the coronary flow reserve (CFR) (Figure 7).

In transplanted patients, the exact value of FFR to determine epicardial disease is difficult to establish and results have been inconsistent between series[56,57]. In a publication by Hirohata *et al*[20], FFR improved as the microvascular disease deteriorated and therefore, due to the particular interaction between microvascular and epicardial disease that occurs in CAV, FFR might not be the best reflection of epicardial affectation in this situation.

CFR reflects the ability of the myocardium to increase blood flow in response to maximal exercise or stress. It is expressed by the ratio of the myocardial blood flow at peak stress, or maximal vasodilatation, to the flow at rest. Decrease in CFR, after Adenosine administration to achieve maximum vasodilation, in the absence of significant epicardial stenosis (normal fractional flow reserve) indicates microvascular dysfunction[58]. If the significance of decreased CFR is well established in the atherosclerotic population[59,60]. Although theoretically more important for CAV, the exact significance of CFR measurement remains to be determined. Using acetylcholine-mediated, endothelium-dependent, coronary vasodilatation measurement of CFR, Hollenberg *et al*[61] showed that endothelial microvascular dysfunction was more common in the group suffering adverse outcomes (death or angiographic evidence of CAV) than in those without adverse outcome. However, published data are not consistent between studies. Kübrich *et al*[62], in a larger cohort, found no correlation between epicardial and microvascular disease and found that, whilst microvascular dysfunction demonstrated by CFR was a predictor of outcome (death or adverse cardiovascular event) in the univariate analysis, it did not predict outcome in the multivariate analysis.

The pediatric population offers very limited data for CFR. In a small cohort, a decrease in CFR correlated with microvasculopathy seen in endomyocardial biopsy specimens[63]. The invasive nature of Doppler wire flow measurements to determine CFR makes it an unattractive tool for children.

Several groups have presented data of CFR quantified

by CMRI of the coronary sinus showing good correlation with PET or flow phantoms[64-66]. More recently, Ishida *et al*[67] presented data on CFR as independent predictor of MACE in patients with known or suspected CAD. Kennedy *et al*[68] have translated this idea into the transplant population: they found that CFR determination by Cardiac Magnetic Resonance Imaging (CMRI) in the coronary sinus, was significantly decreased inpatients with severe CAV and therefore, it may be a useful tool in non-invasively evaluating coronary allograft vasculopathy in heart transplant recipients.

Single photon emission computed tomography

Single photon emission computed tomography (SPECT) is a useful clinical tool for myocardial perfusion imaging to detect and risk-stratify of coronary atherosclerotic disease for management guidance[69]. Either exercise or pharmacological stress can be employed and, most commonly, one of theTc-99m-labeled tracers is used. Numerous studies in adult population with coronary atherosclerotic disease have assessed the relative accuracies of stress imaging using nuclear cardiology techniques: for stress SPECT, sensitivity is around 87% with a specificity of 73% (compared to coronary angiography)[70]. Recently, it has been recognized that some patients with non-critical coronary artery stenosis can have abnormal stress perfusion imaging. This is due to microvascular and endothelial dysfunction causing abnormal flow reserve[71].

When applied to CAV, SPECT has a high negative predictive value in adults[72-77]. When using Dobutamine stress and 99m technetium tetrofosmin, abnormal perfusion is associated to a risk ratio of 3.5 in predicting cardiac death[78-80]. A reversible perfusion defect on stress SPECT is an independent predictor of mortality or graft loss[72,81-83] and it seems that stress SPECT at one year post transplantation could be an earlier prognostic indicator[84].

In Pediatrics, the experience with SPECT is largely anecdotal. The small size of the heart might be a limiting technical factor and the radiation related to the technique itself makes it a rather unattractive diagnostic tool.

Positron emission tomography

Positron emission tomography (PET) has established itself as the gold standard for noninvasive assessment of myocardial perfusion measuring myocardial blood flow at rest and during stress. As well as myocardial perfusion reserve, perfusion of the epicardial arteries and the microvasculature can be determined[85,86]. In patients after heart transplantation, myocardial perfusion reserve measured with PET has been performed in a few studies[87-89]. Wu *et al*[88] found good correlation between IVUS and myocardial perfusion reserve even in the absence of angiographic lesions. Published data is very limited even in adults, related to the limited availability of the technique and the expertise required.

Multidetector computed tomography

In the atherosclerotic population, multidetector computed tomography (MDCT) has shown high sensitivity and specificity in the diagnosis of angiographic coronary arteriopathy and characterization of the stenotic disease[90]. Recent studies also indicate that detection and characterization of the plaque is possible although challenging[91,92] increasing potential value as a diagnostic tool.

The literature provides some data regarding the heart transplant population: Sigurdsson *et al*[93] used a 16-detector MDCT to identify coronary stenosis and compared to angiographic disease (defined by luminal stenosis > 95%). Sensitivity, specificity, positive and negative predictive values were 86%, 99%, 81% and 99% respectively, unfortunately only a few subgroup of patients underwent IVUS.

Gregory *et al*[18], on the other hand, did use IVUS to compare 64-slice MDCT results in 20 patients at 1 year post-transplant. They defined CAV as maximal intimal thickness > 0.5 mm and found that MDCT has a sensitivity of 70% and a specificity of 92% with a positive predictive value of 89% and negative predictive value of 77%. However, in this study, slightly less than 20% of coronary segments (mainly distal) could not been analysed due to poor image quality (probably in relation with elevated heart rate)[18].

Recent studies showed that dual source MDCT allows good image quality of vessel lumen[94,95] and, when validated against IVUS, high diagnostic accuracy[96]. A small study, just under 20 patients, demonstrated that MDCT, using 64-slices, was superior to angiography for the identification of non-obstructive vessel wall disease. However, they did not use IVUS for comparison[94]. Schepis *et al*[96] compared 64 channels dual source MDCT with IVUS to look at vessel wall thickness. Defining CAV as intimal thickness > 0.5 mm on IVUS they established sensitivity, specificity, negative predictive value and positive predictive value of MDCT of 85%, 84%, 76% and 91% respectively.

Therefore, MDCT appears to be a useful tool for CAV screening. Although not as sensitive as IVUS, it is non-invasive and clearly superior to angiography. However, the elevated heart rate post-transplantation, especially in pediatric patients, compromises image quality and the need for potentially nephro-toxic contrast agent adds concern for heart transplant recipients, for whom renal impairment is a frequent comorbidity[97,98].

There is preliminary data available in children using MDCT compared to angiography and IVUS to identify coronary luminal stenosis, although the size of the series was very small[99] and results would require further studies to be validated.

Again, the implied repeated radiation dosage makes it a less attractive screening option in children.

Optical coherence tomography

Optical coherence tomography (OCT) is an intravascular high resolution imaging modality that measures reflected light waves intensity and converts these into a high resolution tomographic image[100]. In CAD patients, OCT has been used to characterize plaque composition and differentiate between intimal hyperplasia, fibrous plaque, lipid-rich plaque or calcifications[101,102] (Figure 8).
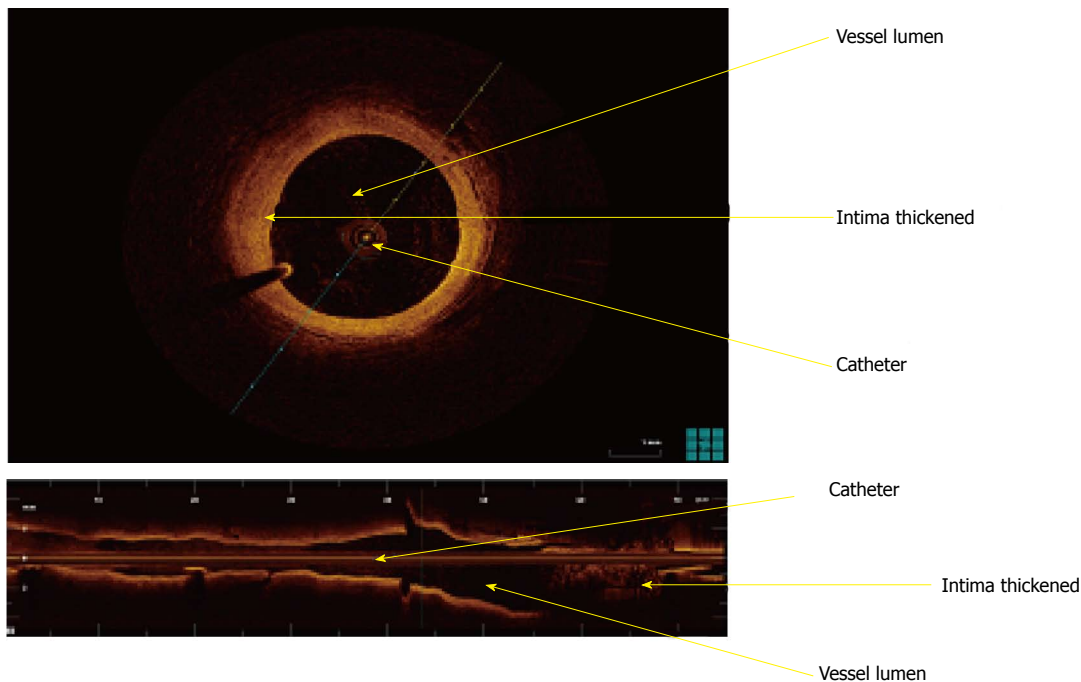
Figure 8 Optical coherence tomography images showing intima hyperplasia. The superior image shows a transverse cut of the coronary. On the inferior part of the image, longitudinal cut.

Recent studies have evaluated the use of OCT in heart transplant recipients with promising results. The OCTAV study demonstrated, in 15 patients early post-transplant (with no angiographic evidence of CAV), that early quantification of intima-media ratio and characterization of the plaque is possible. There was no IVUS performed for comparison[103]. Garrido *et al*[104] compared OCT to IVUS in 21 patients, later post-transplant, and not only found good correlation with IVUS but also postulated that OCT offers better plaque characterization and less inter-observer variability.

Cassar *et al*[105] compared OCT to IVUS and angiography in 53 patients, showing that OCT was superior to angiography but not to IVUS. IVUS and OCT were strongly correlated with 100% agreement.

Further prospective and larger studies are needed to define the exact role of OCT in the diagnosis of CAV and, more importantly, to define its prognostic implications.

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMRI) coronary angiography in the context of CAD has proved its capacity to detect atherosclerotic plaque and proximal to mid-coronary artery stenoses[106-108]. Uribe *et al*[109] have demonstrated the feasibility and accuracy of MR coronary angiography in the detection of coronary anomalies in children, despite elevated heart rates with whole heart dual phase cardiac imaging[109,110]. Greil *et al*[111], have also previously shown the utility of coronary magnetic resonance angiography (CMRA) in patients with Kawasaki disease.

These studies undoubtedly open the door for the application of CMRA in CAV including in pediatric cohorts. Unfortunately, when compared to MDCT, CMRA does not seem to be as sensitive or robust in the detection of coronary stenoses, although limited studies have been done. CMRI offers several advantages: it provides functional information on myocardial characterization and contractility as well as wall motion performance; it allows quantitative measurements of ventricular volumes and it is radiation-free, which is especially valuable in a population already exposed to repeated X-ray angiography.

In conventional atherosclerosis, perfusion imaging has shown to be effective in detecting myocardial ischemia and to assess microvascular dysfunction as it detects downstream microvacular blood flow within the myocardium. The MR-IMPACT study demonstrated that CMRI is superior to SPECT in identifying perfusion defects within the myocardium for atherosclerotic patients[112]. Perfusion stress with adenosine also provides prognostic data: a normal CMR stress perfusion scan showed 99% event free survival at 3 years[113].

The use of adenosine for myocardium stress perfusion after heart transplantation has not been widely reported. Nevertheless, Muehling *et al*[114] showed a reduced myocardial perfusion reserve in patients with CAV with good correlation between MRI and invasive measurements. Unfortunately, microvascular disease in this study could not be assessed. They also demonstrated that patients with CAV have a reduced myocardial perfusion even during rest conditions[114].

In regards to CMR tissue characterization, Steen *et al*[115] showed that more than 80% of patients with severe angiographic CAV had a late gadolinium enhancement pattern suggesting subendocardial infarction with a distribution consistent with the angiographic pattern. Furthermore, he was able to identify silent myocardial
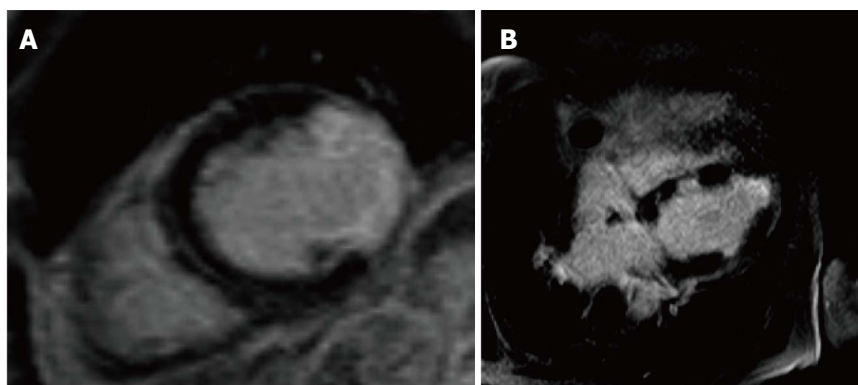
Figure 9 Late gadolinium enhancement scar imaging. A: Typical infarct pattern Late enhancement with > 75% transmurality; B: Atypical pattern with diffuse pattern of late enhancement.
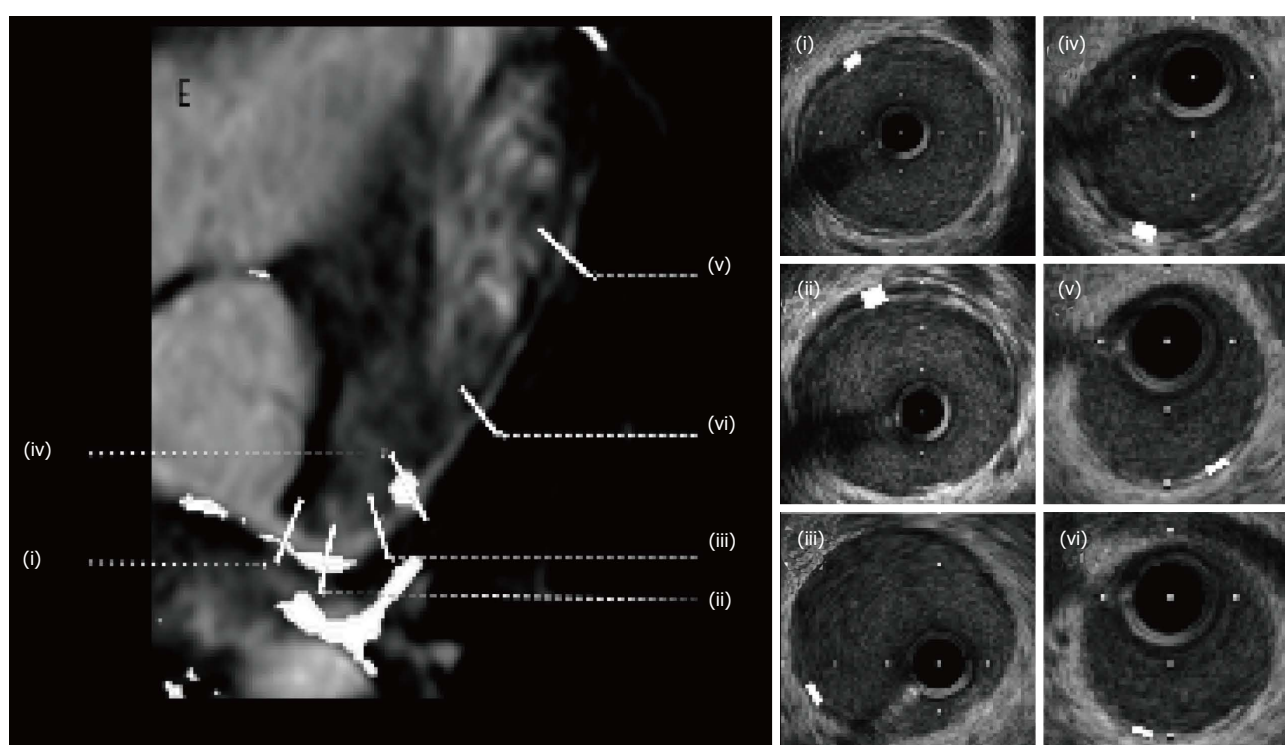

Figure 10 Late gadolinium enhancement in the coronary vessel wall showing corresponding positions for intravascular ultrasound: Illustrates intimal thickening corresponding to enhancement on overlay picture on the left.

infraction in otherwise apparently event-free patients (Figure 9). In a more recent publication, the same group looked at infarct-atypical myocardial involvement that they were not able to correlate with coronary angiographic pattern in the prior study. According to their findings, within the 4 different patterns of infarct-atypical LGE-CMR, only the diffuse form was significantly higher patients early post transplantation, but they could not establish a definite reason for the findings[116].

Hussain *et al*[117], have taken this technique further showing that high resolution late gadolinium enhancement (LGE) can be used to show vessel wall disease in CAV with good correlation with IVUS Figure 10. LGE scores correlated well with the maximal intimal thickness and mean intimal index [Pearson coefficient 0.80 ($P < 0.001$) and 0.92 ($P < 0.001$), respectively]. An enhancement diameter > 7.5 mm gave promising sensitivity and specificity values of 86% and 93%, respectively, for the detection of significant CAV.

A recently published paper, evaluated in 48 transplanted patients both epicardial and microvacular disease concomitantly. The patients underwent coronary angiography, invasive coronary physiological assessment, IVUS and multi-parametric cardiac MRI that includes, tissue characterization, perfusion analysis and tissue tagging. They found that cardiac MRI-based myocardial perfusion reserve was independently predictive of both epicardial and microvacular components of CAV and furthermore that diagnostic performance was significantly higher than angiography[118].

More studies are needed to establish CMRI as a reliable non-invasive tool for CAV diagnostic but certainly the latest

data are encouraging and more work needs to be achieved in this direction.

PREVENTION AND TREATMENT

Rapid progression of CAV within the first year post transplant is a strong indicator of severe CAV, graft loss and mortality[17]. Therefore, prophylactic strategies are paramount and must been introduced early to improve long-term outcomes and prognosis.

Similar to native coronary disease, primary prevention includes control of traditional cardiovascular risk factors such as hypertension, smoking, diabetes and hyperlipidemia. This can be challenging, as many of these factors are also side effects of the immunosuppressive therapy. Tobacco should be avoided and care should be taken to avoid passive smoking in children. Modifications of specific risk factors related to the transplant include prevention and aggressive treatment in case of cytomegalovirus (CMV) sero-conversion[118]. In addition, it is essential to treat any episode of rejection early and aggressively.

Psychological care

Psychological support is crucial in transplanted children and their families throughout all the transplant journey: Leaving with a reduced life expectancy when compared to peers is often complicated and despite good quality of life can be a source of distress for the recipients. In the context of CAV psychological support is especially important: Prevention is paramount; and, if it is essential to treat aggressively any rejection episode, it is also vital for the patients to be compliant with the antirejection therapy. However, it is well known that often therapy compliance declines in adolescence and case of sudden death have been reported related to antirejection treatment discontinuation. In these patients psychological support is essential to ensure therapy obeisance. In cases of advanced CAV the ineluctability of the graft loss and its implication lead to severe depression and negation that also frequently required psychological input.

Statins

Most transplant protocols nowadays include statin, independently of the lipid level. Several studies have highlighted their benefits beyond lipid lowering effects[119-121]; including reduced incidence of severe rejection episodes, reduced CAV progression and improved long term survival[122-124]. Consensus guidelines unequivocally recommend statin therapy[125].

CMV

CMV infection results in acceleration of CAV as the result of the host immune response. Aggressive treatment with ganciclovir reduces progression of CAV[126] and the lack of prophylaxis is associated with increased lumen loss[127]. Our institution, as with most of the transplant centers, uses acyclovir for CMV prophylaxis during the first 3 mo post-transplantation.

Vasodilators

A few reports indicate a potential role for vasodilators in preventing and slowing CAV progression. Calcium channels blockers and ACE inhibitors have been reported in the literature to be beneficial but large prospective trials are needed to determine their exact role[128-130]. Most transplant institutions use both of these to treat hypertension, which develops frequently as side effect of calcineurin inhibitors therapy.

Immunosuppression

Most of the data are from adult studies with limited evidence in the pediatric population.

Calcineurin inhibitors

Tacrolimus not only offers better protection against acute rejection compared to cyclosporine[131-133], but it is also superior against CAV[134]. Moreover, Petrakopoulou *et al*[135] showed that tacrolimus is better than cyclosporine in the prevention of microvascular endothelial dysfunction.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) has demonstrated superiority to azathioprine in mortality and graft loss[136]. In the re-analysis of the same study, it also showed less intimal thickening and wider lumen area[137]. Finally, Kaczmarek *et al*[138], in 2006 demonstrated that MMF decreased CAV incidence.

Proliferation signal inhibitors

Contrary to Calcineurin Inhibitors (CNIs) that blocks T-cell activation and proliferation by suppressing lymphokines production, proliferation signal inhibitors (PSIs) inhibit Tcell and B cell proliferation by impairing their response to growth promoting lymphokines[139]. In addition, PSIs have also a significant cytostatic effect on the immune system[134,140]. In 2003, Eisen *et al*[141] published the first data in favor of PSIs, using everolimus de novo after heart transplantation. They showed preservation of the coronary lumen at 1 year with significant lower incidence of CAV in the everolimus group compared to the aziathropine group. A sub-study published in 2007 confirmed the results at 24 mo[142] and the same group has also shown reduced incidence of cardiovascular events in the everolimus group[143]. Nevertheless, despite the promising results of these studies, all of them compared PSIs to Azathioprine, which is not used as first line therapy anymore and known to be associated to higher rate of rejection than newer immunosuppressive agents. The results of an eagerly awaited clinical trial comparing everolimus de novo to cyclosporine has been recently published showing marked improvement in renal function at 12 mo in the everolimus group without increased of adverse events as well as demonstrated, *via* IVUS, significantly reduced CAV progression at 12 mo in the everolimus group[144].

Mancini *et al*[145], in a randomized study reported that sirolimus (as a secondary immunosuppressant) slows progression of CAV and reduces the incidence of clinically significant events, such as death or graft failure. Keogh *et al*[146], using randomized de novo treatment between

sirolimus or azathioprine reported significantly reduced progression in intimal and medial proliferation at 6 mo post-transplant and a reduction in the number of acute rejection episodes of around 50%. The effect was sustained at 2 years post transplant using IVUS to quantify vessel wall proliferation.

Although a combined regime CNIs + PSIs appears to be attractive in preventing and slowing CAV, serious concerns with this regimen should be raised regarding nephrotoxicity. PSIs have shown in several studies to increase side effects of CNIs, especially for nephropathy[147-151].

Raichlin *et al*[152] have published encouraging data with sirolimus-based immunosuppression, and even postulated that a CNI free regimen would be safe, well tolerated and associated with less CAV progression, coronary events and graft failure, when initiated beyond the first year (and within the first 2 years).

In a more recent study, the same group showed that early conversion to sirolimus attenuated plaque progression, improved overall survival, and increased freedom from cardiac events. However, the retrospective nature of the design and the differences in criteria for the therapy changes, make the results less generalizable[153]. Moreover, a recent study reported that late conversion to PSIs is associated with necrotic plaque core and calcification of the plaque[154].

Hence, safety of early CNI withdrawal with PSI conversion remains uncertain, especially in the first year post-transplant with concerns also raised about acute rejection. Therefore, many continue to recommend against withdrawal of CNIs during the first 12 mo post transplantation[155].

Side effects from PSIs are not infrequent: anemia, dyslipidemia, increased incidence of bacterial infections, peripheral oedema, pericardial or pleural effusion, pneumonitis and delayed wound closure. They seem to be dose-related and reversed by discontinuation of the drug, although most can be controlled with dose adjustments[155].

PSIs have also been attributed with a reduction in CMV infections and an inhibition of Epstein-Barr virus-infected tumorigenic cell lines[156-158]. In the Pediatric population, PSI use is still limited to a rescue therapy for post-transplant complications such as CAV or renal impairment secondary to therapy.

Coronary revascularization
In contrast to native coronary disease, CAV is progressive and revascularization procedures are only palliative with no survival benefit[159,160]. Moreover, the concentric, diffuse and distal nature of CAV precludes the majority of patients for revascularization procedures.

Percutaneous interventions
Percutaneous intervention in transplanted patients are characterized by good short term results but high restenosis rates[160-165].

Unfortunately, stents do not offer better long-term results with a late re-stenosis rate around 70%. Drug eluting stents appear to have slightly better results with less restenosis[57,166]. However, only the minority of CAV lesions are amenable for percutaneous revascularization as outlined above and stent angioplasty might only be an option in selected patients.

Bypass grafting
Surgical revascularization is associated with a very high mortality (up to 40%)[162,167,168] and limited success. Indication is then reserved to highly selected patients.

Re-transplantation
Re-transplantation is the only definitive treatment for CAV. Unfortunately it is associated with lower survival than with the primary graft[169] (relative risk for 10 years mortality according to ISHLT 2012 data is 1.56) and the probability of CAV recurrence is higher (50% at 3 years)[167,170].

The scarcity of donors, and prior antigen sensitization means that, in practice, re-transplantation occurs infrequently.

CONCLUSION
Despite a wide range of new diagnostic techniques, angiography remains, to date, the most commonly used diagnostic tool for CAV. Not only is it invasive, costly and radiation-prone but it also fails to identify the disease in its early phase. IVUS is the most sensitive technique but requires trained operators and it is, again, an invasive technique requiring ionizing radiation.

Overall, the available published evidence support a role for MDCT or DSE as non-invasive screening test to reduce the number of invasive angiograms (and IVUS). However, an accurate and reproducible non-invasive diagnostic tool is yet to be widely established. CMR offers anatomical, histological and physiological assessments and, in the future, it could be valuable in the detection and grading of CAV.

Early detection is paramount but remains challenging. It may allow us to identify those requiring modification in immunosuppression, such as early introduction of PSIs for those with more aggressive CAV.

Unfortunately CAV remains the primary cause of graft failure after the first year post-transplantation and the only definitive treatment is re-transplantation.

REFERENCES
1   **Pahl E**, Naftel DC, Kuhn MA, Shaddy RE, Morrow WR, Canter CE, Kirklin J. The impact and outcome of transplant coronary artery disease in a pediatric population: a 9-year multi-institutional study. *J Heart Lung Transplant* 2005; **24**: 645-651 [PMID: 15949722 DOI: 10.1016/j.healun.2004.03.021]
2   **Kirk R**, Edwards LB, Kucheryavaya AY, Aurora P, Christie JD, Dobbels F, Rahmel AO, Stehlik J, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: thirteenth official pediatric heart transplantation report--2010. *J Heart Lung Transplant* 2010; **29**: 1119-1128 [PMID: 20870166 DOI: 10.1016/j.healun.2010.08.009]
3   **Rahmani M**, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. *Circ Res* 2006; **99**: 801-815 [PMID: 17038650 DOI: 10.1161/01.RES.0000246086.93555.f3]

4   **Colvin-Adams M**, Harcourt N, Duprez D. Endothelial dysfunction and cardiac allograft vasculopathy. *J Cardiovasc Transl Res* 2013; **6**: 263-277 [PMID: 23135991 DOI: 10.1007/s12265-012-9414-3]

5   **Valantine H**. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. *J Heart Lung Transplant* 2004; **23**: S187-S193 [PMID: 15093804 DOI: 10.1016/j.healun.2004.03.009]

6   **Raichlin ER**, McConnell JP, Lerman A, Kremers WK, Edwards BS, Kushwaha SS, Clavell AL, Rodeheffer RJ, Frantz RP. Systemic inflammation and metabolic syndrome in cardiac allograft vasculopathy. *J Heart Lung Transplant* 2007; **26**: 826-833 [PMID: 17692787 DOI: 10.1016/j.healun.2007.05.008]

7   **Conway J**, Dipchand AI. Heart transplantation in children. *Pediatr Clin North Am* 2010; **57**: 353-373, table of contents [PMID: 20371041 DOI: 10.1016/j.pcl.2010.01.009]

8   **Dent CL**, Canter CE, Hirsch R, Balzer DT. Transplant coronary artery disease in pediatric heart transplant recipients. *J Heart Lung Transplant* 2000; **19**: 240-248 [PMID: 10713248]

9   **Kobayashi D**, Du W, L'ecuyer TJ. Predictors of cardiac allograft vasculopathy in pediatric heart transplant recipients. *Pediatr Transplant* 2013; **17**: 436-440 [PMID: 23714284 DOI: 10.1111/petr.12095]

10 **Kirk R**, Edwards LB, Aurora P, Taylor DO, Christie JD, Dobbels F, Kucheryavaya AY, Rahmel AO, Stehlik J, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: Twelfth Official Pediatric Heart Transplantation Report-2009. *J Heart Lung Transplant* 2009; **28**: 993-1006 [PMID: 19782282 DOI: 10.1016/j.healun.2009.08.008]

11 **Gao SZ**, Schroeder JS, Hunt SA, Billingham ME, Valantine HA, Stinson EB. Acute myocardial infarction in cardiac transplant recipients. *Am J Cardiol* 1989; **64**: 1093-1097 [PMID: 2816760]

12 **Schmauss D**, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008; **117**: 2131-2141 [PMID: 18427143 DOI: 10.1161/CIRCULATIONAHA.107.711911]

13 **Mehra MR**, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, Madsen J, Parameshwar J, Starling RC, Uber PA. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010; **29**: 717-727 [PMID: 20620917 DOI: 10.1016/j.healun.2010.05.017]

14 **St Goar FG**, Pinto FJ, Alderman EL, Valantine HA, Schroeder JS, Gao SZ, Stinson EB, Popp RL. Intracoronary ultrasound in cardiac transplant recipients. In vivo evidence of "angiographically silent" intimal thickening. *Circulation* 1992; **85**: 979-987 [PMID: 1537134 DOI: 10.1161/01.CIR.85.3.979]

15 **O'Neill BJ**, Pflugfelder PW, Singh NR, Menkis AH, McKenzie FN, Kostuk WJ. Frequency of angiographic detection and quantitative assessment of coronary arterial disease one and three years after cardiac transplantation. *Am J Cardiol* 1989; **63**: 1221-1226 [PMID: 2653018]

16 **Johnson DE**, Alderman EL, Schroeder JS, Gao SZ, Hunt S, DeCampli WM, Stinson E, Billingham M. Transplant coronary artery disease: histopathologic correlations with angiographic morphology. *J Am Coll Cardiol* 1991; **17**: 449-457 [PMID: 1991903]

17 **Tuzcu EM**, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, Magyar WA, Hobbs RE, Starling RC, Young JB, McCarthy P, Nissen SE. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. *J Am Coll Cardiol* 2005; **45**: 1538-1542 [PMID: 15862431 DOI: 10.1016/j.jacc.2004.12.076]

18 **Gregory SA**, Ferencik M, Achenbach S, Yeh RW, Hoffmann U, Inglessis I, Cury RC, Nieman K, McNulty IA, Laffan JA, Pomerantsev EV, Brady TJ, Semigran MJ, Jang IK. Comparison of sixty-four-slice multidetector computed tomographic coronary angiography to coronary angiography with intravascular ultrasound for the detection of transplant vasculopathy. *Am J Cardiol* 2006; **98**: 877-884 [PMID: 16996866 DOI: 10.1016/j.amjcard.2006.04.027]

19 **Störk S**, Behr TM, Birk M, Uberfuhr P, Klauss V, Spes CH, Angermann CE. Assessment of cardiac allograft vasculopathy late after heart transplantation: when is coronary angiography necessary? *J Heart Lung Transplant* 2006; **25**: 1103-1108 [PMID: 16962473 DOI: 10.1016/j.healun.2006.05.009]

20 **Hirohata A**, Nakamura M, Waseda K, Honda Y, Lee DP, Vagelos RH, Hunt SA, Valantine HA, Yock PG, Fitzgerald PJ, Yeung AC, Fearon WF. Changes in coronary anatomy and physiology after heart transplantation. *Am J Cardiol* 2007; **99**: 1603-1607 [PMID: 17531589 DOI: 10.1016/j.amjcard.2007.01.039]

21 **Glagov S**, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 1987; **316**: 1371-1375 [PMID: 3574413 DOI: 10.1056/NEJM198705283162204]

22 **Tsutsui H**, Schoenhagen P, Ziada KM, Crowe TD, Klingensmith JD, Vince DG, Bott-Silverman C, Starling R, Hobbs RE, Young J, Nissen SE, Tuzcu EM. Early constriction or expansion of the external elastic membrane area determines the late remodeling response and cumulative lumen loss in transplant vasculopathy: an intravascular ultrasound study with 4-year follow-up. *J Heart Lung Transplant* 2003; **22**: 519-525 [PMID: 12742413]

23 **Tsutsui H**, Ziada KM, Schoenhagen P, Iyisoy A, Magyar WA, Crowe TD, Klingensmith JD, Vince DG, Rincon G, Hobbs RE, Yamagishi M, Nissen SE, Tuzcu EM. Lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling: results from a 5-year serial intravascular ultrasound study. *Circulation* 2001; **104**: 653-657 [PMID: 11489770 DOI: 10.1161/hc3101.093867]

24 **Wong C**, Ganz P, Miller L, Kobashigawa J, Schwarzkopf A, Valantine von Kaeper H, Wilensky R, Ventura H, Yeung AC. Role of vascular remodeling in the pathogenesis of early transplant coronary artery disease: a multicenter prospective intravascular ultrasound study. *J Heart Lung Transplant* 2001; **20**: 385-392 [PMID: 11295575]

25 **Costanzo MR**, Naftel DC, Pritzker MR, Heilman JK, Boehmer JP, Brozena SC, Dec GW, Ventura HO, Kirklin JK, Bourge RC, Miller LW. Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. Cardiac Transplant Research Database. *J Heart Lung Transplant* 1998; **17**: 744-753 [PMID: 9730422]

26 **Barbir M**, Lazem F, Banner N, Mitchell A, Yacoub M. The prognostic significance of non-invasive cardiac tests in heart transplant recipients. *Eur Heart J* 1997; **18**: 692-696 [PMID: 9129903]

27 **Nicolas RT**, Kort HW, Balzer DT, Trinkaus K, Dent CL, Hirsch R, Canter CE. Surveillance for transplant coronary artery disease in infant, child and adolescent heart transplant recipients: an intravascular ultrasound study. *J Heart Lung Transplant* 2006; **25**: 921-927 [PMID: 16890112 DOI: 10.1016/j.healun.2006.03.022]

28 **Rickenbacher PR**, Pinto FJ, Lewis NP, Hunt SA, Alderman EL, Schroeder JS, Stinson EB, Brown BW, Valantine HA. Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. *Circulation* 1995; **92**: 3445-3452 [PMID: 8521566]

29 **Kobashigawa JA**, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valantine HA, Yeung AC, Mehra MR, Anzai H, Oeser BT, Abeywickrama KH, Murphy J, Cretin N. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005; **45**: 1532-1537 [PMID: 15862430 DOI: 10.1016/j.jacc.2005.02.035]

30 **Clausell N**, Butany J, Molossi S, Lonn E, Gladstone P, Rabinovitch M, Daly PA. Abnormalities in intramyocardial arteries detected in cardiac transplant biopsy specimens and

lack of correlation with abnormal intracoronary ultrasound or endothelial dysfunction in large epicardial coronary arteries. *J Am Coll Cardiol* 1995; **26**: 110-119 [PMID: 7541058]

31   **Kapadia SR**, Ziada KM, L'Allier PL, Crowe TD, Rincon G, Hobbs RE, Bott-Silverman C, Young JB, Nissen SE, Tuzcu EM. Intravascular ultrasound imaging after cardiac transplantation: advantage of multi-vessel imaging. *J Heart Lung Transplant* 2000; **19**: 167-172 [PMID: 10703693]

32   **Koning G**, Dijkstra J, von Birgelen C, Tuinenburg JC, Brunette J, Tardif JC, Oemrawsingh PW, Sieling C, Melsa S, Reiber JH. Advanced contour detection for three-dimensional intracoronary ultrasound: a validation--in vitro and in vivo. *Int J Cardiovasc Imaging* 2002; **18**: 235-248 [PMID: 12123316]

33   **Smart FW**, Ballantyne CM, Cocanougher B, Farmer JA, Sekela ME, Noon GP, Young JB. Insensitivity of noninvasive tests to detect coronary artery vasculopathy after heart transplant. *Am J Cardiol* 1991; **67**: 243-247 [PMID: 1990786]

34   **Derumeaux G**, Redonnet M, Soyer R, Cribier A, Letac B. Assessment of the progression of cardiac allograft vasculopathy by dobutamine stress echocardiography. *J Heart Lung Transplant* 1998; **17**: 259-267 [PMID: 9563602]

35   **Spes CH**, Klauss V, Mudra H, Schnaack SD, Tammen AR, Rieber J, Siebert U, Henneke KH, Uberfuhr P, Reichart B, Theisen K, Angermann CE. Diagnostic and prognostic value of serial dobutamine stress echocardiography for noninvasive assessment of cardiac allograft vasculopathy: a comparison with coronary angiography and intravascular ultrasound. *Circulation* 1999; **100**: 509-515 [PMID: 10430765]

36   **Bacal F**, Moreira L, Souza G, Rodrigues AC, Fiorelli A, Stolf N, Bocchi E, Bellotti G, Ramires JA. Dobutamine stress echocardiography predicts cardiac events or death in asymptomatic patients long-term after heart transplantation: 4-year prospective evaluation. *J Heart Lung Transplant* 2004; **23**: 1238-1244 [PMID: 15539121 DOI: 10.1016/j.healun.2003.09.007]

37   **Larsen RL**, Applegate PM, Dyar DA, Ribeiro PA, Fritzsche SD, Mulla NF, Shirali GS, Kuhn MA, Chinnock RE, Shah PM. Dobutamine stress echocardiography for assessing coronary artery disease after transplantation in children. *J Am Coll Cardiol* 1998; **32**: 515-520 [PMID: 9708485]

38   **Lewis JF**, Selman SB, Murphy JD, Mills RM, Geiser EA, Conti CR. Dobutamine echocardiography for prediction of ischemic events in heart transplant recipients. *J Heart Lung Transplant* 1997; **16**: 390-393 [PMID: 9154948]

39   **Pahl E**, Crawford SE, Swenson JM, Duffy CE, Fricker FJ, Backer CL, Mavroudis C, Chaudhry FA. Dobutamine stress echocardiography: experience in pediatric heart transplant recipients. *J Heart Lung Transplant* 1999; **18**: 725-732 [PMID: 10452350]

40   **Eroglu E**, D'hooge J, Sutherland GR, Marciniak A, Thijs D, Droogne W, Herbots L, Van Cleemput J, Claus P, Bijnens B, Vanhaecke J, Rademakers F. Quantitative dobutamine stress echocardiography for the early detection of cardiac allograft vasculopathy in heart transplant recipients. *Heart* 2008; **94**: e3 [PMID: 17916666 DOI: 10.1136/hrt.2007.119693]

41   **Akosah KO**, McDaniel S, Hanrahan JS, Mohanty PK. Dobutamine stress echocardiography early after heart transplantation predicts development of allograft coronary artery disease and outcome. *J Am Coll Cardiol* 1998; **31**: 1607-1614 [PMID: 9626841]

42   **Jackson PA**, Akosah KO, Kirchberg DJ, Mohanty PK, Minisi AJ. Relationship between dobutamine-induced regional wall motion abnormalities and coronary flow reserve in heart transplant patients without angiographic coronary artery disease. *J Heart Lung Transplant* 2002; **21**: 1080-1089 [PMID: 12398873]

43   **Dipchand AI**, Bharat W, Manlhiot C, Safi M, Lobach NE, McCrindle BW. A prospective study of dobutamine stress echocardiography for the assessment of cardiac allograft vasculopathy in pediatric heart transplant recipients. *Pediatr Transplant* 2008; **12**: 570-576 [PMID: 18363614 DOI: 10.1111/j.1399-3046.2007.00861.x]

44   **Di Filippo S**, Semiond B, Roriz R, Sassolas F, Raboisson MJ, Bozio A. Non-invasive detection of coronary artery disease by dobutamine-stress echocardiography in children after heart transplantation. *J Heart Lung Transplant* 2003; **22**: 876-882 [PMID: 12909467 DOI: 10.1016/S1053-2498(02)00664-2]

45   **Tona F**, Caforio AL, Montisci R, Angelini A, Ruscazio M, Gambino A, Ramondo A, Thiene G, Gerosa G, Iliceto S. Coronary flow reserve by contrast-enhanced echocardiography: a new noninvasive diagnostic tool for cardiac allograft vasculopathy. *Am J Transplant* 2006; **6**: 998-1003 [PMID: 16611336 DOI: 10.1111/j.1600-6143.2006.01298.x]

46   **Tona F**, Osto E, Tarantini G, Gambino A, Cavallin F, Feltrin G, Montisci R, Caforio AL, Gerosa G, Iliceto S. Coronary flow reserve by transthoracic echocardiography predicts epicardial intimal thickening in cardiac allograft vasculopathy. *Am J Transplant* 2010; **10**: 1668-1676 [PMID: 20642688 DOI: 10.1111/j.1600-6143.2010.03160.x]

47   **Rodrigues AC**, Frimm Cde C, Bacal F, Andreolli V, Tsutsui JM, Bocchi EA, Mathias W, Lage SG. Coronary flow reserve impairment predicts cardiac events in heart transplant patients with preserved left ventricular function. *Int J Cardiol* 2005; **103**: 201-206 [PMID: 16080981 DOI: 10.1016/j.ijcard.2004.12.017]

48   **Dandel M**, Hummel M, Müller J, Wellnhofer E, Meyer R, Solowjowa N, Ewert R, Hetzer R. Reliability of tissue Doppler wall motion monitoring after heart transplantation for replacement of invasive routine screenings by optimally timed cardiac biopsies and catheterizations. *Circulation* 2001; **104**: I184-I191 [PMID: 11568053]

49   **Dandel M**, Wellnhofer E, Hummel M, Meyer R, Lehmkuhl H, Hetzer R. Early detection of left ventricular dysfunction related to transplant coronary artery disease. *J Heart Lung Transplant* 2003; **22**: 1353-1364 [PMID: 14672750 DOI: 10.1016/S1053-2498(03)00055-X]

50   **Fyfe DA**, Ketchum D, Lewis R, Sabatier J, Kanter K, Mahle W, Vincent R. Tissue Doppler imaging detects severely abnormal myocardial velocities that identify children with pre-terminal cardiac graft failure after heart transplantation. *J Heart Lung Transplant* 2006; **25**: 510-517 [PMID: 16678028 DOI: 10.1016/j.healun.2005.11.453]

51   **Savage A**, Hlavacek A, Ringewald J, Shirali G. Evaluation of the myocardial performance index and tissue doppler imaging by comparison to near-simultaneous catheter measurements in pediatric cardiac transplant patients. *J Heart Lung Transplant* 2010; **29**: 853-858 [PMID: 20471861 DOI: 10.1016/j.healun.2010.03.014]

52   **Collings CA**, Pinto FJ, Valantine HA, Popylisen S, Puryear JV, Schnittger I. Exercise echocardiography in heart transplant recipients: a comparison with angiography and intracoronary ultrasonography. *J Heart Lung Transplant* 1994; **13**: 604-613 [PMID: 7947876]

53   **Chen MH**, Abernathey E, Lunze F, Colan SD, O'Neill S, Bergersen L, Geva T, Blume ED. Utility of exercise stress echocardiography in pediatric cardiac transplant recipients: a single-center experience. *J Heart Lung Transplant* 2012; **31**: 517-523 [PMID: 22301421 DOI: 10.1016/j.healun.2011.12.014]

54   **Hollenberg SM**, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Oberoi M, Johnson MR, Costanzo MR. Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. *Circulation* 2001; **104**: 3091-3096 [PMID: 11748106]

55   **Wijns W**, Kolh P, Danchin N, Di Mario C, Falk V, Folliguet T, Garg S, Huber K, James S, Knuuti J, Lopez-Sendon J, Marco J, Menicanti L, Ostojic M, Piepoli MF, Pirlet C, Pomar JL, Reifart N, Ribichini FL, Schalij MJ, Sergeant P, Serruys PW, Silber S, Sousa Uva M, Taggart D. Guidelines on myocardial revascularization. *Eur Heart J* 2010; **31**: 2501-2555 [PMID: 20802248 DOI: 10.1093/eurheartj/ehq277]

56   **Fearon WF**, Hirohata A, Nakamura M, Luikart H, Lee DP, Vagelos RH, Hunt SA, Valantine HA, Fitzgerald PJ, Yock PG, Yeung AC. Discordant changes in epicardial and microvascular

coronary physiology after cardiac transplantation: Physiologic Investigation for Transplant Arteriopathy II (PITA II) study. *J Heart Lung Transplant* 2006; **25**: 765-771 [PMID: 16818118 DOI: 10.1016/j.healun.2006.03.003]

57   **Lee CM**, Wu YW, Jui HY, Yen RF, Tzen KY, Chou NK, Wang SS. Intravascular ultrasound correlates with coronary flow reserve and predicts the survival in angiographically normal cardiac transplant recipients. *Cardiology* 2008; **109**: 93-98 [PMID: 17664873 DOI: 10.1159/000105548]

58   **Fearon WF**, Nakamura M, Lee DP, Rezaee M, Vagelos RH, Hunt SA, Fitzgerald PJ, Yock PG, Yeung AC. Simultaneous assessment of fractional and coronary flow reserves in cardiac transplant recipients: Physiologic Investigation for Transplant Arteriopathy (PITA Study). *Circulation* 2003; **108**: 1605-1610 [PMID: 12963639 DOI: 10.1161/01.CIR.0000091116.84926.6F]

59   **Suwaidi JA**, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000; **101**: 948-954 [PMID: 10704159]

60   **Schächinger V**, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; **101**: 1899-1906 [PMID: 10779454]

61   **Hollenberg SM**, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Bromet D, Satran A, Costanzo MR. Changes in coronary endothelial function predict progression of allograft vasculopathy after heart transplantation. *J Heart Lung Transplant* 2004; **23**: 265-271 [PMID: 15019634 DOI: 10.1016/S1053-2498(03)00150-5]

62   **Kübrich M**, Petrakopoulou P, Kofler S, Nickel T, Kaczmarek I, Meiser BM, Reichart B, von Scheidt W, Weis M. Impact of coronary endothelial dysfunction on adverse long-term outcome after heart transplantation. *Transplantation* 2008; **85**: 1580-1587 [PMID: 18551063 DOI: 10.1097/TP.0b013e318170b4cd]

63   **Schubert S**, Abdul-Khaliq H, Wellnhofer E, Hiemann NE, Ewert P, Lehmkuhl HB, Meyer R, Miera O, Peters B, Hetzer R, Berger F. Coronary flow reserve measurement detects transplant coronary artery disease in pediatric heart transplant patients. *J Heart Lung Transplant* 2008; **27**: 514-521 [PMID: 18442717 DOI: 10.1016/j.healun.2008.02.005]

64   **van Rossum AC**, Visser FC, Hofman MB, Galjee MA, Westerhof N, Valk J. Global left ventricular perfusion: noninvasive measurement with cine MR imaging and phase velocity mapping of coronary venous outflow. *Radiology* 1992; **182**: 685-691 [PMID: 1535881]

65   **Koskenvuo JW**, Sakuma H, Niemi P, Toikka JO, Knuuti J, Laine H, Komu M, Kormano M, Saraste M, Hartiala JJ. Global myocardial blood flow and global flow reserve measurements by MRI and PET are comparable. *J Magn Reson Imaging* 2001; **13**: 361-366 [PMID: 11241807]

66   **Schwitter J**, DeMarco T, Kneifel S, von Schulthess GK, Jörg MC, Arheden H, Rühm S, Stumpe K, Buck A, Parmley WW, Lüscher TF, Higgins CB. Magnetic resonance-based assessment of global coronary flow and flow reserve and its relation to left ventricular functional parameters: a comparison with positron emission tomography. *Circulation* 2000; **101**: 2696-2702 [PMID: 10851206]

67   **Ishida M**, Ito T, Shiraishi Y, Kitagawa K, Dohi K, Nakajima H, Ito M, Sakuma H. Impaired coronary flow reserve determined by MR measurement of coronary sinus flow predicts adverse outcome in patients with known or suspected coronary artery disease. *J Cardiovasc Magn Reson* 2013; **15** (Suppl 1): O91 [DOI: 10.1186/1532-429X-15-S1-O91]

68   **Kennedy K**, Dick A, Drangova M, Raval A, Mahoney C, Karlik S, Pflugfelder PW. Magnetic resonance measurements of coronary flow reserve in heart transplant recipients: an exploratory study of the relationship to coronary angiographic findings. *J Cardiovasc Magn Reson* 2007; **9**: 701-707 [PMID: 17578727 DOI: 10.1080/10976640701198980]

69   **Shaw LJ**, Berman DS, Maron DJ, Mancini GB, Hayes SW, Hartigan PM, Weintraub WS, O'Rourke RA, Dada M, Spertus JA, Chaitman BR, Friedman J, Slomka P, Heller GV, Germano G, Gosselin G, Berger P, Kostuk WJ, Schwartz RG, Knudtson M, Veledar E, Bates ER, McCallister B, Teo KK, Boden WE. Optimal medical therapy with or without percutaneous coronary intervention to reduce ischemic burden: results from the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial nuclear substudy. *Circulation* 2008; **117**: 1283-1291 [PMID: 18268144 DOI: 10.1161/CIRCULATIONAHA.107.743963]

70   **Klocke FJ**, Baird MG, Lorell BH, Bateman TM, Messer JV, Berman DS, O'Gara PT, Carabello BA, Russell RO, Cerqueira MD, St John Sutton MG, DeMaria AN, Udelson JE, Kennedy JW, Verani MS, Williams KA, Antman EM, Smith SC, Alpert JS, Gregoratos G, Anderson JL, Hiratzka LF, Faxon DP, Hunt SA, Fuster V, Jacobs AK, Gibbons RJ, Russell RO. ACC/AHA/ASNC guidelines for the clinical use of cardiac radionuclide imaging--executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASNC Committee to Revise the 1995 Guidelines for the Clinical Use of Cardiac Radionuclide Imaging). *Circulation* 2003; **108**: 1404-1418 [PMID: 12975245 DOI: 10.1161/01.CIR.0000080946.42225.4D]

71   **Camici PG**, Crea F. Coronary microvascular dysfunction. *N Engl J Med* 2007; **356**: 830-840 [PMID: 17314342 DOI: 10.1056/NEJMra061889]

72   **Wu YW**, Yen RF, Lee CM, Ho YL, Chou NK, Wang SS, Huang PJ. Diagnostic and prognostic value of dobutamine thallium-201 single-photon emission computed tomography after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 544-550 [PMID: 15896751 DOI: 10.1016/j.healun.2003.12.004]

73   **Carlsen J**, Toft JC, Mortensen SA, Arendrup H, Aldershvile J, Hesse B. Myocardial perfusion scintigraphy as a screening method for significant coronary artery stenosis in cardiac transplant recipients. *J Heart Lung Transplant* 2000; **19**: 873-878 [PMID: 11008077]

74   **Ciliberto GR**, Mangiavacchi M, Banfi F, Massa D, Danzi G, Cataldo G, Cipriani M, Piccalò G, Dabalà A, Gronda E. Coronary artery disease after heart transplantation: non-invasive evaluation with exercise thallium scintigraphy. *Eur Heart J* 1993; **14**: 226-229 [PMID: 8449198]

75   **Rodney RA**, Johnson LL, Blood DK, Barr ML. Myocardial perfusion scintigraphy in heart transplant recipients with and without allograft atherosclerosis: a comparison of thallium-201 and technetium 99m sestamibi. *J Heart Lung Transplant* 1994; **13**: 173-180 [PMID: 8031797]

76   **Howarth DM**, Forstrom LA, Samudrala V, Sinak LJ, McGregor CG, Rodeheffer RJ. Evaluation of 201Tl SPET myocardial perfusion imaging in the detection of coronary artery disease after orthotopic heart transplantation. *Nucl Med Commun* 1996; **17**: 105-113 [PMID: 8778633]

77   **Ambrosi P**, Habib G, Kreitman B, Metras D, Riberi A, Faugère G, Bernard P, Luccioni R. Thallium perfusion and myocardial hypertrophy in transplanted heart recipients with normal or near-normal coronary arteriograms. *Eur Heart J* 1994; **15**: 1119-1123 [PMID: 7988605]

78   **Elhendy A**, Sozzi FB, van Domburg RT, Vantrimpont P, Valkema R, Krenning EP, Roelandt JR, Maat LP, Balk AH. Accuracy of dobutamine tetrofosmin myocardial perfusion imaging for the noninvasive diagnosis of transplant coronary artery stenosis. *J Heart Lung Transplant* 2000; **19**: 360-366 [PMID: 10775817]

79   **Verhoeven PP**, Lee FA, Ramahi TM, Franco KL, Mendes de Leon C, Amatruda J, Gorham NA, Mattera JA, Wackers FJ. Prognostic value of noninvasive testing one year after orthotopic cardiac transplantation. *J Am Coll Cardiol* 1996; **28**: 183-189 [PMID: 8752812]

80   **Elhendy A**, van Domburg RT, Vantrimpont P, Poldermans D, Bax JJ, van Gelder T, Baan CC, Schinkel A, Roelandt JR, Balk AH. Prediction of mortality in heart transplant recipients

by stress technetium-99m tetrofosmin myocardial perfusion imaging. *Am J Cardiol* 2002; **89**: 964-968 [PMID: 11950436]

81   **Ciliberto GR**, Ruffini L, Mangiavacchi M, Parolini M, Sara R, Massa D, De Maria R, Gronda E, Vitali E, Parodi O. Resting echocardiography and quantitative dipyridamole technetium-99m sestamibi tomography in the identification of cardiac allograft vasculopathy and the prediction of long-term prognosis after heart transplantation. *Eur Heart J* 2001; **22**: 964-971 [PMID: 11428820 DOI: 10.1053/euhj.2000.2422]

82   **Hacker M**, Tausig A, Romüller B, Hoyer X, Klauss V, Stempfle U, Reichart B, Hahn K, Tiling R. Dobutamine myocardial scintigraphy for the prediction of cardiac events after heart transplantation. *Nucl Med Commun* 2005; **26**: 607-612 [PMID: 15942481]

83   **Manrique A**, Bernard M, Hitzel A, Bubenheim M, Tron C, Agostini D, Cribier A, Véra P, Bessou JP, Redonnet M. Diagnostic and prognostic value of myocardial perfusion gated SPECT in orthotopic heart transplant recipients. *J Nucl Cardiol* 2010; **17**: 197-206 [PMID: 20151240 DOI: 10.1007/s12350-009-9166-x]

84   **Lenihan DJ**, Rosenbaum AF, Burwinkel P, Tseng CY, Bhat G, Wagoner L, Walsh RA, Gerson MC. Prediction of human transplantation arteriopathy and coronary events with lung/heart count ratios during intravenous dipyridamole thallium-201 imaging. *Am Heart J* 1999; **137**: 942-948 [PMID: 10220645]

85   **Bengel FM**, Higuchi T, Javadi MS, Lautamäki R. Cardiac positron emission tomography. *J Am Coll Cardiol* 2009; **54**: 1-15 [PMID: 19555834 DOI: 10.1016/j.jacc.2009.02.065]

86   **Uren NG**, Melin JA, De Bruyne B, Wijns W, Baudhuin T, Camici PG. Relation between myocardial blood flow and the severity of coronary-artery stenosis. *N Engl J Med* 1994; **330**: 1782-1788 [PMID: 8190154 DOI: 10.1056/NEJM199406233302503]

87   **Allen-Auerbach M**, Schöder H, Johnson J, Kofoed K, Einhorn K, Phelps ME, Kobashigawa J, Czernin J. Relationship between coronary function by positron emission tomography and temporal changes in morphology by intravascular ultrasound (IVUS) in transplant recipients. *J Heart Lung Transplant* 1999; **18**: 211-219 [PMID: 10328146]

88   **Wu YW**, Chen YH, Wang SS, Jui HY, Yen RF, Tzen KY, Chen MF, Lee CM. PET assessment of myocardial perfusion reserve inversely correlates with intravascular ultrasound findings in angiographically normal cardiac transplant recipients. *J Nucl Med* 2010; **51**: 906-912 [PMID: 20484427 DOI: 10.2967/jnumed.109.073833]

89   **Preumont N**, Berkenboom G, Vachiery J, Jansens J, Antoine M, Wikler D, Damhaut P, Degré S, Lenaers A, Goldman S. Early alterations of myocardial blood flow reserve in heart transplant recipients with angiographically normal coronary arteries. *J Heart Lung Transplant* 2000; **19**: 538-545 [PMID: 10867333]

90   **Mollet NR**, Cademartiri F, van Mieghem CA, Runza G, McFadden EP, Baks T, Serruys PW, Krestin GP, de Feyter PJ. High-resolution spiral computed tomography coronary angiography in patients referred for diagnostic conventional coronary angiography. *Circulation* 2005; **112**: 2318-2323 [PMID: 16203914 DOI: 10.1161/CIRCULATIONAHA.105.533471]

91   **Leber AW**, Knez A, von Ziegler F, Becker A, Nikolaou K, Paul S, Wintersperger B, Reiser M, Becker CR, Steinbeck G, Boekstegers P. Quantification of obstructive and nonobstructive coronary lesions by 64-slice computed tomography: a comparative study with quantitative coronary angiography and intravascular ultrasound. *J Am Coll Cardiol* 2005; **46**: 147-154 [PMID: 15992649 DOI: 10.1016/j.jacc.2005.03.071]

92   **Hoffmann U**, Moselewski F, Nieman K, Jang IK, Ferencik M, Rahman AM, Cury RC, Abbara S, Joneidi-Jafari H, Achenbach S, Brady TJ. Noninvasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and stable lesions in stable

angina by multidetector computed tomography. *J Am Coll Cardiol* 2006; **47**: 1655-1662 [PMID: 16631006 DOI: 10.1016/j.jacc.2006.01.041]

93   **Sigurdsson G**, Carrascosa P, Yamani MH, Greenberg NL, Perrone S, Lev G, Desai MY, Garcia MJ. Detection of transplant coronary artery disease using multidetector computed tomography with adaptative multisegment reconstruction. *J Am Coll Cardiol* 2006; **48**: 772-778 [PMID: 16904548 DOI: 10.1016/j.jacc.2006.04.082]

94   **Iyengar S**, Feldman DS, Cooke GE, Leier CV, Raman SV. Detection of coronary artery disease in orthotopic heart transplant recipients with 64-detector row computed tomography angiography. *J Heart Lung Transplant* 2006; **25**: 1363-1366 [PMID: 17097502 DOI: 10.1016/j.healun.2006.06.018]

95   **Romeo G**, Houyel L, Angel CY, Brenot P, Riou JY, Paul JF. Coronary stenosis detection by 16-slice computed tomography in heart transplant patients: comparison with conventional angiography and impact on clinical management. *J Am Coll Cardiol* 2005; **45**: 1826-1831 [PMID: 15936614 DOI: 10.1016/j.jacc.2005.02.069]

96   **Schepis T**, Achenbach S, Weyand M, Raum P, Marwan M, Pflederer T, Daniel WG, Tandler R, Kondruweit M, Ropers D. Comparison of dual source computed tomography versus intravascular ultrasound for evaluation of coronary arteries at least one year after cardiac transplantation. *Am J Cardiol* 2009; **104**: 1351-1356 [PMID: 19892049 DOI: 10.1016/j.amjcard.2009.06.060]

97   **Bogot NR**, Durst R, Shaham D, Admon D. Cardiac CT of the transplanted heart: indications, technique, appearance, and complications. *Radiographics* 2007; **27**: 1297-1309 [PMID: 17848692 DOI: 10.1148/rg.275065107]

98   **Nunoda S**, Machida H, Sekikawa A, Shitakura K, Okajima K, Kubo Y, Ueno E, Otsuka K. Evaluation of cardiac allograft vasculopathy by multidetector computed tomography and whole-heart magnetic resonance coronary angiography. *Circ J* 2010; **74**: 946-953 [PMID: 20215697]

99   **Bae KT**, Hong C, Takahashi N, Gutierrez F, Sharkey AM, Hirsch R, Canter CE. Multi-detector row computed tomographic angiography in pediatric heart transplant recipients: Initial observations. *Transplantation* 2004; **77**: 599-602 [PMID: 15084942]

100   **Pinto TL**, Waksman R. Clinical applications of optical coherence tomography. *J Interv Cardiol* 2006; **19**: 566-573 [PMID: 17107374 DOI: 10.1111/j.1540-8183.2006.00201.x]

101   **Suter MJ**, Nadkarni SK, Weisz G, Tanaka A, Jaffer FA, Bouma BE, Tearney GJ. Intravascular optical imaging technology for investigating the coronary artery. *JACC Cardiovasc Imaging* 2011; **4**: 1022-1039 [PMID: 21920342 DOI: 10.1016/j.jcmg.2011.03.020]

102   **Tearney GJ**, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, Bouma B, Bruining N, Cho JM, Chowdhary S, Costa MA, de Silva R, Dijkstra J, Di Mario C, Dudek D, Falk E, Feldman MD, Fitzgerald P, Garcia-Garcia HM, Gonzalo N, Granada JF, Guagliumi G, Holm NR, Honda Y, Ikeno F, Kawasaki M, Kochman J, Koltowski L, Kubo T, Kume T, Kyono H, Lam CC, Lamouche G, Lee DP, Leon MB, Maehara A, Manfrini O, Mintz GS, Mizuno K, Morel MA, Nadkarni S, Okura H, Otake H, Pietrasik A, Prati F, Räber L, Radu MD, Rieber J, Riga M, Rollins A, Rosenberg M, Sirbu V, Serruys PW, Shimada K, Shinke T, Shite J, Siegel E, Sonoda S, Suter M, Takarada S, Tanaka A, Terashima M, Thim T, Uemura S, Ughi GJ, van Beusekom HM, van der Steen AF, van Es GA, van Soest G, Virmani R, Waxman S, Weissman NJ, Weisz G. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. *J Am Coll Cardiol* 2012; **59**: 1058-1072 [PMID: 22421299]

103   **Khandhar SJ**, Yamamoto H, Teuteberg JJ, Shullo MA, Bezerra

HG, Costa MA, Selzer F, Lee JS, Marroquin OC, McNamara DM, Mulukutla SR, Toma C. Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTCAV study). *J Heart Lung Transplant* 2013; **32**: 596-602 [PMID: 23499356 DOI: 10.1016/j.healun.2013.02.005]

104   **Garrido IP**, García-Lara J, Pinar E, Pastor-Pérez F, Sánchez-Mas J, Valdés-Chavarri M, Pascual-Figal DA. Optical coherence tomography and highly sensitivity troponin T for evaluating cardiac allograft vasculopathy. *Am J Cardiol* 2012; **110**: 655-661 [PMID: 22640973 DOI: 10.1016/j.amjcard.2012.04.047]

105   **Cassar A**, Matsuo Y, Herrmann J, Li J, Lennon RJ, Gulati R, Lerman LO, Kushwaha SS, Lerman A. Coronary atherosclerosis with vulnerable plaque and complicated lesions in transplant recipients: new insight into cardiac allograft vasculopathy by optical coherence tomography. *Eur Heart J* 2013; **34**: 2610-2617 [PMID: 23801824 DOI: 10.1093/eurheartj/eht236]

106   **Kim WY**, Danias PG, Stuber M, Flamm SD, Plein S, Nagel E, Langerak SE, Weber OM, Pedersen EM, Schmidt M, Botnar RM, Manning WJ. Coronary magnetic resonance angiography for the detection of coronary stenoses. *N Engl J Med* 2001; **345**: 1863-1869 [PMID: 11756576 DOI: 10.1056/NEJMoa010866]

107   **Botnar RM**, Kim WY, Börnert P, Stuber M, Spuentrup E, Manning WJ. 3D coronary vessel wall imaging utilizing a local inversion technique with spiral image acquisition. *Magn Reson Med* 2001; **46**: 848-854 [PMID: 11675634]

108   **Botnar RM**, Stuber M, Kissinger KV, Kim WY, Spuentrup E, Manning WJ. Noninvasive coronary vessel wall and plaque imaging with magnetic resonance imaging. *Circulation* 2000; **102**: 2582-2587 [PMID: 11085960]

109   **Uribe S**, Hussain T, Valverde I, Tejos C, Irarrazaval P, Fava M, Beerbaum P, Botnar RM, Razavi R, Schaeffter T, Greil GF. Congenital heart disease in children: coronary MR angiography during systole and diastole with dual cardiac phase whole-heart imaging. *Radiology* 2011; **260**: 232-240 [PMID: 21493790 DOI: 10.1148/radiol.11101659]

110   **Tangcharoen T**, Bell A, Hegde S, Hussain T, Beerbaum P, Schaeffter T, Razavi R, Botnar RM, Greil GF. Detection of coronary artery anomalies in infants and young children with congenital heart disease by using MR imaging. *Radiology* 2011; **259**: 240-247 [PMID: 21325034 DOI: 10.1148/radiol.10100828]

111   **Greil GF**, Stuber M, Botnar RM, Kissinger KV, Geva T, Newburger JW, Manning WJ, Powell AJ. Coronary magnetic resonance angiography in adolescents and young adults with kawasaki disease. *Circulation* 2002; **105**: 908-911 [PMID: 11864916]

112   **Schwitter J**, Wacker CM, van Rossum AC, Lombardi M, Al-Saadi N, Ahlstrom H, Dill T, Larsson HB, Flamm SD, Marquardt M, Johansson L. MR-IMPACT: comparison of perfusion-cardiac magnetic resonance with single-photon emission computed tomography for the detection of coronary artery disease in a multicentre, multivendor, randomized trial. *Eur Heart J* 2008; **29**: 480-489 [PMID: 18208849 DOI: 10.1093/eurheartj/ehm617]

113   **Jahnke C**, Nagel E, Gebker R, Kokocinski T, Kelle S, Manka R, Fleck E, Paetsch I. Prognostic value of cardiac magnetic resonance stress tests: adenosine stress perfusion and dobutamine stress wall motion imaging. *Circulation* 2007; **115**: 1769-1776 [PMID: 17353441 DOI: 10.1161/CIRCULATIONAHA.106.652016]

114   **Muehling OM**, Wilke NM, Panse P, Jerosch-Herold M, Wilson BV, Wilson RF, Miller LW. Reduced myocardial perfusion reserve and transmural perfusion gradient in heart transplant arteriopathy assessed by magnetic resonance imaging. *J Am Coll Cardiol* 2003; **42**: 1054-1060 [PMID: 13678930]

115   **Steen H**, Merten C, Refle S, Klingenberg R, Dengler T, Giannitsis E, Katus HA. Prevalence of different gadolinium enhancement patterns in patients after heart transplantation.

J Am Coll Cardiol 2008; **52**: 1160-1167 [PMID: 18804744 DOI: 10.1016/j.jacc.2008.05.059]

116   **Braggion-Santos MF**, Andre F, Lossnitzer D, Hofmann E, Simpfendörfer J, Dösch A, Katus HA, Steen H. Prevalence of different forms of infarct-atypical late gadolinium enhancement in patients early and late after heart transplantation. *Clin Res Cardiol* 2014; **103**: 57-63 [PMID: 24122145 DOI: 10.1007/s00392-013-0623-9]

117   **Hussain T**, Burch M, Fenton MJ, Whitmore PM, Rees P, Elliott M, Aurora P. Positive pretransplantation cytomegalovirus serology is a risk factor for cardiac allograft vasculopathy in children. *Circulation* 2007; **115**: 1798-1805 [PMID: 17353448 DOI: 10.1161/CIRCULATIONAHA.106.627570]

118   **Miller CA**, Sarma J, Naish JH, Yonan N, Williams SG, Shaw SM, Clark D, Pearce K, Stout M, Potluri R, Borg A, Coutts G, Chowdhary S, McCann GP, Parker GJ, Ray SG, Schmitt M. Multiparametric cardiovascular magnetic resonance assessment of cardiac allograft vasculopathy. *J Am Coll Cardiol* 2014; **63**: 799-808 [PMID: 24355800 DOI: 10.1016/j.jacc.2013.07.119]

119   **Blum A**, Shamburek R. The pleiotropic effects of statins on endothelial function, vascular inflammation, immunomodulation and thrombogenesis. *Atherosclerosis* 2009; **203**: 325-330 [PMID: 18834985 DOI: 10.1016/j.atherosclerosis.2008.08.022]

120   **Anderson TJ**, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995; **26**: 1235-1241 [PMID: 7594037]

121   **Treasure CB**, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med* 1995; **332**: 481-487 [PMID: 7830728 DOI: 10.1056/NEJM199502233320801]

122   **Wenke K**, Meiser B, Thiery J, Nagel D, von Scheidt W, Krobot K, Steinbeck G, Seidel D, Reichart B. Simvastatin initiated early after heart transplantation: 8-year prospective experience. *Circulation* 2003; **107**: 93-97 [PMID: 12515749]

123   **Mehra MR**, Uber PA, Vivekananthan K, Solis S, Scott RL, Park MH, Milani RV, Lavie CJ. Comparative beneficial effects of simvastatin and pravastatin on cardiac allograft rejection and survival. *J Am Coll Cardiol* 2002; **40**: 1609-1614 [PMID: 12427413]

124   **Mehra MR**, Raval NY. Metaanalysis of statins and survival in de novo cardiac transplantation. *Transplant Proc* 2004; **36**: 1539-1541 [PMID: 15251380 DOI: 10.1016/j.transproceed.2004.05.036]

125   **Costanzo MR**, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010; **29**: 914-956 [PMID: 20643330 DOI: 10.1016/j.healun.2010.05.034]

126   **Valentine VG**, Weill D, Gupta MR, Raper B, Laplace SG, Lombard GA, Bonvillain RW, Taylor DE, Dhillon GS. Ganciclovir for cytomegalovirus: a call for indefinite prophylaxis in lung transplantation. *J Heart Lung Transplant* 2008; **27**: 875-881 [PMID: 18656801 DOI: 10.1016/j.healun.2008.05.009]

127   **Fearon WF**, Potena L, Hirohata A, Sakurai R, Yamasaki M, Luikart H, Lee J, Vana ML, Cooke JP, Mocarski ES, Yeung AC, Valantine HA. Changes in coronary arterial dimensions early

after cardiac transplantation. *Transplantation* 2007; **83**: 700-705 [PMID: 17414701 DOI: 10.1097/01.tp.0000256335.84363.9b]

128 **Mehra MR**, Ventura HO, Smart FW, Collins TJ, Ramee SR, Stapleton DD. An intravascular ultrasound study of the influence of angiotensin-converting enzyme inhibitors and calcium entry blockers on the development of cardiac allograft vasculopathy. *Am J Cardiol* 1995; **75**: 853-854 [PMID: 7717300]

129 **Schroeder JS**, Gao SZ, Alderman EL, Hunt SA, Johnstone I, Boothroyd DB, Wiederhold V, Stinson EB. A preliminary study of diltiazem in the prevention of coronary artery disease in heart-transplant recipients. *N Engl J Med* 1993; **328**: 164-170 [PMID: 8417382 DOI: 10.1056/NEJM199301213280303]

130 **Erinc K**, Yamani MH, Starling RC, Crowe T, Hobbs R, Bott-Silverman C, Rincon G, Young JB, Feng J, Cook DJ, Smedira N, Tuzcu EM. The effect of combined Angiotensin-converting enzyme inhibition and calcium antagonism on allograft coronary vasculopathy validated by intravascular ultrasound. *J Heart Lung Transplant* 2005; **24**: 1033-1038 [PMID: 16102438 DOI: 10.1016/j.healun.2004.06.005]

131 **Meiser BM**, Groetzner J, Kaczmarek I, Landwehr P, Müller M, Jung S, Uberfuhr P, Fraunberger P, Stempfle HU, Weis M, Reichart B. Tacrolimus or cyclosporine: which is the better partner for mycophenolate mofetil in heart transplant recipients? *Transplantation* 2004; **78**: 591-598 [PMID: 15446320]

132 **Grimm M**, Rinaldi M, Yonan NA, Arpesella G, Arizón Del Prado JM, Pulpón LA, Villemot JP, Frigerio M, Rodriguez Lambert JL, Crespo-Leiro MG, Almenar L, Duveau D, Ordonez-Fernandez A, Gandjbakhch J, Maccherini M, Laufer G. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients--a large European trial. *Am J Transplant* 2006; **6**: 1387-1397 [PMID: 16686762 DOI: 10.1111/j.1600-6143.2006.01300.x]

133 **Kobashigawa JA**, Miller LW, Russell SD, Ewald GA, Zucker MJ, Goldberg LR, Eisen HJ, Salm K, Tolzman D, Gao J, Fitzsimmons W, First R. Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant* 2006; **6**: 1377-1386 [PMID: 16686761 DOI: 10.1111/j.1600-6143.2006.01290.x]

134 **Segovia J**, Gómez-Bueno M, Alonso-Pulpón L. Treatment of allograft vasculopathy in heart transplantation. *Expert Opin Pharmacother* 2006; **7**: 2369-2383 [PMID: 17109612 DOI: 10.1517/14656566.7.17.2369]

135 **Petrakopoulou P**, Anthopoulou L, Muscholl M, Klauss V, von Scheidt W, Uberfuhr P, Meiser BM, Reichart B, Weis M. Coronary endothelial vasomotor function and vascular remodeling in heart transplant recipients randomized for tacrolimus or cyclosporine immunosuppression. *J Am Coll Cardiol* 2006; **47**: 1622-1629 [PMID: 16631000 DOI: 10.1016/j.jacc.2005.10.073]

136 **Eisen HJ**, Kobashigawa J, Keogh A, Bourge R, Renlund D, Mentzer R, Alderman E, Valantine H, Dureau G, Mancini D, Mamelok R, Gordon R, Wang W, Mehra M, Constanzo MR, Hummel M, Johnson J. Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. *J Heart Lung Transplant* 2005; **24**: 517-525 [PMID: 15896747 DOI: 10.1016/j.healun.2005.02.002]

137 **Kobashigawa JA**, Tobis JM, Mentzer RM, Valantine HA, Bourge RC, Mehra MR, Smart FW, Miller LW, Tanaka K, Li H, Gjertson DW, Gordon RD. Mycophenolate mofetil reduces intimal thickness by intravascular ultrasound after heart transplant: reanalysis of the multicenter trial. *Am J Transplant* 2006; **6**: 993-997 [PMID: 16611335 DOI: 10.1111/j.1600-6143.2006.01297.x]

138 **Kaczmarek I**, Ertl B, Schmauss D, Sadoni S, Knez A, Daebritz S, Meiser B, Reichart B. Preventing cardiac allograft vasculopathy: long-term beneficial effects of mycophenolate mofetil. *J Heart Lung Transplant* 2006; **25**: 550-556 [PMID: 16678034 DOI: 10.1016/j.healun.2006.01.003]

139 **Sehgal SN**. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc* 2003; **35**: 7S-14S [PMID: 12742462]

140 **Raichlin E**, Kushwaha SS. Proliferation signal inhibitors and cardiac allograft vasculopathy. *Curr Opin Organ Transplant* 2008; **13**: 543-550 [PMID: 19060540 DOI: 10.1097/MOT.0b013e32830fdf70]

141 **Eisen HJ**, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sørensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med* 2003; **349**: 847-858 [PMID: 12944570 DOI: 10.1056/NEJMoa022171]

142 **Viganò M**, Tuzcu M, Benza R, Boissonnat P, Haverich A, Hill J, Laufer G, Love R, Parameshwar J, Pulpón LA, Renlund D, Abeywickrama K, Cretin N, Starling RC, Eisen HJ. Prevention of acute rejection and allograft vasculopathy by everolimus in cardiac transplants recipients: a 24-month analysis. *J Heart Lung Transplant* 2007; **26**: 584-592 [PMID: 17543781 DOI: 10.1016/j.healun.2007.03.005]

143 **Eisen H**. Long-term cardiovascular risk in transplantation--insights from the use of everolimus in heart transplantation. *Nephrol Dial Transplant* 2006; **21** Suppl 3: iii9-ii13 [PMID: 16815858 DOI: 10.1093/ndt/gfl295]

144 **Andreassen AK,** Andersson B, Gustafsson F, Eiskjaer H, Rådegran G, Gude E, Jansson K, Solbu D, Sigurdardottir V, Arora S, Dellgren G, Gullestad L. Everolimus Initiation and Early Calcineurin Inhibitor Withdrawal in Heart Transplant Recipients: A Randomized Trial. *Am J Transplant* 2014; **14**: 1828-1838 [PMID: 25041227 DOI: 10.1111/ajt.12809]

145 **Mancini D**, Pinney S, Burkhoff D, LaManca J, Itescu S, Burke E, Edwards N, Oz M, Marks AR. Use of rapamycin slows progression of cardiac transplantation vasculopathy. *Circulation* 2003; **108**: 48-53 [PMID: 12742978 DOI: 10.1161/01.CIR.0000070421.38604.2B]

146 **Keogh A**, Richardson M, Ruygrok P, Spratt P, Galbraith A, O'Driscoll G, Macdonald P, Esmore D, Muller D, Faddy S. Sirolimus in de novo heart transplant recipients reduces acute rejection and prevents coronary artery disease at 2 years: a randomized clinical trial. *Circulation* 2004; **110**: 2694-2700 [PMID: 15262845 DOI: 10.1161/01.CIR.0000136812.90177.94]

147 **Lyster H**, Panicker G, Leaver N, Banner NR. Initial experience with sirolimus and mycophenolate mofetil for renal rescue from cyclosporine nephrotoxicity after heart transplantation. *Transplant Proc* 2004; **36**: 3167-3170 [PMID: 15686720 DOI: 10.1016/j.transproceed.2004.10.062]

148 **Rothenburger M**, Teerling E, Bruch C, Lehmkuhl H, Suwelack B, Bara C, Wichter T, Hinder F, Schmid C, Stypmann J. Calcineurin inhibitor-free immunosuppression using everolimus (Certican) in maintenance heart transplant recipients: 6 months' follow-up. *J Heart Lung Transplant* 2007; **26**: 250-257 [PMID: 17346627 DOI: 10.1016/j.healun.2007.01.017]

149 **Groetzner J**, Kaczmarek I, Meiser B, Müller M, Daebritz S, Reichart B. Sirolimus and mycophenolate mofetil as calcineurin inhibitor-free immunosuppression in a cardiac transplant patient with chronic renal failure. *J Heart Lung Transplant* 2004; **23**: 770-773 [PMID: 15366440]

150 **Groetzner J**, Kaczmarek I, Landwehr P, Mueller M, Daebritz S, Lamm P, Meiser B, Reichart B. Renal recovery after conversion to a calcineurin inhibitor-free immunosuppression in late cardiac transplant recipients. *Eur J Cardiothorac Surg* 2004; **25**: 333-341 [PMID: 15019657 DOI: 10.1016/j.ejcts.2003.11.030]

151 **Hunt J**, Lerman M, Magee MJ, Dewey TM, Herbert M, Mack MJ. Improvement of renal dysfunction by conversion from calcineurin inhibitors to sirolimus after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 1863-1867 [PMID: 16297793 DOI: 10.1016/j.healun.2005.02.018]

152 **Raichlin E**, Bae JH, Khalpey Z, Edwards BS, Kremers WK, Clavell AL, Rodeheffer RJ, Frantz RP, Rihal C, Lerman A, Kushwaha SS. Conversion to sirolimus as primary immunosuppression

attenuates the progression of allograft vasculopathy after cardiac transplantation. *Circulation* 2007; **116**: 2726-2733 [PMID: 18025531 DOI: 10.1161/CIRCULATIONAHA.107.692996]

153 **Topilsky Y**, Hasin T, Raichlin E, Boilson BA, Schirger JA, Pereira NL, Edwards BS, Clavell AL, Rodeheffer RJ, Frantz RP, Maltais S, Park SJ, Daly RC, Lerman A, Kushwaha SS. Sirolimus as primary immunosuppression attenuates allograft vasculopathy with improved late survival and decreased cardiac events after cardiac transplantation. *Circulation* 2012; **125**: 708-720 [PMID: 22207715 DOI: 10.1161/CIRCULATIONAHA.111.040360]

154 **Arora S**, Erikstad I, Ueland T, Sigurdardottir V, Ekmehag B, Jansson K, Eiskjaer H, Bøtker HE, Mortensen SA, Saunamaki K, Gude E, Ragnarsson A, Solbu D, Aukrust P, Gullestad L. Virtual histology assessment of cardiac allograft vasculopathy following introduction of everolimus--results of a multicenter trial. *Am J Transplant* 2012; **12**: 2700-2709 [PMID: 22958738 DOI: 10.1111/j.1600-6143.2012.04234.x]

155 **Zuckermann A**, Manito N, Epailly E, Fiane A, Bara C, Delgado JF, Lehmkuhl H, Ross H, Eisen H, Chapman J, Valantine H. Multidisciplinary insights on clinical guidance for the use of proliferation signal inhibitors in heart transplantation. *J Heart Lung Transplant* 2008; **27**: 141-149 [PMID: 18267219 DOI: 10.1016/j.healun.2007.08.014]

156 **Demopoulos L**, Polinsky M, Steele G, Mines D, Blum M, Caulfield M, Adamkovic A, Liu Q, Harler MB, Hahn C, Singh A. Reduced risk of cytomegalovirus infection in solid organ transplant recipients treated with sirolimus: a pooled analysis of clinical trials. *Transplant Proc* 2008; **40**: 1407-1410 [PMID: 18589118 DOI: 10.1016/j.transproceed.2008.03.084]

157 **Hill JA**, Hummel M, Starling RC, Kobashigawa JA, Perrone SV, Arizón JM, Simonsen S, Abeywickrama KH, Bara C. A lower incidence of cytomegalovirus infection in de novo heart transplant recipients randomized to everolimus. *Transplantation* 2007; **84**: 1436-1442 [PMID: 18091519 DOI: 10.1097/01.tp.0000290686.68910.bd]

158 **Nepomuceno RR**, Balatoni CE, Natkunam Y, Snow AL, Krams SM, Martinez OM. Rapamycin inhibits the interleukin 10 signal transduction pathway and the growth of Epstein Barr virus B-cell lymphomas. *Cancer Res* 2003; **63**: 4472-4480 [PMID: 12907620]

159 **Benza RL**, Zoghbi GJ, Tallaj J, Brown R, Kirklin JK, Hubbard M, Rayburn B, Foley B, McGiffin DC, Pinderski LJ, Misra V, Bourge RC. Palliation of allograft vasculopathy with transluminal angioplasty: a decade of experience. *J Am Coll Cardiol* 2004; **43**: 1973-1981 [PMID: 15172400 DOI: 10.1016/j.jacc.2004.02.045]

160 **Simpson L**, Lee EK, Hott BJ, Vega DJ, Book WM. Long-term results of angioplasty vs stenting in cardiac transplant recipients with allograft vasculopathy. *J Heart Lung Transplant* 2005; **24**:

1211-1217 [PMID: 16143235 DOI: 10.1016/j.healun.2004.10.005]

161 **Jonas M**, Fang JC, Wang JC, Giri S, Elian D, Har-Zahav Y, Ly H, Seifert PA, Popma JJ, Rogers C. In-stent restenosis and remote coronary lesion progression are coupled in cardiac transplant vasculopathy but not in native coronary artery disease. *J Am Coll Cardiol* 2006; **48**: 453-461 [PMID: 16875968 DOI: 10.1016/j.jacc.2006.01.081]

162 **Halle AA**, DiSciascio G, Massin EK, Wilson RF, Johnson MR, Sullivan HJ, Bourge RC, Kleiman NS, Miller LW, Aversano TR. Coronary angioplasty, atherectomy and bypass surgery in cardiac transplant recipients. *J Am Coll Cardiol* 1995; **26**: 120-128 [PMID: 7797740]

163 **Schnetzler B**, Drobinski G, Dorent R, Camproux AC, Ghossoub J, Thomas D, Gandjbakhch I. The role of percutaneous transluminal coronary angioplasty in heart transplant recipients. *J Heart Lung Transplant* 2000; **19**: 557-565 [PMID: 10867336]

164 **Doshi R**, Rogers J, Kern MJ, Hauptman PJ. Effectiveness of percutaneous coronary intervention in cardiac allograft vasculopathy. *Am J Cardiol* 2004; **93**: 90-92 [PMID: 14697475]

165 **Fernández-Yáñez J**, Palomo J, Elízaga J, Moreno J, Sánchez A, Almendral J. Results of percutaneous revascularization in cardiac allograft vascular disease. *Transplant Proc* 2003; **35**: 1996-1998 [PMID: 12962874]

166 **Bader FM**, Kfoury AG, Gilbert EM, Barry WH, Humayun N, Hagan ME, Thomas H, Renlund D. Percutaneous coronary interventions with stents in cardiac transplant recipients. *J Heart Lung Transplant* 2006; **25**: 298-301 [PMID: 16507423 DOI: 10.1016/j.healun.2005.09.016]

167 **Musci M**, Loebe M, Wellnhofer E, Meyer R, Pasic M, Hummel M, Bocksch W, Grauhan O, Weng Y, Hetzer R. Coronary angioplasty, bypass surgery, and retransplantation in cardiac transplant patients with graft coronary disease. *Thorac Cardiovasc Surg* 1998; **46**: 268-274 [PMID: 9885117 DOI: 10.1055/s-2007-1010237]

168 **Patel VS**, Radovancevic B, Springer W, Frazier OH, Massin E, Benrey J, Kadipasaoglu K, Cooley DA. Revascularization procedures in patients with transplant coronary artery disease. *Eur J Cardiothorac Surg* 1997; **11**: 895-901 [PMID: 9196306]

169 **Topkara VK**, Dang NC, John R, Cheema FH, Barbato R, Cavallo M, Liu JF, Liang LM, Liberman EA, Argenziano M, Oz MC, Naka Y. A decade experience of cardiac retransplantation in adult recipients. *J Heart Lung Transplant* 2005; **24**: 1745-1750 [PMID: 16297775 DOI: 10.1016/j.healun.2005.02.015]

170 **Srivastava R**, Keck BM, Bennett LE, Hosenpud JD. The results of cardiac retransplantation: an analysis of the Joint International Society for Heart and Lung Transplantation/United Network for Organ Sharing Thoracic Registry. *Transplantation* 2000; **70**: 606-612 [PMID: 10972218]

MINIREVIEWS

Role of liver transplantation in the management of hepatoblastoma in the pediatric population

Saira Khaderi, Jacfranz Guiteau, Ronald T Cotton, Christine O'Mahony, Abbas Rana, John A Goss

Saira Khaderi, Jacfranz Guiteau, Ronald T Cotton, Christine O'Mahony, Abbas Rana, John A Goss, Division of Abdominal Transplantation, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX 77030, United States
Author contributions: Khaderi S and Guiteau J wrote the manuscript; Cotton RT and Rana A performed the statistical analysis and assisting in writing the manuscript; O'Mahony C and Goss JA provided expertise regarding pediatric transplantation and assisted in writing the manuscript.
Correspondence to: Saira Khaderi, MD, MPH, Assistant Professor of Surgery, Division of Abdominal Transplantation, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, 6620 Main Street, Suite 1425, Houston, TX 77030, United States. khaderi@bcm.edu
Telephone: +1-832-3551400   Fax: +1-713-6102479
Received: August 12, 2014   Revised: October 21, 2014
Accepted: October 28, 2014
Published online: December 24, 2014

Abstract

Hepatoblastoma (HB) is the most common primary liver tumor in children and accounts for two-thirds of all malignant liver neoplasms in the pediatric population. For patients with advanced HB (unresectable or unresponsive to chemotherapy), combined treatment with chemotherapy and liver transplantation is an excellent option. The etiology of HB is mostly obscure because of its extreme rarity although some inherited syndromes and very low birth weight have been associated with it. The prognosis for children with HB has significantly improved in the past three decades thanks to advancements in chemotherapy, surgical resection and postoperative care. In 2002 a surgical staging system called pretreatment extent of disease (PRETEXT) was designed to allow a universal, multidisciplinary approach to patients with HB. Between one-third to two-thirds of patients initially present with unresectable tumors or distant metastases, but up to 85% of these tumors become operable after neoadjuvant chemotherapy. Patients with PRETEXT categories 1, 2, and some 3 are referred for neoadjuvant chemotherapy followed by surgical resection with the goal of complete tumor removal. Classic treatments regimens include a combination of cisplatin, fluorouracil, and vincristine or cisplatin and doxorubicin. Liver transplantation is the only treatment option for unresectable HB. In 2010 the pediatric end-stage liver disease, a pediatric-specific scoring system that determines a patient's ranking on the liver transplant list, began to award additional "exception" points for patients with HB. We analyzed the Standard Transplant Analysis and Research dataset to assess the impact of changes in exception point criteria for HB on outcomes after liver transplantation at Texas Children's Hospital in Houston, Texas. We found that patients who were listed for transplantation with current HB exception criteria experienced a shorter waitlist time but survival was similar between the two eras.

Core tip: Hepatoblastoma (HB) is the most common primary liver tumor in children. Between one-third to two-thirds of patients present with unresectable tumors or distant metastases, but up to 85% of these tumors become operable after neoadjuvant chemotherapy. Liver transplantation is the only treatment option for unresectable HB. In 2010 the pediatric end-stage liver disease scoring system began to award additional "exception" points for patients with HB. We analyzed the Standard Transplant Analysis and Research dataset and found that patients who were listed for transplantation with current HB exception criteria experienced a shorter waitlist time but survival was similar between the two eras.

Khaderi S, Guiteau J, Cotton RT, O'Mahony C, Rana A, Goss JA. Role of liver transplantation in the management of hepatoblastoma in the pediatric population. *World J Transplant* 2014; 4(4): 294-298 Available from: URL: http://www.wjgnet.com/2220-3230/full/v4/

i4/294.htm DOI: http://dx.doi.org/10.5500/wjt.v4.i4.294

INTRODUCTION

Hepatoblastoma (HB) is the most common primary liver tumor in children and accounts for two-thirds of all malignant liver neoplasms in the pediatric population[1]. Standard treatment of HB includes neoadjuvant chemotherapy and surgical resection followed by adjuvant chemotherapy. For patients with advanced HB (unresectable or unresponsive to chemotherapy), combined treatment with chemotherapy and liver transplantation is an excellent option[2]. This article briefly reviews the epidemiology and treatment of HB in the pediatric population with an emphasis on the role of orthotopic liver transplantation (OLT).

EPIDEMIOLOGY

The etiology of HB is mostly obscure because of its extreme rarity. The rate of HB in the United States Surveillance, Epidemiology, and End Results (SEER) from 2002-2008 was 10.5 cases per million in children less than one year of age and 5.2 cases per million in children 1 through 4 years of age[3]. It is assumed the tumor originates *in utero* for two reasons. Histologically HB cells resemble embryonal liver cells and the incidence is highest at birth suggesting the process is initiated during gestation[4].

Some inherited syndromes have been associated with HB. Incidence of HB among children with Familial Adenomatous Polyposis was found to be 847 times the incidence in the SEER population[5]. Those with the Beckwith-Wiedemann overgrowth syndrome had an incidence 2280 times that of the United States population of the same age[6]. Although these inherited conditions raise the risk of HB, they account for only a few cases overall.

Very low birth weight (< 1500 g) increases the risk of HB in children 20-fold and moderate low birth weight (1500-2500 g) doubles the risk[7]. The association of low birth weight and HB has two explanations. HB may be initiated or promoted by iatrogenic hazards in the neonatal intensive care units[8] in combination with decreased antioxidant defense mechanisms of pre-term infants[9]. Alternatively, HB and very low birth weight may share a common mechanism and the increase in survival of these patients has made the association more apparent.

TREATMENT

The prognosis for children with HB has significantly improved in the past three decades thanks to advancements in chemotherapy, surgical resection and postoperative care[10]. Prior to the discovery of effective chemotherapy, cure was limited to completely resectable tumors and overall survival was dismal[10].

Early experiences with successful cure were sporadic at best and were limited to lesions that could be completely resected. In 2002 a staging system called the PRETreatment EXTent of disease (PRETEXT) was designed to allow a universal, multidisciplinary approach to patients with HB (Figure 1). The main aim of PRETEXT grouping was to identify patients in whom complete tumor resection was possible with a partial hepatectomy. Physicians placed patients in one of four PRETEXT categories based on the extent of their tumor on imaging. The liver is divided into four sectors in the PRETEXT system - anterior and posterior on the right and a medial and lateral sector on the left. Four groups were identified based on tumor extension: PRETEXT I, tumor only in one sector; PRETEXT II, tumor involves two sectors; PRETEXT III, tumor involves three sectors or two non-adjoining sectors; and PRETEXT 4, tumor involves all four sectors[11]. These categories are further characterized by describing extrahepatic spread: V for involvement of the hepatic veins and/or inferior vena cava, P for involvement of the portal vein, E for extrahepatic tumor extension, and M for distant metastases[11].

Between one-third to two-thirds of patients initially present with unresectable tumors or distant metastases, but up to 85% of these tumors become operable after neoadjuvant chemotherapy[12]. Preoperative chemotherapy has many advantages. It is responsible for making tumors smaller and more demarcated from the surrounding liver. Most surgeons agree that operating on tumors that shrink with chemotherapy is easier because the tumor is more defined and less prone to bleeding. It also exposes metastases (both visible and micrometastases) to chemotherapy earlier. In one trial, up to 52% of patients with initial lung metastases achieved complete remission with chemotherapy alone[13]. Classic treatment regimens include a combination of cisplatin, fluorouracil, and vincristine or cisplatin and doxorubicin. Although an effective agent, patients treated with doxorubicin can have a higher incidence of treatment complications and toxic death-especially from heart failure[14]. More recent studies have shown the effectiveness of single-agent cisplatin treatment in both standard and high-risk patients with HB[15,16] decreasing the likelihood of chemotherapy-induced toxicity.

Patients with PRETEXT categories 1, 2, and some 3 are referred for neoadjuvant chemotherapy followed by surgical resection with the goal of complete tumor removal. Current chemotherapy at our institution consists of cisplatin, 5-fluorouracil, and vincristine or vincristine and doxorubicin. Patients will undergo four rounds of chemotherapy prior to resection and two rounds after resection. Disease-free survival following partial liver resection under these circumstances has been reported to be greater than 70%[2]. It has been argued that tumors with favorable prognostic factors, such as pure fetal histology and low mitotic rate, may not require toxic chemotherapy and should be treated with surgical resection only[17]. However, the treatment regimen at our institution closely follows the precedent set forth in European studies which emphasize the use of neoadjuvant chemotherapy in all HB patients because of the high frequency of HB chemosensitivity[11,18].
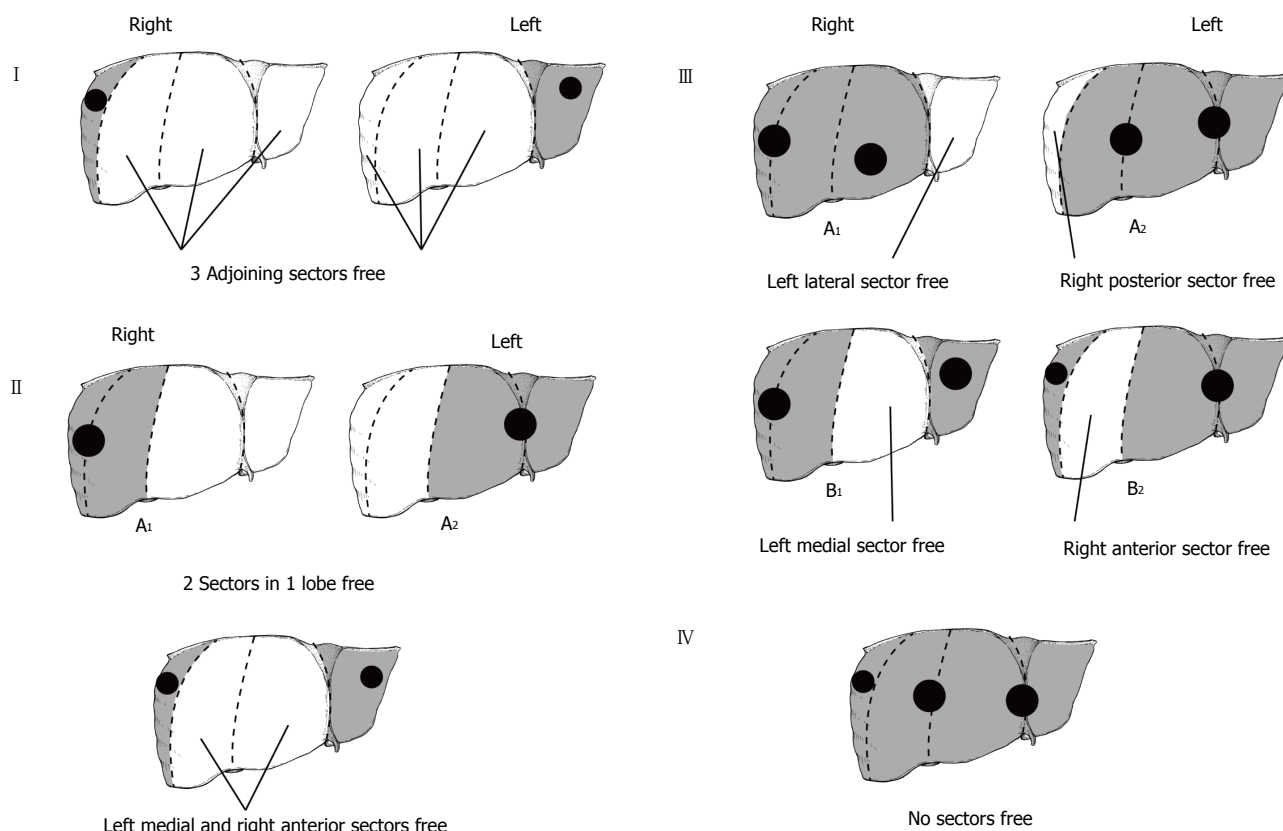
Figure 1 Pretreatment (pretreatment extent of disease) grouping system. Printed with permission from Baylor College of Medicine.

Liver transplant

Liver transplantation is the only treatment option for unresectable HB. Transplant should be considered in the following cases: multifocal disease (PRETEXT Ⅳ), PRETEXT Ⅲ with the tumor in close proximity to major vessels, and tumor extension into major vessels. Patients that fall into these categories at our institution are listed for OLT immediately after the diagnosis of HB is confirmed and undergo chemotherapy while they await transplantation. Overall patient survival at 6 years has been reported to be over 80% making OLT the preferred treatment modality in this group[19]. Patients with intrahepatic recurrence or residual tumor after resection are rarely candidates for transplant because of poor outcomes[19].

There are few contraindications to OLT for unresectable HB. Patients with persistent pulmonary metastases despite neoadjuvant therapy and those with viable extrahepatic tumor not amenable to resection are not candidates for OLT. Patients that present with lung metastases are candidates for OLT if their lung metastases resolve with chemotherapy or with resection. Those with extrahepatic disease that remains viable after full chemotherapy and not amenable to surgical resection represent the only absolute contraindication to OLT in patients with HB[19].

Organ allocation rules for children with HB have changed over the past decade. The pediatric end-stage liver disease (PELD) is a pediatric-specific scoring system that was adopted in 2002 to help determine a patient's ranking on the liver transplant list. The effect of the system has been to decrease the rate of death and removal from the transplant list and increase the percentage of children who receive a deceased donor organ. The score is based on total bilirubin, coagulopathy, serum albumin, age < 1 year and growth failure, but additional "exception" points may be awarded for risk factors not represented by the PELD equation. For example, patients with unresectable HB are listed with a PELD score of 30 for 30 d and are increased to status 1B if they have not been transplanted.

We analyzed the Standard Transplant Analysis and Research dataset to assess the impact of changes in exception point criteria for HB on outcomes after liver transplantation at Texas Children's Hospital in Houston, Texas. Patients who underwent orthotopic liver transplant in our center from 1987-2014 with recipient diagnosis of either HB, cirrhosis post-resection of HB, or for whom a MELD exception was granted for non-metastatic HB were selected for analysis. Patients were grouped based on date of initial listing for transplantation. The 1987-2009 era preceded the current policy for HB exception while the 2010-2014 era followed its implementation. Differences in age at listing, recipient gender, waitlist time, and post-transplant patient survival between the two groups were calculated. To examine the difference between the number of patients listed in each era, a one-sample binomial test was used. Independent samples Mann-Whitney U testing was performed to compute differences in means between the two groups, while Pearson's Chi-Squared was employed for

Table 1 Pediatric patients transplanted for hepatoblastoma at our center before (Era 1) and after (Era 2) implementation of pediatric end-stage liver disease exception points for hepatoblastoma			
	Era 1 (1987-2009)	Era 2 (2010-2014)	Significance (*P*-value)
Total patients listed for transplantation	7	14	0.189
Gender			0.557
Male	57.10%	35.70%	
Female	42.90%	64.30%	
Age at listing (yr)	5.4	2	0.094
Age at transplant (yr)	5.6	2.1	0.110
Waitlist time (d)	45.6	25.4	0.025

Figure 2 Patient survival by Era of transplantation.

differences in frequencies. Actuarial survival was assessed *via* the Kaplan-Meier Method. All statistical computations were performed with SPSS version 22 (IBM Armonk, New York).

Descriptive statistics for patients transplanted in each era are displayed in Table 1. A statistically similar number of patients were transplanted in each group (7 *vs* 14, *P* = 0.189). Similarly, there was no significant difference in gender, age at listing, and age at transplantation between the two eras. Patients listed for transplantation with the current HB exception criteria experienced a shorter waitlist time (45.5 d *vs* 25.4 d, *P* = 0.025).

Figure 2 demonstrates patient survival in our center before and after implementation of the revised HB exception policy. From 1987-2009, 30-d, one-year, and five-year survival following liver transplant in our center was 98.6%, 87.0%, and 77.4%, respectively. In comparison, 30-d, one-year survival following transplantation from 2010-2014 was 97.1% and 90.5%. Statistically, patient survival is similar between the two eras (*P* = 0.7).
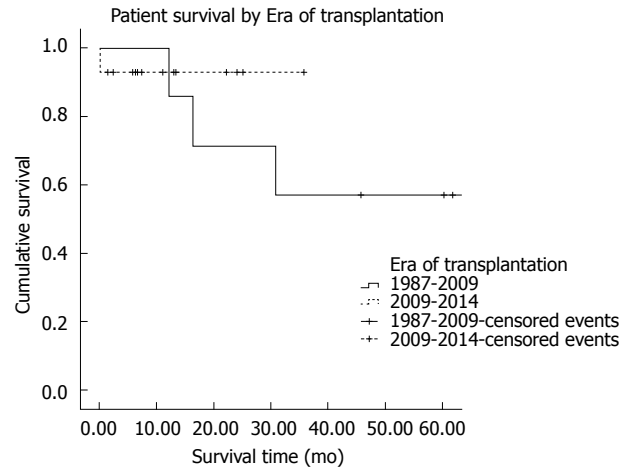
CONCLUSION

In conclusion, standard treatment with neoadjuvant chemotherapy, surgical resection followed by adjuvant chemotherapy is a good option for most pediatric and adolescent patients with HB. For those with tumors that are unresectable or unresponsive to chemotherapy, combined treatment with chemotherapy and liver transplantation is an excellent option. PELD exception points for HB have decreased the wait time for most patients listed for transplant but it is too soon to determine if this translates into increased survival for the group.

ACKNOWLEDGMENTS

REFERENCES

1   **Ries LAG**, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, Bunin GR (eds). Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975-1995. National Cancer Institute, SEER Program. Bethesda, MD, NIH. Pub. 1999: 99-4649 Available from: URL: http://seer.cancer.gov/archive/publications/childhood/
2   **Ismail H**, Broniszczak D, Kaliciński P, Dembowska-Bagińska B, Perek D, Teisseyre J, Kluge P, Kościesza A, Lembas A, Markiewicz M. Changing treatment and outcome of children with hepatoblastoma: analysis of a single center experience over the last 20 years. *J Pediatr Surg* 2012; **47**: 1331-1339 [PMID: 22813792 DOI: 10.1016/j.jpedsurg.2011.11.073]
3   **Howlader N,** Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). National Cancer Institute. Based on November 2011 SEER data submission, posted to the SEER web site, April 2012 [Updated August 20, 2012]. Available from: URL: http://seer.cancer.gov/archive/csr/1975_2009_pops09/
4   **Litten JB**, Tomlinson GE. Liver tumors in children. *Oncologist* 2008; **13**: 812-820 [PMID: 18644850 DOI: 10.1634/theoncologist.2008-0011]
5   **Giardiello FM**, Offerhaus GJ, Krush AJ, Booker SV, Tersmette AC, Mulder JW, Kelley CN, Hamilton SR. Risk of hepatoblastoma in familial adenomatous polyposis. *J Pediatr* 1991; **119**: 766-768 [PMID: 1658283]
6   **DeBaun MR**, Tucker MA. Risk of cancer during the first four years of life in children from The Beckwith-Wiedemann Syndrome Registry. *J Pediatr* 1998; **132**: 398-400 [PMID: 9544889 DOI: 10.1016/S0022-3476(98)70008-3]
7   **Spector LG**, Birch J. The epidemiology of hepatoblastoma. *Pediatr Blood Cancer* 2012; **59**: 776-779 [PMID: 22692949 DOI: 10.1002/pbc.24215]
8   **Lai TT**, Bearer CF. Iatrogenic environmental hazards in the neonatal intensive care unit. *Clin Perinatol* 2008; **35**: 163-181, ix [PMID: 18280881 DOI: 10.1016/j.clp.2007.11.003]
9   **Weinberger B**, Laskin DL, Heck DE, Laskin JD. Oxygen toxicity in premature infants. *Toxicol Appl Pharmacol* 2002; **181**: 60-67 [PMID: 12030843 DOI: 10.1006/taap.2002.9387]
10   **Davies JQ**, de la Hall PM, Kaschula RO, Sinclair-Smith CC, Hartley P, Rode H, Millar AJ. Hepatoblastoma--evolution of management and outcome and significance of histology of the resected tumor. A 31-year experience with 40 cases. *J Pediatr Surg* 2004; **39**: 1321-1327 [PMID: 15359384 DOI: 10.1016/j.jpedsurg.2004.05.020]
11   **Brown J**, Perilongo G, Shafford E, Keeling J, Pritchard J, Brock P, Dicks-Mireaux C, Phillips A, Vos A, Plaschkes J. Pretreatment prognostic factors for children with hepatoblastoma-- results

from the International Society of Paediatric Oncology (SIOP) study SIOPEL 1. *Eur J Cancer* 2000; **36**: 1418-1425 [PMID: 10899656 DOI: 10.1016/S0959-8049(00)00074-5]

12 **Pham TH**, Iqbal CW, Grams JM, Zarroug AE, Wall JC, Ishitani MB, Nagorney DM, Moir C. Outcomes of primary liver cancer in children: an appraisal of experience. *J Pediatr Surg* 2007; **42**: 834-839 [PMID: 17502194 DOI: 10.1016/j.jpedsurg.2006.12.065]

13 **Zsíros J**, Maibach R, Shafford E, Brugieres L, Brock P, Czauderna P, Roebuck D, Childs M, Zimmermann A, Laithier V, Otte JB, de Camargo B, MacKinlay G, Scopinaro M, Aronson D, Plaschkes J, Perilongo G. Successful treatment of childhood high-risk hepatoblastoma with dose-intensive multiagent chemotherapy and surgery: final results of the SIOPEL-3HR study. *J Clin Oncol* 2010; **28**: 2584-2590 [PMID: 20406943 DOI: 10.1200/JCO.2009.22.4857]

14 **Ortega JA**, Douglass EC, Feusner JH, Reynolds M, Quinn JJ, Finegold MJ, Haas JE, King DR, Liu-Mares W, Sensel MG, Krailo MD. Randomized comparison of cisplatin/vincristine/fluorouracil and cisplatin/continuous infusion doxorubicin for treatment of pediatric hepatoblastoma: A report from the Children's Cancer Group and the Pediatric Oncology Group. *J Clin Oncol* 2000; **18**: 2665-2675 [PMID: 10894865]

15 **Perilongo G**, Maibach R, Shafford E, Brugieres L, Brock P, Morland B, de Camargo B, Zsiros J, Roebuck D, Zimmermann A, Aronson D, Childs M, Widing E, Laithier V, Plaschkes J, Pritchard J, Scopinaro M, MacKinlay G, Czauderna P. Cisplatin versus cisplatin plus doxorubicin for standard-risk hepatoblastoma. *N Engl J Med* 2009; **361**: 1662-1670 [PMID: 19846851 DOI: 10.1056/NEJMoa0810613]

16 **Zhang YT**, Feng LH, Zhong XD, Wang LZ, Chang J. Single-agent cisplatin treatment of children with high-risk hepatoblastoma. *J Pediatr Hematol Oncol* 2014; **36**: 271-275 [PMID: 24309602 DOI: 10.1097/MPH.0000000000000072]

17 **Finegold MJ**. Chemotherapy for suspected hepatoblastoma without efforts at surgical resection is a bad practice. *Med Pediatr Oncol* 2002; **39**: 484-486 [PMID: 12228904]

18 **Carceller A**, Blanchard H, Champagne J, St-Vil D, Bensoussan AL. Surgical resection and chemotherapy improve survival rate for patients with hepatoblastoma. *J Pediatr Surg* 2001; **36**: 755-759 [PMID: 11329582 DOI: 10.1053/jpsu.2001.22953]

19 **Otte JB**, Pritchard J, Aronson DC, Brown J, Czauderna P, Maibach R, Perilongo G, Shafford E, Plaschkes J. Liver transplantation for hepatoblastoma: results from the International Society of Pediatric Oncology (SIOP) study SIOPEL-1 and review of the world experience. *Pediatr Blood Cancer* 2004; **42**: 74-83 [PMID: 14752798 DOI: 10.1002/pbc.10376]

RESEARCH REPORT

1400W reduces ischemia reperfusion injury in an *ex-vivo* porcine model of the donation after circulatory death kidney donor

Sarah A Hosgood, Phillip J Yates, Michael L Nicholson

Sarah A Hosgood, Phillip J Yates, Michael L Nicholson, Department of Infection, Immunity and Inflammation, Transplant Group, University of Leicester, LE5 4PW Leicester, United Kingdom

Sarah A Hosgood, Michael L Nicholson, Leicester General Hospital, University Hospitals of Leicester, LE5 4PW Leicester, United Kingdom

Author contributions: Hosgood SA, Yates PJ and Nicholson ML designed the study; Hosgood SA and Yates PJ carried out the experiments; Hosgood SA and Yates PJ analysed the data; Hosgood SA and Yates PJ wrote the manuscript; Nicholson ML revised the manuscript and approved the final version.

Correspondence to: Dr. Sarah A Hosgood, Department of Infection, Immunity and Inflammation, Transplant Group. University of Leicester, Gwendolen Road, LE5 4PW Leicester, United Kingdom. sarahhosgood@hotmail.com

Telephone: +44-116-2584658 Fax: +44-116-2490064

Received: May 28, 2014   Revised: July 8, 2014
Accepted: October 31, 2014
Published online: December 24, 2014

Abstract

AIM: To investigate the effects of 1400W-a selective inducible nitric oxide synthase (iNOS) inhibitor in a model of donation after circulatory death (DCD) kidneys.

METHODS: Porcine kidneys were retrieved after 25 min warm ischemia. They were then stored on ice for 18 h before being reperfused *ex vivo* with oxygenated autologous blood on an isolated organ perfusion system. The selective iNOS inhibitor 1400W (10 mg/kg) was administered before reperfusion ($n = 6$) vs control group ($n = 7$). Creatinine (1000 μmol/L) was added to the system, renal and tubular cell function and the level of ischemia reperfusion injury were assessed over 3 h of reperfusion using plasma, urine and tissue samples.

RESULTS: Kidneys treated with 1400W had a higher level of creatinine clearance (CrCl) [area under the curve (AUC) CrCl: 2.37 ± 0.97 mL/min per 100 g *vs* 0.96 ± 0.32 mL/min per 100 g, $P = 0.004$] and urine output [Total: 320 ± 96 mL *vs* 156 ± 82 mL, $P = 0.008$]. There was no significant difference in levels of fractional excretion of sodium (AUC, Fr ex Na+: Control, 186.3% ± 81.7%.h *vs* 1400W, 153.4% ± 12.1%.h, $P = 0.429$). Levels of total protein creatinine ratio were significantly lower in the 1400W group after 1 h of reperfusion (1h Pr/Cr: 1400W 9068 ± 6910 mg/L/mmol/L *vs* Control 21586 ± 5464 mg/L/mmol/L, $P = 0.026$). Levels of 8-isoprostane were significantly lower in the 1400W group [8-iso/creatinine ratio: Control 239 ± 136 pg/L/mmol/L *vs* 1400W 139 ± 47 pg/L/mmol/L, $P = 0.041$].

CONCLUSION: This study demonstrated that 1400W reduced ischaemia reperfusion injury in this porcine kidney model of DCD donor. Kidneys had improved renal function and reduced oxidative stress.

Core tip: It is important to examine the effects of therapies that can reduce ischemia reperfusion injury particularly in donation after circulatory death donor kidneys. The biological role of inducible nitric oxide synthase (iNOS) is somewhat controversial. This study uses a large animal *ex vivo* model to assess the effects of 1400W, an iNOS inhibitor. The model provides a functional assessment of each kidney, providing a close simulation to clinical transplantation. The study found that 1400W improved early renal function and reduced oxidative stress.

Hosgood SA, Yates PJ, Nicholson ML. 1400W reduces ischemia reperfusion injury in an *ex-vivo* porcine model of the donation af-

INTRODUCTION

The pathophysiology of ischemia reperfusion (I/R) injury is a complex action involving many intercellular and molecular processes. It is characterised by the up-regulation of inflammatory processes, activation of endothelial cells, generation and release of reactive oxygen species (ROS), migration of inflammatory leucocytes, cellular oedema, cell membrane damage, apoptosis and necrosis[1-3]. Severe I/R injury causes significant disruption to the microcirculation and is associated with high rates of delayed graft function, primary non function and acute rejection after kidney transplantation[4,5]. This is of particular significance in kidneys from marginal or donation after circulatory death (DCD) donors that sustain both a period of warm and cold ischemic injury prior to transplantation. It is therefore important to investigate therapies to alleviate injury to improve the outcome of DCD transplantation.

Nitric oxide (NO) is an important mediator of normal biological processes. It is a free radical produced by all mammalian cells from the synthesis of L-arginine and oxygen, by the enzyme NO synthase (NOS)[6]. It is capable of regulating local blood flow, scavenging free radicals and inhibiting platelet and leukocyte activation[6,7]. There are three different isoforms of NO; neuronal, endothelial (eNOS) and inducible (iNOS)[8].

The biological role of iNOS is somewhat controversial[9]. iNOS is known to be up-regulated by certain disease states such as inflammation, ischemia and during reperfusion after transplantation[10]. Although NO is generally regarded as cytoprotective, excess NO derived from iNOS during these states can contribute to the injury process[11,12]. NO can augment I/R injury by reacting with superoxide generated by excess ROS to form peroxynitrite, causing severe oxidative damage ands cellular injury[10]. It also has a role in the mediation of neutrophil activation, although the processes are not fully understood[9].

Evidence suggests that the effects and role of iNOS are influenced by the microenvironment and bioavailability of the other forms of NO[9] and iNOS inhibitors have been shown to reduce I/R injury[11-13]. However, these have principally been studied in small animal models after a sole period of warm ischemic injury and reperfusion. The aim of this study was to assess the effects of 1400W a selective iNOS inhibitor on I/R injury in a model of the DCD donor using porcine kidneys.

MATERIALS AND METHODS

Under Home Office regulations (Scientific Act 1986, Schedule 1 procedure) female large white pigs (60-70 kg) were killed by electrocution followed by exsanguination. Approximately 2 L of blood was collected into a sterile receptacle containing 25000 units of heparin (Multiparin®; CP Pharmaceuticals, Wrexham, United Kingdom). The blood was then transferred into CPDA-1 blood bags (Baxter Healthcare, Thetford, United Kingdom) for storage at 4 ℃.

The kidneys were retrieved after 25 min of *in situ* warm ischemia and flushed with 500 mL of hyperosmolar citrate (Soltran; Baxter Healthcare) at 4 ℃ infused at a hydrostatic pressure of 100 cm H_2O. Kidneys were then placed in ice for a period of 18 h.

Reperfusion

After the preservation period kidneys were prepared for *ex vivo* reperfusion. The renal artery, vein and ureter were cannulated and kidneys flushed with Ringer's at 4 ℃ (Baxter Healthcare, United Kingdom) to remove the preservation solution before being placed immediately on the isolated organ preservation system. They were then reperfused with oxygenated autologous blood for 3 h at a temperature of 38 ℃ and set mean arterial pressure of 85 mmHg. The system has been previously described[14]. Creatinine (Sigma-Aldrich, Steinheim, Germany) was added to the perfusate to achieve an initial circulating concentration of 1000 μmol/L.

1400W (Sigma-Aldrich) -a highly selective iNOS inhibitor was prepared before use and stored at -20 ℃ until required.

Experimental design

Kidneys were divided into two groups; Control ($n = 7$) and 1400W at a dose of 10 mg/kg per kidney weight ($n = 6$). 1400W was added as a bolus to the arterial arm of the circuit 15 min before reperfusion of the kidney.

Parameters

Renal blood flow (RBF) and mean arterial pressure (MAP) were recorded continuously and intrarenal resistance (IRR) calculated (MAP/RBF). Urine output was also measured during reperfusion.

Biochemical analysis of serum and urine samples was carried out at hourly intervals. The following parameters were calculated:

Creatinine clearance (urinary creatinine × urinary volume/plasma creatinine), fractional excretion of sodium [(urinary sodium × urine volume) / (glomerular filtration rate × plasma sodium) × 100)] and the urinary total protein (mg/L) to creatinine (mmol/L) ratio.

Blood gas analysis was used to record P_aO_2, P_vO_2 and acid-base homeostasis. Oxygen consumption [(P_aO_2-venous P_vO_2) × flow rate/weight] was calculated

8-Isoprostane

Urine samples were taken at 1 and 3 h of reperfusion and stored at -80 ℃ until analyses. Levels of urine 8-isoprostane were determined by ELISA (Cayman Chemical Co, MI, United States). Urine samples were centrifuged at 10000 *g* for 2 min and the supernatant taken for analysis. Samples were diluted 10 fold prior to analysis. The sample and

standards were added in duplicate to the ELISA plate together with an 8-isoprostane-acetylcholinesterase (AChE) conjugate and incubated for 18 h at 4 ℃. During incubation 8-isoprostane present in the sample competed with the 8-isoprostane AChE conjugate for the 8-isoprostane rabbit antiserum binding sites on the pre-coated plate. The plate was then washed and developed by the addition of the substrate to AChE. The plate was read at 405 nm after colour development for 90 min.

Total nitric oxide

Plasma samples were taken pre and 3 h after reperfusion and urine samples taken at 1 and 3 h of reperfusion and stored at -80 ℃ until analyses. Urine levels of NO were quantified using the total NO test kit (Assay Designs, MI, United States) according to the manufacturers' instructions. This assay is based on the conversion of NO to nitrate and the subsequent conversion of nitrate to nitrite by the enzyme nitrate reductase. Nitrite is then detected colorimetrically at 540 nm as an azodye product of the Griess reaction. Briefly, plasma and urine sample were centrifuged at 10000 *g* and the supernatant withdrawn. Fifty μL of each sample were added in duplicate to a micro titre test plate. Twenty-five μL NADH and 25 μL nitrate reductase were added to each well and incubated at 37 ℃ for 30 min. One hundred μL Griess reagents (sulphanilamide and N-(l-Naphthyl) ethylenediamine in 2M HCl) were then added and incubated at room temperature for 10 min. Optical density was then read at 540 nm using a spectrophotometer and the concentration calculated using standards.

Histology

Wedge biopsies were taken after 25 min warm ischemia and after 3 h of reperfusion, fixed in 10% formal saline, dehydrated and embedded in paraffin wax. Sections of 4 μm were cut and stained with haematoxylin and eosin for evaluation using light microscopy. Sections were scored over five fields, assessing changes in four morphological variables; Tubular dilation, Tubular debris, vaculolation and interstitial oedema. Samples were scored from 0 to 3 according to the level of damage; 0 representing normal, 1 representing mild, 2 representing moderate and 3 representing severe morphological changes.

Myeloperoxidase activity

Immunohistochemical staining of MPO, a marker mainly for neutrophil granulocytes, was undertaken on post reperfusion paraffin sections using a DAKO ChemMate EnVision™ Detection Kit (DAKO, Glostrup, Denmark). The sections were digested by 40 μg/mL proteinase K for 15 min at 37 ℃ then blocked by peroxidase-blocking reagent. The sections were labelled by an anti-MPO antibody (1:600, DAKO) at 4 ℃ overnight. The antibody binding was revealed by 3'-amino-9-ethylcarbazole. MPO+ cells in the tubular, interstitial and glomeruli were semi-quantitatively scored by counting the number of positive cells in 20 fields at 400 × magnification.

Statistical analysis

Values are presented as mean ± SD. Levels of continuous variables such as RBF were plotted against time and the area under the curve (AUC) for individual perfusion experiments was calculated using Excel® software (Microsoft, Reading, United Kingdom) and Graphpad Prism (GraphPad Software, San Diego California, United States).

Mean AUC values were compared using Mann Whitney *U*-Test (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, United States). Correlations between parameters were made with Spearman's non parametric rank correlation. $P < 0.050$ was taken as statistically significant.

RESULTS

Renal function

There was a significant fall in the level of RBF and an increase in intra-renal resistance in the 1400W group after 10 and 15 min of reperfusion compared to the control kidneys (RBF, $P = 0.002$ and 0.005, respectively; IRR, $P = 0.005$ and 0.014, respectively; Figure 1A and B). RBF then recovered and IRR fell with no significant difference between the groups throughout the rest of the reperfusion period (AUC, RBF: Control 270 ± 86 mL/min/100 g.h *vs* 1400W 274 ± 143 mL/min/100 g.h, $P = 0.999$; IRR: Control 13.4 ± 7.3 mmHg/min.h *vs* 1400W 17.8 ± 8.5 mmHg/min.h, $P = 0.234$). The level of oxygen consumption after reperfusion was higher in the 1400W group after 3 h of reperfusion but this did not reach statistical significance (3 h: Control 28.0 ± 13.9 mL/min/g *vs* 1400W 36.7 ± 22.8 mL/min/g, $P = 0.731$).

Levels of creatinine clearance were significantly higher after 1 and 2 h of reperfusion in the 1400W group compared to the control ($P = 0.026$ and 0.009 respectively; Figure 2A) and the AUC creatinine clearance was significantly higher (AUC, CrCl: 1400W 2.37 ± 0.97 mL/min/100 g.h *vs* Control 0.96 ± 0.32 mL/min/100 g.h, $P = 0.004$). Levels of serum creatinine fell more quickly in the 1400W group but the difference with controls was only marginally significant at the end of reperfusion ($P = 0.073$; Figure 2B).

Tubular function

There was no significant difference in levels of fractional excretion of sodium (AUC, Fr ex Na+: Control 186.3% ± 81.7%.h *vs* 1400W 153.4% ± 12.1%.h, $P = 0.429$), although total urine output was significantly higher in the 1400W group (Total urine output: 1400W 320 ± 96 *vs* Control, 156 ± 83 mL, $P = 0.008$).

Levels of total protein creatinine ratio were significantly lower in the 1400W group after 1 h of reperfusion (1h Pr/Cr: 1400W 9068 ± 6910 mg/L/mmol/L *vs* Control 21586 ± 5464 mg/L/mmol/L, $P = 0.026$). There was no further difference in the levels between the groups after 2 and 3 h of reperfusion ($P = 0.662$ and 0.628, respectively).

Acid base balance

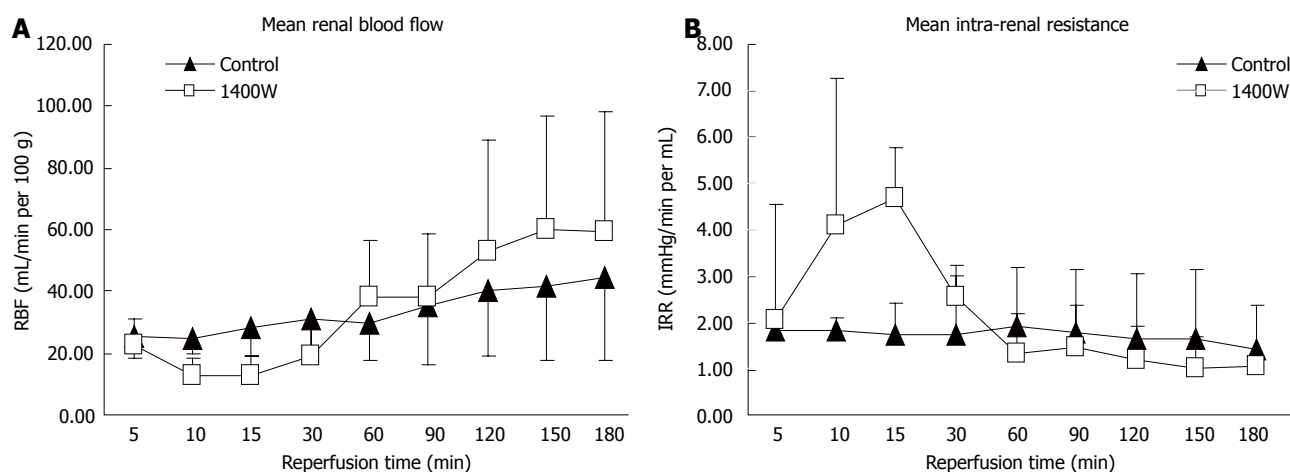Levels of pH fell significantly in both groups with no

Figure 1 Mean renal blood flow over 3 h of reperfusion in the Control and 1400W groups. Area under the curve (AUC), *P* = 0.999 (A); Mean intrarenal resistance over 3 h of reperfusion in the Control and 1400W groups, AUC, *P* = 0.234 (B). Mann Whitney *U*-test. RBF: Mean renal blood flow.
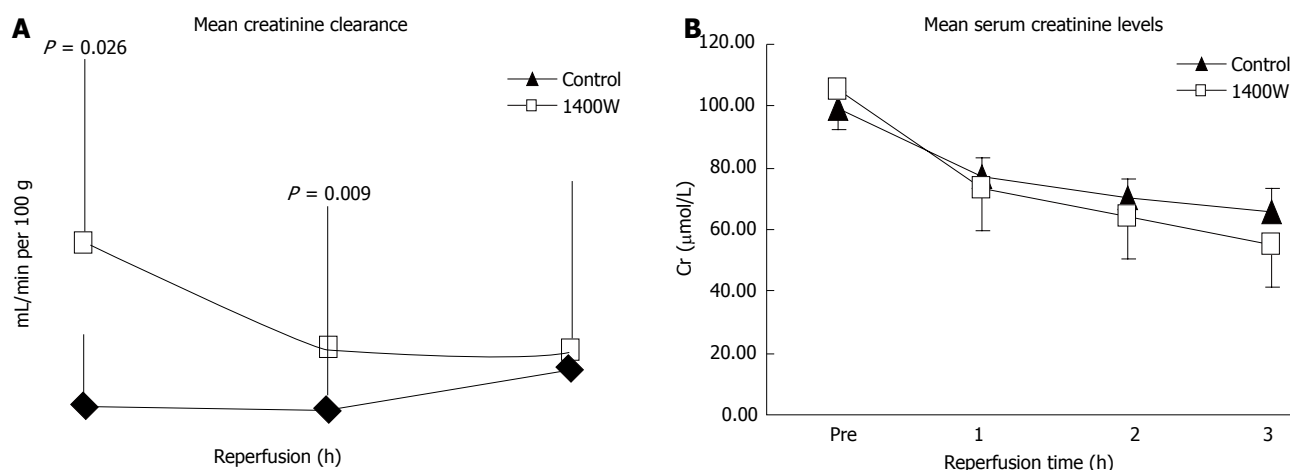

Figure 2 Mean creatinine clearance over 3 h of reperfusion in the Control and 1400W groups. (*P* = 0.026 and 0.009 after 1 and 2 h, respectively) (A); Mean serum creatinine levels over 3 h of reperfusion in the Control and 1400W groups, *P* > 0.050 between groups (B). Mann Whitney *U*-test.

Table 1 Acid base balance, levels of pH, bicarbonate and potassium pre and 3 h after reperfusion

	Control		1400W	
	Pre	3 h	Pre	3 h
pH	7.43 ± 0.03	7.30 ± 0.08	7.47 ± 0.04	7.24 ± 0.04
Bicarbonate (mmol/L)	21.2 ± 1.4	17.3 ± 3.0	23.4 ± 1.6	17.6 ± 2.1
Potassium (mmol/L)	5.5 ± 0.3[a]	10.7 ± 1.3	5.9 ± 0.2	11.9 ± 0.3

[a]*P* < 0.05 between groups. Mann Whitney *U*-test.

significant difference between groups at 3 h (*P* = 0.100; Table 1). There was also no significant difference in levels of bicarbonate or potassium after 3 h (*P* = 0.628 and 0.295, respectively; Table 1). Pre levels of potassium were significantly lower but within normal range in the control group compared to 1400W (*P* = 0.002; Table 1).

Oxidative damage/inflammation
Urinary levels of 8-isoprostane were significantly lower in the 1400W group after 3 h of reperfusion compared to

the control group (*P* = 0.041; Figure 3A).

There was no significant difference in the pre or 3 h reperfusion plasma concentrations of total NO (Pre: Control 73.6 ± 43.1 pg/mL, 1400W 79.9 ± 14.5 pg/mL; 3h: Control 48.7 ± 21.7 pg/mL, 1400W 63.9 ± 20.2 pg/mL). Urinary levels of total nitric oxide were significantly higher in the 1400W group after 1 and 3 h of reperfusion (*P* = 0.002 and 0.002, respectively; Figure 3B).

There was a significantly higher amount of MPO positive cells in the control group compared to the 1400W (*P* = 0.002; Figure 3C). Positive cells were largely localised in the interstitium.

Histology
Baseline biopsies showed an increased level of tubular dilatation in the 1400W group compared to the control (*P* = 0.001; Table 2) and a higher level of interstitial oedema in the control group compared to the 1400W (*P* = 0.032; Table 2). After 3 h of reperfusion there was a significant increase in tubular dilatation and vacuolation in the control group (*P* = 0.0003 and 0.033, respectively; Table 2) and
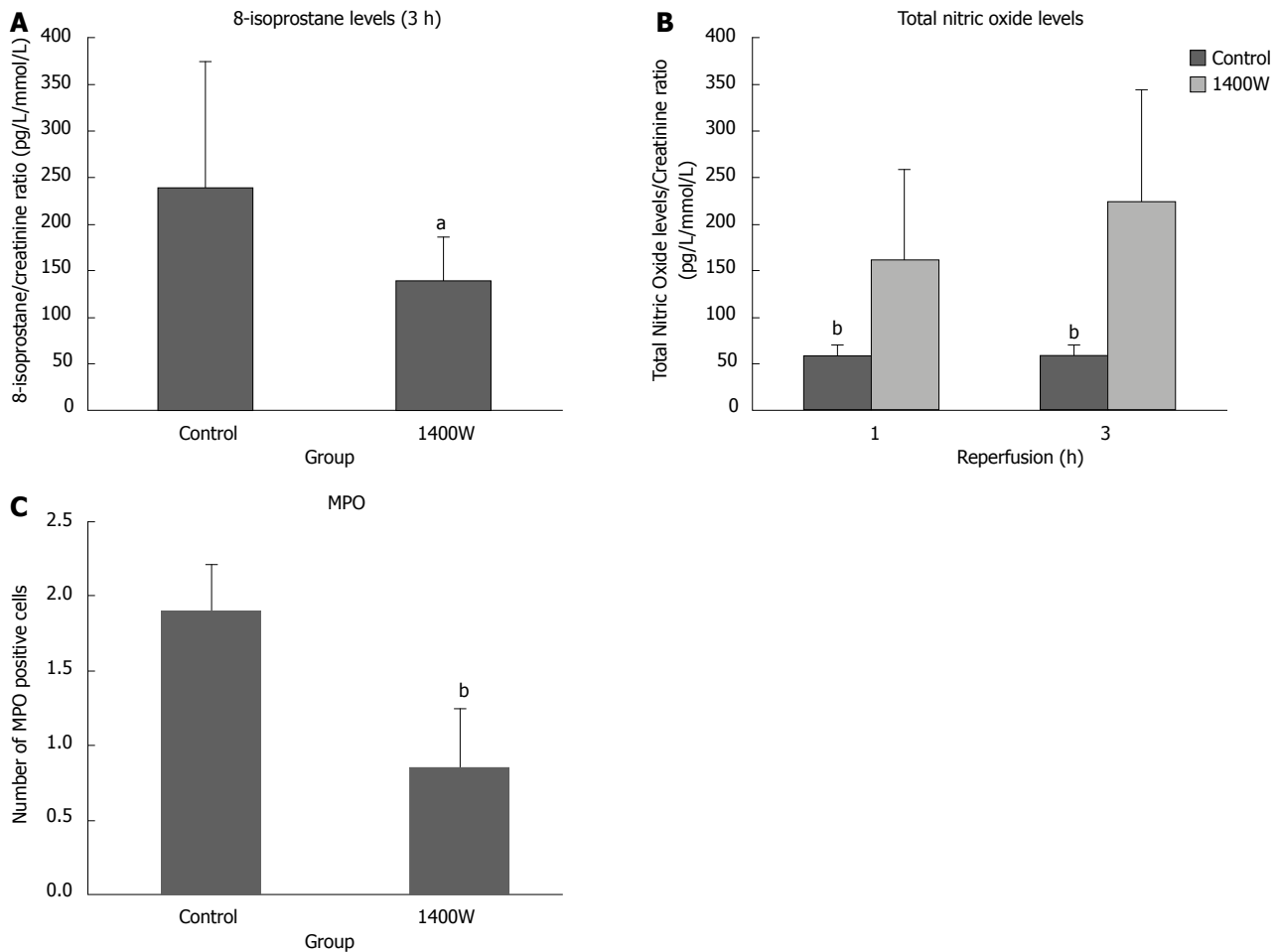
Figure 3 Urinary levels of 8-isoprostane after 3 h of reperfusion in the Control and 1400W groups (^aP = 0.041 between groups) (A); Levels of total nitric oxide in the urine after 1 and 3 h of reperfusion Control and 1400W groups (^bP = 0.002 and 0.002, respectively) between groups (B); Myeloperoxidase score after 3 h of reperfusion in the Control and 1400W (^bP = 0.002) between groups (C). Mann Whitney *U*-test, scored by counting the number of positive cells in 20 fields (× 400). MPO: Myeloperoxidase.

Table 2 Histology score

	Control		1400W	
	Pre	**Post**	**Pre**	**Post**
Tubular dilatation	0.91 ± 0.68	1.60 ± 0.60[a]	2.03 ± 0.80[b]	1.63 ± 0.81
Tubular debris	1.44 ± 0.50	1.14 ± 0.73	1.47 ± 0.50	2.07 ± 0.74[a,b]
Vacuolation	0.52 ± 0.76	1.09 ± 1.091	0.50 ± 0.60	1.00 ± 0.91[a]
Interstitial oedema	1.33 ± 0.48[b]	1.29 ± 0.52	0.93 ± 0.70	1.47 ± 0.82[a]

Pre and post reperfusion biopsies in the control and 1400W groups. Biopsies were scored over 5 fields assessing tubular dilatation, tubular debris, vacuolation and interstitial oedema. $^aP \leqslant 0.05$ between time points, $^bP \leqslant 0.05$ between groups. Mann Whitney *U*-test.

tubular debris, vacuolation and interstitial oedema in the 1400W group (P = 0.003, 0.040 and 0.011, respectively; Table 2). The 1400W group had a significantly higher level of tubular debris after reperfusion compared to the control (P = 0.0001; Table 2).

DISCUSSION

This study demonstrated that the administration of

1400W, a selective iNOS inhibitor, reduced the level I/R injury in porcine kidneys that were subjected to warm and cold ischemic injury. Kidneys had a higher level of creatinine clearance, reduced oxidative stress and neutrophil infiltration during reperfusion compared to untreated kidneys.

NO is generally regarded as cytoprotective: scavenging free radicals, relaxing the endothelium, inhibiting platelet aggregation and reducing neutrophil adherence[6,15].

However, the biological effects of NO derived from iNOS can be either deleterious or beneficial, depending on the disease state[9]. iNOS is known to be upregulated during ischemia and reperfusion and is widely expressed throughout the vasculature, tubule cells and glomeruli in the kidney. It is also expressed on monocytes, macrophages and neutrophils[16].

Warm and cold ischemic injury sustained before transplantation exacerbates the level of I/R injury[4,14]. The anoxic conditions, depletion of adenosine triphosphate (ATP) and accumulation of toxic substances results in severe cellular disruption[5]. The level of warm and cold ischemic injury in this porcine kidney model of the DCD donor was sufficient to cause severe renal dysfunction, alteration of acid base homeostasis and histological change during reperfusion. Kidneys treated with 1400W showed some ameliorate of injury with higher levels of creatinine clearance, urine output and reduced levels of protein excretion and oxidative stress compared to untreated kidneys. However, iNOS inhibition did not improve tubular cell function, acid base balance or reduce the level of histological injury.

1400W is a selective inhibitor of iNOS. It is relatively long acting and has been used successfully in several rat I/R injury models to reduce injury[13,17]. Mark *et al*[17] found that 1400W administered 20 min before ischemia, improved renal function and reduced the level of tubular dysfunction. Another study compared the effects of 1400W and melatonin: an antioxidant, iNOS inhibitor and scavenger of peroxynitrite[13]. They found that both agents reduced the level of oxidative damage, albeit melatonin to a greater extent due to its scavenging properties. Other selective iNOS inhibitors such as, L-N6-(L-iminoethyl) lysine (L-NIL)[16] and the novel iNOS inhibitor GW274150 have also been used to improved glomerular and tubular function and reduce levels of NO in rat models of I/R injury[12] and FR260330 in Vervet monkeys[18].

A key role of NO is the modulation of blood flow and NO derived from eNOS is thought to be particularly important during early reperfusion[6-8]. In this present study there was a marked reduction in renal blood flow and increase in intra-renal resistance during the first 15 min of reperfusion with iNOS inhibition. This warrants further investigation but was possibly due to low levels of NO derived from eNOS during the early reperfusion phase as a result of the level of ischemic injury and inhibition of iNOS. This suggests an important role for iNOS in the control of homeostasis during this acute phase.

The activation of neutrophils during reperfusion is a principle mediator of I/R injury causing microcirculatory disruption and release of superoxide[19]. NO can inhibit the expression of P-selectin on endothelial cells, preventing rolling, and expression of intercellular and vascular cell adhesion molecules-1 (ICAM-1, VCAM-1) reducing neutrophil adhesion and infiltration[11,17]. NO derived from iNOS is thought to enhance endothelial-leukocyte activation and inhibitors have demonstrated a reduction in neutrophil activation[12]. Contrary to this, in a model of endotoxic shock, NO released by cNOS and iNOS reduced neutrophil migration due to decreased rolling and adhesion[19]. Levels of neutrophil infiltration were reduced by almost half after iNOS inhibition in this present study. Hickey *et al*[9] suggested that the role of iNOS varies according to the cell type and location in which it is expressed, and that leukocyte recruitment could alter according to the type of inflammatory response. Evidence from this study supports the findings of others that iNOS inhibition prevents neutrophil infiltration during I/R injury, although the exact mechanisms are still to be elucidated. Nonetheless, the activation of neutrophils has also an important role in regeneration and repair and it is likely that a balance is needed to ensure optimal graft function[20].

Plasma concentrations of total NO were not affected by iNOS inhibition in this study and perhaps real time analysis of NO or the measurement of eNOS and iNOS expression may have provided more information on the significance and bioavailability of NO in this model. Urinary levels of total NO were however, significantly increased during reperfusion after iNOS inhibition possibly indicating a higher level of proximal tubular cell injury. Nonetheless, high levels were not associated with tubular cell dysfunction. Urinary levels of 8-isoprostane, a marker of lipid peroxidation, generated by free radical catalyzed attack on arachidonic acid, were significantly lower after the administration of 1400W[21]. Lower levels of lipid peroxidation suggest less oxidative damage and formation of peroxynitrite during reperfusion possibly due to less neutrophil infiltration.

In conclusion, the administration of 1400W a selective inhibitor of iNOS improved renal function, reduced oxidative stress and neutrophil infiltration in this porcine kidney model of the DCD. This study supports the evidence of the deleterious effects of iNOS during I/R injury.

COMMENTS

Background
The shortage of organ donors has led to increasing use of marginal donors. Although a valuable source of kidneys for transplantation these kidneys have more injury and a high percentage do not function immediately after transplantation. This injury is in part, mediated by an inflammatory action immediately after transplantation: ischaemia reperfusion injury. Targeting this inflammatory process by using therapies may improve early graft function. Despite an abundance of research into such therapeutic agents, none are used clinically as part of standard practice.

Research Frontiers
Inducible nitric oxide synthase (iNOS) is produced naturally by the body and thought to play a role in the injury process after transplantation. 1400W is an iNOS inhibitor that has been shown to reduce injury and improve graft function. However, the research hotspot is that it has not been trialed in a clinically relevant model such as the porcine kidney with similar ischaemic insults that human kidneys are subject to.

Innovations and breakthroughs
iNOS inhibitors such as 1400W have previously been used to reduce injury and improve renal function. However, some studies have found no benefit in inhibiting iNOS. Furthermore, most of these studies have used small animal models which do not necessarily represent the effect in humans. In this present study the authors used a porcine model with similar periods of ischaemic injury to

assess the effects of 1400W. Porcine kidneys have similar anatomy to human kidneys and their physiological response to ischaemic injury is also comparable. The authors found that 1400W significantly reduced the injury processes and improved renal function. This suggests that iNOS plays an important role in the injury process after transplantation.

Applications
This study suggests that iNOS inhibitors are a potential therapy for reducing renal ischaemia reperfusion injury after transplantation.

Terminology
Ischaemia reperfusion injury is a natural inflammatory like reaction that a transplanted organ suffers. It involves a cascade of events that can cause irreversible cellular damage. This can reduce renal function and also limit graft survival. Nitric oxide synthase (NOS) is a gaseous molecule that is produced naturally in the body. There are three different forms of NOS. Generally it has a protective role however iNOS is associated with inflammatory disease states.

Peer review
Ischaemia reperfusion injury is a critical problem in the transplant field. This study reported that 1400W reduced ischaemia reperfusion injury in a porcine model of the donation after circulatory death donor. This paper is well written and the results of renal function, oxidative stress and histology in 1400W reveal the protection from I/R injury.

REFERENCES

1   **Ponticelli C**. Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant* 2014; **29**: 1134-1140 [PMID: 24335382 DOI: 10.1093/ndt/gft488]

2   **de Vries DK**, Lindeman JH, Tsikas D, de Heer E, Roos A, de Fijter JW, Baranski AG, van Pelt J, Schaapherder AF. Early renal ischemia-reperfusion injury in humans is dominated by IL-6 release from the allograft. *Am J Transplant* 2009; **9**: 1574-1584 [PMID: 19459788 DOI: 10.1111/j.1600-6143.2009.02675.x]

3   **Frangogiannis NG**. Chemokines in ischemia and reperfusion. *Thromb Haemost* 2007; **97**: 738-747 [PMID: 17479184]

4   **Gok MA**, Shenton BK, Pelsers M, Whitwood A, Mantle D, Cornell C, Peaston R, Rix D, Jaques BC, Soomro NA, Manas DM, Talbot D. Ischemia-reperfusion injury in cadaveric nonheart beating, cadaveric heart beating and live donor renal transplants. *J Urol* 2006; **175**: 641-647 [PMID: 16407015 DOI: 10.1016/S0022-5347(05)00170-9]

5   **Perico N**, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* 2004; **364**: 1814-1827 [PMID: 15541456 DOI: 10.1016/S0140-6736(04)17406-0]

6   **Vos IH**, Joles JA, Rabelink TJ. The role of nitric oxide in renal transplantation. *Semin Nephrol* 2004; **24**: 379-388 [PMID: 15252777 DOI: 10.1016/j.semnephrol.2004.04.009]

7   **Yates PJ**, Hosgood SA, Nicholson ML. A biphasic response to nitric oxide donation in an ex vivo model of donation after cardiac death renal transplantation. *J Surg Res* 2012; **175**: 316-321 [PMID: 21592523 DOI: 10.1016/j.jss.2011.03.073]

8   **Joubert J**, Malan SF. Novel nitric oxide synthase inhibitors: a patent review. *Expert Opin Ther Pat* 2011; **21**: 537-560 [PMID: 21338328 DOI: 10.1517/13543776.2011.556619]

9   **Hickey MJ**, Granger DN, Kubes P. Inducible nitric oxide synthase (iNOS) and regulation of leucocyte/endothelial cell interactions: studies in iNOS-deficient mice. *Acta Physiol Scand* 2001; **173**: 119-126 [PMID: 11678734 DOI: 10.1046/j.1365-201X.2001.00892.x]

10 **Joles JA**, Vos IH, Gröne HJ, Rabelink TJ. Inducible nitric oxide synthase in renal transplantation. *Kidney Int* 2002; **61**: 872-875 [PMID: 11849440 DOI: 10.1046/j.1523-1755.2002.00235.x]

11 **Korkmaz A**, Kolankaya D. Inhibiting inducible nitric oxide synthase with rutin reduces renal ischemia/reperfusion injury. *Can J Surg* 2013; **56**: 6-14 [PMID: 23187035 DOI: 10.1503/cjs.004811]

12 **Chatterjee PK**, Patel NS, Sivarajah A, Kvale EO, Dugo L, Cuzzocrea S, Brown PA, Stewart KN, Mota-Filipe H, Britti D, Yaqoob MM, Thiemermann C. GW274150, a potent and highly selective inhibitor of iNOS, reduces experimental renal ischemia/reperfusion injury. *Kidney Int* 2003; **63**: 853-865 [PMID: 12631066 DOI: 10.1046/j.1523-1755.2003.00802.x]

13 **Ersoz N**, Guven A, Cayci T, Uysal B, Turk E, Oztas E, Akgul EO, Korkmaz A, Cetiner S. Comparison of the efficacy of melatonin and 1400W on renal ischemia/reperfusion injury: a role for inhibiting iNOS. *Ren Fail* 2009; **31**: 704-710 [PMID: 19814638 DOI: 10.3109/08860220903085989]

14 **Hosgood SA**, Bagul A, Yang B, Nicholson ML. The relative effects of warm and cold ischemic injury in an experimental model of nonheartbeating donor kidneys. *Transplantation* 2008; **85**: 88-92 [PMID: 18192917 DOI: 10.1097/01.tp.0000296055.76452.1b]

15 **Lo Faro ML**, Fox B, Whatmore JL, Winyard PG, Whiteman M. Hydrogen sulfide and nitric oxide interactions in inflammation. *Nitric Oxide* 2014; **41**: 38-47 [PMID: 24929214 DOI: 10.1016/j.niox.2014.05.014]

16 **Legrand M**, Almac E, Mik EG, Johannes T, Kandil A, Bezemer R, Payen D, Ince C. L-NIL prevents renal microvascular hypoxia and increase of renal oxygen consumption after ischemia-reperfusion in rats. *Am J Physiol Renal Physiol* 2009; **296**: F1109-F1117 [PMID: 19225052 DOI: 10.1152/ajprenal.90371.2008]

17 **Mark LA**, Robinson AV, Schulak JA. Inhibition of nitric oxide synthase reduces renal ischemia/reperfusion injury. *J Surg Res* 2005; **129**: 236-241 [PMID: 16140334 DOI: 10.1016/j.jss.2005.06.019]

18 **Qi S**, Xu D, Ma A, Zhang X, Chida N, Sudo Y, Tamura K, Daloze P, Chen H. Effect of a novel inducible nitric oxide synthase inhibitor, FR260330, in prevention of renal ischemia/reperfusion injury in vervet monkeys. *Transplantation* 2006; **81**: 627-631 [PMID: 16495814 DOI: 10.1097/01.tp.0000199282.05021.0c]

19 **Dal Secco D**, Paron JA, de Oliveira SH, Ferreira SH, Silva JS, Cunha Fde Q. Neutrophil migration in inflammation: nitric oxide inhibits rolling, adhesion and induces apoptosis. *Nitric Oxide* 2003; **9**: 153-164 [PMID: 14732339 DOI: 10.1016/j.niox.2003.11.001]

20 **Ysebaert DK**, De Greef KE, Vercauteren SR, Ghielli M, Verpooten GA, Eyskens EJ, De Broe ME. Identification and kinetics of leukocytes after severe ischaemia/reperfusion renal injury. *Nephrol Dial Transplant* 2000; **15**: 1562-1574 [PMID: 11007823 DOI: 10.1093/ndt/15.10.1562]

21 **Waller HL**, Harper SJ, Hosgood SA, Bagul A, Yang B, Kay MD, Kaushik M, Nicholson ML. Biomarkers of oxidative damage to predict ischaemia-reperfusion injury in an isolated organ perfusion model of the transplanted kidney. *Free Radic Res* 2006; **40**: 1218-1225 [PMID: 17050175 DOI: 10.1080/10715760600907368]

Baishideng®

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com